SMART FOOD FOR HEALTHY, SUSTAINABLE AND RESILIENT FOOD SYSTEMS

EDITED BY: Stefania Grando, Seetha Anitha, Ramadjita Tabo, Taku Tsusaka, Ian Givens and Tshilidzi Madzivhandila
PUBLISHED IN: Frontiers in Sustainable Food Systems and Frontiers in Nutrition

frontiers Research Topics

ISSN 1664-8714
ISBN 978-2-88976-263-7
DOI 10.3389/978-2-88976-263-7

About Frontiers

Frontiers is more than just an open-access publisher of scholarly articles: it is a pioneering approach to the world of academia, radically improving the way scholarly research is managed. The grand vision of Frontiers is a world where all people have an equal opportunity to seek, share and generate knowledge. Frontiers provides immediate and permanent online open access to all its publications, but this alone is not enough to realize our grand goals.

Frontiers Journal Series

The Frontiers Journal Series is a multi-tier and interdisciplinary set of open-access, online journals, promising a paradigm shift from the current review, selection and dissemination processes in academic publishing. All Frontiers journals are driven by researchers for researchers; therefore, they constitute a service to the scholarly community. At the same time, the Frontiers Journal Series operates on a revolutionary invention, the tiered publishing system, initially addressing specific communities of scholars, and gradually climbing up to broader public understanding, thus serving the interests of the lay society, too.

Dedication to Quality

Each Frontiers article is a landmark of the highest quality, thanks to genuinely collaborative interactions between authors and review editors, who include some of the world's best academicians. Research must be certified by peers before entering a stream of knowledge that may eventually reach the public - and shape society; therefore, Frontiers only applies the most rigorous and unbiased reviews.
Frontiers revolutionizes research publishing by freely delivering the most outstanding research, evaluated with no bias from both the academic and social point of view. By applying the most advanced information technologies, Frontiers is catapulting scholarly publishing into a new generation.

What are Frontiers Research Topics?

Frontiers Research Topics are very popular trademarks of the Frontiers Journals Series: they are collections of at least ten articles, all centered on a particular subject. With their unique mix of varied contributions from Original Research to Review Articles, Frontiers Research Topics unify the most influential researchers, the latest key findings and historical advances in a hot research area! Find out more on how to host your own Frontiers Research Topic or contribute to one as an author by contacting the Frontiers Editorial Office: frontiersin.org/about/contact

SMART FOOD FOR HEALTHY, SUSTAINABLE AND RESILIENT FOOD SYSTEMS

Topic Editors:
Stefania Grando, Consultant, Italy
Seetha Anitha, International Crops Research Institute for the Semi-Arid Tropics (ICRISAT), India
Ramadjita Tabo, International Crops Research Institute for the Semi-Arid Tropics (Mali), Mali
Taku Tsusaka, Ostrom, Thailand
Ian Givens, University of Reading, United Kingdom
Tshilidzi Madzivhandila, Agriculture and Natural Resources Policy Analysis Network, South Africa

Citation: Grando, S., Anitha, S., Tabo, R., Tsusaka, T., Givens, I., Madzivhandila, T., eds. (2022). Smart Food for Healthy, Sustainable and Resilient Food Systems. Lausanne: Frontiers Media SA. doi: 10.3389/978-2-88976-263-7

Table of Contents

frontiers in Nutrition

MINI REVIEW
published: 25 June 2021
doi: 10.3389/fnut.2021.683678

The Mediterranean and Nordic Diet: A Review of Differences and Similarities of Two Sustainable, Health-Promoting Dietary Patterns

Željko Krznarić [1,2], Irena Karas [1], Dina Ljubas Kelečić [1] and Darija Vranešić Bender [1]*

[1] University Hospital Zagreb, Department of Internal Medicine, Clinical Unit of Clinical Nutrition, Zagreb, Croatia, [2] Zagreb School of Medicine, Zagreb, Croatia

The Mediterranean diet (MD) and the Nordic diet (ND) share more similarities than differences. Both diets are based on typical local and seasonal foods, share similar nutritional recommendations based on plant-based dietary principles, and are both now orienting toward environmental protection and sustainability. The main difference between the two diets is the primary fat source. Olive oil is the synonym for MD while the ND uses more rapeseed/canola oil. While longitudinal epidemiological studies support adherence to MD as a way to prevent chronic diseases, ND still needs more such studies because the current results are discrepant. Notably, studies that assessed the association between both diets and lower risks of chronic diseases, disability, and mortality from specific and all causes, implied that ND could also have an advantageous effect as MD. Hopefully, there will be more longitudinal and large prospective studies in the future that will provide more evidence-based recommendations.

Keywords: Mediterranean diet, Nordic diet, olive oil, rapeseed oil, plant-based, chronic disease prevention

OPEN ACCESS

Edited by:
Stefania Grando,
Consultant, Ascoli Piceno, Italy

Reviewed by:
Alexandru Rusu,
Biozoon, Germany
Cinzia Barbieri,
University of Turin, Italy

***Correspondence:**
Željko Krznarić
zeljko.krznaric1@zg.t-com.hr

Specialty section:
This article was submitted to Nutrition and Sustainable Diets, a section of the journal Frontiers in Nutrition

Received: *21 March 2021*
Accepted: *26 May 2021*
Published: *25 June 2021*

Citation:
Krznarić Ž, Karas I, Ljubas Kelečić D and Vranešić Bender D (2021) The Mediterranean and Nordic Diet: A Review of Differences and Similarities of Two Sustainable, Health-Promoting Dietary Patterns. Front. Nutr. 8:683678. doi: 10.3389/fnut.2021.683678

INTRODUCTION

In countries around the world, balanced diets that are based on practical, sustainable, and health-promoting dietary guidelines, which support locally available food, represent typical preventive policies (1, 2). Since the Food and Agriculture Organization (FAO) predicts that food production will increase by at least 60% by 2050, radical changes in food production and consumption are needed to conserve natural resources for future generations while providing sufficient food for a growing global population (3).

Both the Mediterranean diet (MD) and the Nordic diet (ND) have similar dietary recommendations, are considered to be plant-based, and are oriented toward environmental protection and sustainability. The objective of this mini-review is to show that there are more similarities to the two diets than differences. Although the north and the south of Europe differ significantly in their climate, which undoubtedly affects the types of food that can be grown, both diets are so similar in their general guidelines that the main notable difference between the two is in the oil that is used in everyday consumption. Since longitudinal and large prospective studies for ND are still lacking, it is still not so easy to provide evidence-based recommendations just for the ND. We reviewed the studies that compared the effects of both diets on human health, which showed that greater adherence to ND is associated with lower all-cause and cardiovascular mortality and lower incidence of disability. These studies indicate that ND could have as beneficial effects as the MD.

With this in mind, we believe that both diets can be implemented and perhaps even alternated as a part of a healthy lifestyle.

COMPOSITION AND CONCEPT OF THE ND AND MD

The Nordic Diet is based on traditional ways of eating in the Nordic countries: Denmark, Norway, Sweden, Finland, Greenland, and Iceland. Certain foods and preparation methods are relatively the same in mentioned countries and include berries (e.g., lingonberry), cabbage, apples, pears, root vegetables, oats, rye, and fermented milk. All of these traditional Nordic foods have been associated with beneficial health effects (4, 5). As meat is the least environmentally friendly food, ND recommends an increased intake of legumes which helps increase the amount of protein in the diet but also has a beneficial effect on reducing the pressure on the environment (6). Moreover, Nordic countries have a rich marine archipelago, which is a great source of fish and other seafood that represent an important part of the diet (1, 6). The most common source of added fats in the ND is rapeseed (also known as canola oil) which is produced from the Rapeseed plant (*Brassica napus*), a member of the Cruciferae family. This can probably be ascribed to the geographical location of the countries since the rapeseed plant is predominantly cultivated in its winter form. The collaboration of Nordic countries for several decades has resulted in setting guidelines for dietary composition and reference values for the nutrient intake through the conjoint publication of the Nordic Nutrition Recommendations (NNR). This is one of the most comprehensive regional collaborations of the world, involving Denmark, Finland, Iceland, Norway, Sweden, the Faroe Islands, Greenland, and Åland (7, 8). According to the NNRs, the recommended daily energy distribution (as energy percent, E %) for macronutrients is: 25–40% of total energy intake should be derived from fats, 45–60% from carbohydrates, and 10–20% from protein (7). Since the ND includes several countries, the food-based dietary guidelines (FBDGs) slightly vary for every Nordic country. The daily quantitative recommendations for fruits, vegetables, starchy foods (wholegrain cereals or wholemeal alternatives), fish, and red meat are somewhat similar, while milk and dairy products are not specified in quantitative recommendations for all countries, but all are in concordance that low-fat dairy products should be the preferred choice. All countries advise using "softer and healthier fat" like plant oils and soft margarine, and limitation of salt to 5–6 g/day. An average daily intake of 1.5 L of water or other unsweetened liquids is recommended (9).

The Mediterranean diet incorporates traditional living habits of people from countries surrounding the Mediterranean Sea; it varies by country and region and has a range of definitions, and is the result of the long tradition of a century of sharing goods, food, and culture between countries in the Mediterranean area. Due to contemporary lifestyle and environmental challenges, the traditional MD pattern had to be updated, especially on the topics of local, seasonal, and minimally processed food consumption, sustainability, and cost of food, while adapting to socio-economic, cultural, and geographical contexts. According to MD, everyday main meals and snacks should contain cereals, vegetables, and fruit, while low-fat dairy products should be consumed in moderation. Two or more servings of fish and white meat, with weekly consumption of various plant proteins, is recommended. Red and processed meat should be reduced in quantity and frequency of consumption. A daily average of 1.5–2 L of water or other unsweetened, low-sodium liquids should be ensured. The main source of dietary fat is olive oil, especially extra virgin olive oil (EVOO) (10). Studies vary when trying to define daily energy distribution for macronutrients; according to Davis et al. (11), the MD provides close to 37% energy from total fat, 15% from protein, and at least 43% energy from carbohydrates (11).

The MD and the ND are considered as "plant-based" eating patterns, both recommend choosing more of the proteins from plant sources by recommending a higher intake of fruits, vegetables, grains (especially whole grains), legumes, nuts, and seeds, while limiting consumption of red and processed meat (12). The notable point of difference is the oil used in each diet, while MD is based on olive oil (preferably EVOO), the ND mainly uses rapeseed (canola) oil (RO) (13). The main bioactive components of EVOO, which affect sensory and contribute to its nutritional characteristics are MUFA, PUFA, phytosterols, polyphenols, pigments, tocopherols, squalene, triterpenic acids, and dialcohols. Olive oil has a high content of MUFA, especially oleic acid (18:1 ω-9), whose health-promoting effects came from cardiovascular prevention trials that showed versatile biological effects: modifications of plasma lipids and lipoprotein patterns, impact on membrane composition and fluidity of blood cells, inhibition of coagulation, improvement of glucose homeostasis and blood pressure, and reduction of inflammation and oxidative states in fasting conditions. Olive oil is high in squalene, which besides anticancer and antioxidant properties, also has an essential role in cholesterol metabolism in humans. Over 100 different types of biophenols have been reported in olive products, from which hydroxytyrosol (HT), its metabolites, and oleocanthal (known for its anti-inflammatory and chemotherapeutic properties, neuroprotective and anti-rheumatic effects) have been extensively researched (14–16). Phenols are known for their antioxidant, anti-inflammatory, and antimicrobial activities, and epidemiological and observational studies have shown their effectiveness in the prevention of inflammatory and chronic human conditions such as cardiovascular disease (CVD), cerebral diseases, and cancer (14). RO has a very good lipid profile, it is characterized by a low level of SFA and significant amounts of MUFA and PUFA, and has a notably higher level of α-linolenic acid (ALA) than olive oil, which is connected to cardioprotective benefits (15, 17, 18). RO contains fewer phenolic compounds,

Abbreviations: MD, Mediterranean diet; ND, Nordic diet; NNR, Nordic Nutrition Recommendations; EVOO, extra virgin olive oil; RO, rapeseed oil; MUFA, monounsaturated fatty acids; PUFA, polyunsaturated fatty acids; ALA, α-linolenic acid; CVD, cardiovascular disease; T2D, type 2 diabetes; CRC, colorectal cancer; MI, myocardial infarction; mMED, modified Mediterranean diet score; HNFI, Healthy Nordic Food Index; GHG, greenhouse gas emission; EH, environmental hourglass.

TABLE 1 | Studies that compare the effects of both diets on human health.

Name of the study	Objective	Method	Results
The EPIC-Potsdam study (a prospective cohort based in Germany as part of the European-wide multicenter EPIC study)	The aim was to investigate the association between the ND and the MD with the risk of chronic diseases (type 2 diabetes (T2D), myocardial infarction (MI)	27,548 participants were recruited between 1994 and 1998. After exclusion of prevalent cases, baseline adherence to the diet score was evaluated, with application of Cox regression models to examine the association between the diet scores and the incidence of major chronic diseases	The ND showed a statistically non-significant inverse association with incidence of MI in the overall population and of stroke in men. Adherence to the MD was associated with lower incidence of T2D. In women, the MD score was also inversely associated with MI. No association was observed for any of the scores with cancer
PopGen CRC survivor cohort (prospective cohort study in Northern Germany)	Aim was to investigate the association of the two diets with all-cause mortality in long-term CRC survivors	Diet was assessed at a median time of 6 y after cancer diagnosis in 1,404 CRC survivors (median age: 69 y; 56% men) by using a semiquantitative food-frequency questionnaire. Cox proportional hazard models were used to assess associations of the diets with all-cause mortality	In multivariable-adjusted models, higher adherence to the MD was significantly associated with lower all-cause mortality. Similarly, the ND was inversely associated with all-cause mortality (when the highest was compared with the lowest index quartile) and when modeled as a continuous trait
The Helsinki Birth Cohort Study	Aim was to assess the association of the ND and the MD with incident disability with a follow-up of 10 years	962 home-dwelling men and women, mean age 61.6 years, who were free of disability at baseline, participated in a clinical examination	The likelihood of having mobility limitations and difficulties in self-care activities were lower among those who had better adherence to the ND. Greater adherence to MD was associated with a lower disability incidence (however, the association was not statistically significant)
The Swedish Mammography Cohort	The combined effect of the two diet scores and association with all-cause and cause-specific mortality (cancer, CVD and ischemic heart disease)	The study included 38,428 women (median age of 61 years) where diet and covariate data were collected in a questionnaire	Higher adherence to MD was associated with lower mortality and compared with ND, was more strongly associated with a lower cause-specific mortality. Both MD and ND were inversely associated with all-cause and cardiovascular mortality. Cancer mortality was not independently associated with ND, whereas higher adherence to the MD was associated with lower cancer mortality

but the amount of phytosterols and tocopherols is higher (16, 17). RO also contains pigments (chlorophylls) and other trace elements i.e., ubiquinone (Coenzyme Q10), a compound involved in energy production and prevention of peroxidative damage to membrane phospholipids and free radical-induced oxidation (19, 20). Significant differences between bioactive compounds in EVOO and RO, apart from the basic source material, are the result of different production methods: EVOO is produced by mechanical extraction, while RO production needs more processing (solvent extraction, degumming, neutralization, bleaching, and deodorization). Side effects of this production are depletion of certain phytosterols, tocopherols, and other bioactive compounds that were present in the rapeseeds at the beginning (17, 18).

DISCUSSION

The Mediterranean diet has been extensively assessed in relation to chronic diseases. Because of the recognition that clinical and longitudinal epidemiological studies are needed for evidence-based recommendations, the publications on this subject have increased significantly since 1985. Some of the intervention studies such as the Medi-RIVAGE study in France, the PREDIMED (*Prevencion con DietaMediterranea*) study in Spain, and prospective cohorts such as the European Prospective Investigation into Cancer and Nutrition (EPIC) study and the SUN study have been analyzing the value of MD (20). On the other hand, the effects of the ND on major chronic diseases that were examined through three large prospective cohorts in Denmark, Sweden, and Finland resulted in inconsistent outcome. Still, studies that were based on the comparison of both diets and their potentially beneficial effect on chronic diseases, disability, specific-cause, and all-cause mortality, implied that ND also has a beneficial effect as MD.

The PREDIMED study, a multicenter, randomized prevention trial assessed effects of the low-fat diet, MD rich in olive oil, and MD rich in tree nuts on CVD. The results showed that MD (either with virgin olive oil or a mixture of nuts), compared to a low-fat diet, resulted in lower blood pressure, decreased insulin resistance, reduced concentrations of inflammatory molecules, and improved lipid profiles, after 3 months of follow-up (20). However, even though this is one the most influential randomized trials, in June 2018 the trial was retracted and republished because serious protocol deviations were detected; the original was substituted with a reanalysis that presented PREDIMED as a non-randomized study and in the end gave similar estimates for the primary endpoint (21). The EPIC study showed that greater adherence to MD was associated with a small reduction in the risk of developing type 2 diabetes (T2D) (22), while the EPIC study in Greece on the elderly European population and survival showed that greater adherence to the MD was connected to lower mortality, an inverse association was observed for overall mortality, coronary heart disease, and cancer mortality (20). The UK-based EPIC-Norfolk prospective cohort which examined the relationship between MD and incidence of CVD showed that greater compliance with MD was associated with lower incidence of CVD and mortality (23). The SUN study on MD in the primary prevention of nutrition-related chronic disease that included more than 22,000 participants in Spain from 1999 to February 2018, validated self-reported data on lifestyle, diet, and clinical diagnosis. The study outcomes showed that high adherence to MD is associated with reduced incidence of mortality, CVD, T2D, weight gain, metabolic syndrome, depression, cognitive decline, nephrolithiasis, and data even suggested that it may also enhance fertility (20, 24). The Medi-RIVAGE intervention study compared the effects of MD to a low-fat diet in subjects at high cardiovascular risk, which showed that participants on MD had better improvements in the majority of the cardiovascular risk factors compared to those on a low-fat diet after 3 months of follow-up (20). It also improved postprandial lipaemia in both genders at moderate cardiovascular risk (25, 26).

The Diet, Cancer and Health cohort study in Denmark showed that ND was associated with a lower risk of T2D for both men and women (27), and women who strongly complied with this diet had a lower incidence of colorectal cancer (CRC), but no significant effect was found for men (28). From the same cohort study, men and women with cases of stroke were identified from the Danish National Patient Register and better compliance to the ND was related to a lower risk of stroke, which leads to the conclusion that the ND could be suggested for stroke prevention (29). The Swedish Women's Lifestyle and Health cohort examined the association between ND incidence of overall CVD, CRC, and risk of breast cancer, and found no association between adherence to the ND and the risk of aforementioned diseases (30–32). However, prospective analysis in this cohort that examined the association of ND and total and cause-specific mortality, found that higher adherence to ND was associated with a 6% lower total mortality per 1 point increment in the adjusted models (32). Additionally, a prospective study that included two independent Finnish studies (the Helsinki Birth Cohort Study and the Health 2000 Survey) found no statistically significant association between adherence to the ND and incidence of T2D during 10 years of follow-up and suggested a larger prospective study to get stronger estimates (33, 34).

The EPIC-Potsdam study in Germany (part of the European-wide multicenter EPIC study) was designed to explore the relationship between both diets and the risk of chronic diseases (T2D, myocardial infarction (MI), stroke, and cancer). With a mean of 10.6 years of follow-up, the MD displayed inverse associations with the risk of developing T2D and MI (the latter only among women), while the ND demonstrated an inverse (but not-statistically profound) association with MI risk and stroke (the latter only among men). No relationship was observed for both diets with the incidence of cancer (35). A prospective cohort study in Northern Germany investigated the connection between MD and ND with all-cause mortality in long-term CRC survivors where the key findings showed a statistically significant association of MD with better overall survival, and the same can be implied for ND in the multivariable-adjusted model (36). A longitudinal study on subjects who belonged to the Helsinki Birth Cohort Study that analyzed the relationship between the two diets with the incidence of disability in a 10-year follow-up showed that individuals with greater adherence to the ND had lower mobility limitations and difficulties to perform self-care activities, while those with greater adherence to the MD had lower disability incidence, but with no statistically significant association. The study implies that the ND could decrease the probability of disability incidence and that the MD could also be helpful in the matter, but more longitudinal and intervention studies are needed (37). In a study from the Swedish Mammography Cohort, two diet scores [modified Mediterranean diet score (mMED) and Healthy Nordic Food Index (HNFI)] and their conjoined effect related to all-cause and cause-specific mortality (cancer, CVD, and ischemic heart disease) were assessed. It should be noted that the mMED was adapted to fit the non-Mediterranean settings, and the used HNFI was created in Denmark (consequently, this HNFI may lack certain ND features distinctive for Sweden). This study demonstrated that mMED was strongly associated with lower cause-specific mortality than HNFI, but both were inversely associated with lower total all-cause and cardiovascular mortality. This result may seem to be contrary to the results of The Swedish Women's Lifestyle and Health cohort where the adherence to the ND was not related to the risk of CVD, but presented a 6% lower total mortality per 1 point increment which was limited to cancer and non-CVD causes. Different results may be due to a greater number of CVD cases in this cohort and older subjects with greater BMI. The results of this cohort may indicate a greater advantage to be adherent more to the mMED than HNFI, but it should be taken into account that in this cohort, the HNFI was perhaps not the true embodiment of the ND and may still need improvement (38). The summation of the results of studies comparing the effect of both diets on human health is shown in **Table 1**.

Much was discussed about the health benefits of MD and ND, but both also illustrate how a diet can also have a positive impact on the environment and the nitrogen cycle (39). It is well-known that current food systems have a significant effect on the environment; food production, processing, preservation, and distribution of waste, all consume a significant amount of energy and resources, which contributes to about 32% of total greenhouse gas emissions (GHG) from human activities. A recent study based on MD and ND proposed a new way to assess the environmental impact of the diets, by introducing an environmental hourglass (EH) approach. The idea is to facilitate translating healthy dietary recommendations (that also take the regional context and cultural diversity into account) into practical dietary advice that is both sustainable and environmentally friendly. The EH approach describes the production of GHG through weekly consumption of the recommended dietary intake. The visual concept of the EH depicts the foods that should be consumed frequently every day at the bottom, while the foods that are supposed to be consumed one to three times per week are at the top of the hourglass. Therefore, fruits and vegetables were placed at the bottom, after which came cereals and potatoes, followed by milk and dairy products, and finally at the top are meat, fish, eggs, and legumes. Both MD and ND had similar GHG impact, even though the distribution of food and the contribution of individual food categories differed: vegetables contributed most to the overall impact in MD, and for ND, were the wholegrain/cereals; also, the recommended weekly amount of protein-rich foods, as well as the distribution of specific food items in this group, varied in both diets. The study demonstrated that appropriate food choices in accordance with the MD and ND may reduce some of the adverse effects of food production on the environment (40).

CONCLUSION

Mediterranean diet is not only perceived as a healthy diet but also as a way of life (with country-specific variations). MD and ND share similar nutritional recommendations and prefer seasonal, locally available foods while taking into account cultural heredity, sustainability, and preservation of the environment. We believe that both of these diets can be implemented and perhaps even alternated as a part of a healthy lifestyle, regardless of the geographical location. MD and its effects on health have been vastly studied, but similar longitudinal epidemiological studies for the ND are still lacking. Although some focus only on specific bioactive compounds in the diet, it should probably be better to assess ND as a whole; because the overall impact on health comes not only from one isolated bioactive component but rather a combination of different compounds from food that we eat every day and the whole lifestyle.

AUTHOR CONTRIBUTIONS

ŽK and DV designed the concept of the mini-review. IK and DL interpreted the data. IK and DV drafted the manuscript. All authors performed the critical analysis of the manuscript, approved the final manuscript, read, and agreed to the published version of the manuscript.

ACKNOWLEDGMENTS

The topic was a part of a scientific program and an invited lecture at the European Society of Clinical Nutrition and Metabolism (ESPEN) Congress held in Madrid in 2019; therefore, the authors would like to acknowledge ESPEN for the recognition.

REFERENCES

1. Bere E, Brug J. Towards health-promoting and environmentally friendly regional diets – a Nordic example. *Public Health Nutr.* (2008) 12:91–6. doi: 10.1017/S1368980008001985
2. Roswall N, Eriksson U, Sandin S, Löf M, Olsen A, Skeie G, et al. Adherence to the healthy Nordic food index, dietary composition, and lifestyle among Swedish women. *Food Nutr Res.* (2015) 59:26336. doi: 10.3402/fnr.v59.26336
3. Dernini S, Berry EM, Serra-Majem L, La Vecchia C, Capone R, Medina FX, et al. Med Diet 4.0: the Mediterranean diet with four sustainable benefits. *Public Health Nutr.* (2016) 20:1322–30. doi: 10.1017/S1368980016003177
4. Akesson A, Andersen LF, Kristjánsdóttir AG, Roos E, Trolle E, Voutilainen E, et al. Health effects associated with foods characteristic of the Nordic diet: a systematic literature review. *Food Nutr Res.* (2013) 57:22790. doi: 10.3402/fnr.v57i0.22790
5. Olsen A, Egeberg R, Halkjær J, Christensen J, Overvad K, Tjønneland A. Healthy aspects of the Nordic diet are related to lower total mortality. *J Nutr.* (2011) 141:639–44. doi: 10.3945/jn.110.131375
6. Mithril C, Dragsted LO, Meyer C, Tetens I, Biltoft-Jensen A, Astrup A. Dietary composition and nutrient content of the New Nordic Diet. *Public Health Nutr.* (2012) 16:777–85. doi: 10.1017/S1368980012004521
7. Nordic Council of Ministers. *Nordic Nutrition Recommendations 2012.* Copenhagen: Nordisk Ministerråd (2014). p. 627. doi: 10.6027/Nord2014-002
8. Fogelholm M. New Nordic nutrition recommendations are here. *Food Nutr Res.* (2013) 57:22903. doi: 10.3402/fnr.v57i0.22903
9. European Comission. *Food-Based Dietary Guidelines in Europe*. (2018). Available online at: https://ec.europa.eu/jrc/en/health-knowledge-gateway (accessed December 15, 2020).
10. Bach-Faig A, Berry EM, Lairon D, Reguant J, Trichopoulou A, Dernini S, et al. Mediterranean diet pyramid today. Science and cultural updates. *Public Health Nutr.* (2011) 14:2274–84. doi: 10.1017/S1368980011002515
11. Davis C, Bryan J, Hodgson J Murphy K. Definition of the Mediterranean diet: a literature review. *Nutrients.* (2015) 7:9139–53. doi: 10.3390/nu7115459
12. Hemler EC, Hu FB. Plant-based diets for personal, population, and planetary health. *Adv Nutr.* (2019) 10(Suppl. 4):S275–83. doi: 10.1093/advances/nmy117
13. Lankinen M, Uusitupa M Schwab U. Nordic diet and inflammation—a review of observational and intervention studies. *Nutrients.* (2019) 11:1369. doi: 10.3390/nu11061369
14. Piroddi M, Albini A, Fabiani R, Giovannelli L, Luceri C, Natella F, et al. Nutrigenomics of extra-virgin olive oil: a review. *BioFactors.* (2017) 43:17–41. doi: 10.1002/biof.1318
15. Garcia-Aloy M, Hulshof PJM, Estruel-Amades S, Osté MCJ, Lankinen M, Geleijnse JM, et al. Biomarkers of food intake for nuts and vegetable oils: an extensive literature search. *Genes Nutr.* (2019) 14:7. doi: 10.1186/s12263-019-0628-8
16. Francisco V, Ruiz-Fernández C, Lahera V, Lago F, Pino J, Skaltsounis L, et al. Natural molecules for healthy lifestyles: oleocanthal from extra virgin olive oil. *J Agric Food Chem.* (2019) 67:3845–53. doi: 10.1021/acs.jafc.8b06723
17. Hoffman R, Gerber M. Can rapeseed oil replace olive oil as part of a Mediterranean-style diet? *Br J Nutr.* (2014) 112:1882–95. doi: 10.1017/S0007114514002888
18. Saleem M, Naveed A. Characterization of canola oil extracted by different methods using fluorescence spectroscopy. *PLoS ONE.* (2018) 3:e0208640. doi: 10.1371/journal.pone.0208640
19. Yang R, Deng L, Zhang L, Yue X, Mao J, Ma F, et al. Comparative metabolomic analysis of rapeseeds from three countries. *Metabolites.* (2019) 9:161. doi: 10.3390/metabo9080161
20. Martinez-Gonzalez MA, Bes-Rastrollo M, Serra-Majem L, Lairon D, Estruch R, Trichopoulou A. Mediterranean food pattern and the primary prevention of chronic disease: recent developments. *Nutr Rev.* (2009) 67(Suppl. 1):S111–6. doi: 10.1111/j.1753-4887.2009.00172.x
21. Agarwal A, Ioannidis JPA. PREDIMED trial of Mediterranean diet: retracted, republished, still trusted? *BMJ.* (2019) 364:l341. doi: 10.1136/bmj.l341
22. InterAct Consortium, Romaguera D, Guevara M, Norat T, Langenberg C, Forouhi NG, et al. Mediterranean diet and type 2 diabetes risk in the European Prospective Investigation into Cancer and Nutrition (EPIC) study: theInterAct project. *Diabetes Care.* (2011) 34:1913–8. doi: 10.2337/dc11-0891
23. Tong TY, Wareham NJ, Khaw KT, Imamura F, Forouhi NG. Prospective association of the Mediterranean diet with cardiovascular

disease incidence and mortality and its population impact in a non-Mediterranean population: the EPIC-Norfolk study. *BMC Med.* (2016) 14:135. doi: 10.1186/s12916-016-0677-4
24. Carlos S, De La Fuente-Arrillaga C, Bes-Rastrollo M, Razquin C, Rico-Campà A, Martínez-González MA, et al. Mediterranean diet and health outcomes in the SUN cohort. *Nutrients.* (2018) 10:439. doi: 10.3390/nu10040439
25. Hyson D, Rutledge JC Berglund L. Postprandial lipemia and cardiovascular disease. *Curr Atheroscler Rep.* (2003) 5:437–44. doi: 10.1007/s11883-003-0033-y
26. Defoort C, Vincent-Baudry S, Lairon D. Effects of 3-month Mediterranean-type diet on postprandial TAG and apolipoprotein B48 in the Medi-RIVAGE cohort. *Public Health Nutr.* (2011) 14:2302–8. doi: 10.1017/S1368980011002552
27. Lacoppidan SA, Kyrø C, Loft S, Helnæs A, Christensen J, Plambeck Hansen C, et al. Adherence to a healthy Nordic food index is associated with a lower risk of type-2 diabetes—the Danish diet, cancer and health cohort study. *Nutrients.* (2015) 7:8633–44. doi: 10.3390/nu7105418
28. Kyrø C, Skeie G, Loft S, Overvad K, Christensen J, Tjønneland A, et al. Adherence to a healthy Nordic food index is associated with a lower incidence of colorectal cancer in women: the Diet, Cancer and Health cohort study. *Br J Nutr.* (2013) 109:920–7. doi: 10.1017/S0007114512002085
29. Hansen CP, Overvad K, Kyrø, Olsen A, Tjønneland A, Johnsen SP, et al. Adherence to a healthy Nordic diet and risk of stroke: a Danish cohort study. *Stroke.* (2017) 48:259–64. doi: 10.1161/STROKEAHA.116.015019
30. Roswall N, Sandin S, Scragg R, Löf M, Skeie G, Olsen A, et al. No association between adherence to the healthy Nordic food index and cardiovascular disease amongst Swedish women: a cohort study. *J Intern Med.* (2015) 278:531–41. doi: 10.1111/joim.12378
31. Roswall N, Li Y, Kyrø C, Sandin S, Löf M, Adami HO, et al. No Association between adherence to a healthy Nordic food index and colorectal cancer: results from a Swedish cohort study. *Cancer Epidemiol Biomarkers Prev.* (2015) 24:755–7. doi: 10.1158/1055-9965.EPI-14-1314
32. Li Y, Roswall N, Sandin S, Ström P, Adami HO, Weiderpass E. Adherence to a healthy Nordic food index and breast cancer risk: results from a Swedish cohort study. *Cancer Causes Control.* (2015) 26:893–902. doi: 10.1007/s10552-015-0564-x
33. Roswall N, Sandin S, Löf M, Skeie G, Olsen A, Adami HO, et al. Adherence to the healthy Nordic food index and total and cause-specific mortality among Swedish women. *Eur J Epidemiol.* (2015) 30:509–17. doi: 10.1007/s10654-015-0021-x
34. Kanerva N, Rissanen H, Knekt P, Havulinna AS, Eriksson JG, Männistö S. The healthy Nordic diet and incidence of Type 2 Diabetes—10-year follow-up. *Diabetes Res Clin Pract.* (2014) 106:e34–7. doi: 10.1016/j.diabres.2014.08.016
35. Galbete C, Kröger J, Jannasch F, Iqbal K, Schwingshackl L, Schwedhelm C, et al. Nordic diet, Mediterranean diet, and the risk of chronic diseases: the EPIC-Potsdam study. *BMC Med.* (2018) 16:99. doi: 10.1186/s12916-018-1082-y
36. Ratjen I, Schafmayer C, di Giuseppe R, Waniek S, Plachta-Danielzik S, Koch M, et al. Postdiagnostic Mediterranean and healthy Nordic dietary patterns are inversely associated with all-cause mortality in long-term colorectal cancer survivors. *J Nutr.* (2017) 147:636–44. doi: 10.3945/jn.116.244129
37. Perälä MM, von Bonsdorff MB, Männistö S, Salonen MK, Simonen M, Pohjolainen P, et al. The healthy Nordic diet and Mediterranean diet and incidence of disability 10 years later in home-dwelling old adults. *J Am Med Dir Assoc.* (2019) 20:511–6. doi: 10.1016/j.jamda.2018.09.001
38. Warensjö Lemming E, Byberg L, Wolk A, Michaëlsson K. A comparison between two healthy diet scores, the modified Mediterranean diet score and the Healthy Nordic Food Index, in relation to all-cause and cause-specific mortality. *Br J Nutr.* (2018) 119:836–46. doi: 10.1017/S0007114518000387
39. Leite J, LouroCaldeira S, Watzl B, Wollgast J. Healthy low nitrogen footprint diets. *Glob Food Secur.* (2020) 24:100342. doi: 10.1016/j.gfs.2019.100342
40. Ulaszewska MM, Luzzani G, Pignatelli S, Capri E. Assessment of diet-related GHG emissions using the environmental hourglass approach for the Mediterranean and new Nordic diets. *Sci Total Environ.* (2017) 574:829–36. doi: 10.1016/j.scitotenv.2016.09.039

Conflict of Interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

frontiers in Nutrition

SYSTEMATIC REVIEW
published: 28 July 2021
doi: 10.3389/fnut.2021.687428

A Systematic Review and Meta-Analysis of the Potential of Millets for Managing and Reducing the Risk of Developing Diabetes Mellitus

Seetha Anitha[1]*, Joanna Kane-Potaka[1], Takuji W. Tsusaka[2], Rosemary Botha[3], Ananthan Rajendran[4], D. Ian Givens[5], Devraj J. Parasannanavar[4], Kowsalya Subramaniam[6], Kanaka Durga Veera Prasad[1], Mani Vetriventhan[1] and Raj Kumar Bhandari[7]

[1] Smart Food Initiative, International Crops Research Institute for the Semi-Arid Tropics (ICRISAT), Patancheru, India, [2] Organization for Advanced and Integrated Research, Kobe University, Kobe, Japan, [3] Development Strategy and Governance Division, International Food Policy Research Institute (IFPRI), Lilongwe, Malawi, [4] National Institute of Nutrition (NIN), Hyderabad, India, [5] Institute of Food, Nutrition and Health, University of Reading, Reading, United Kingdom, [6] Food Science and Nutrition, Avinashilingam Institute for Home Science and Higher Education for Women, Coimbatore, India, [7] National Technical Board of Nutrition, Government of India (GoI), New Delhi, India

OPEN ACCESS

Edited by:
Rakesh Bhardwaj,
National Bureau of Plant Genetic Resources (ICAR), India

Reviewed by:
Maharishi Tomar,
Indian Council of Agricultural Research (ICAR), India
Venkateswarlu Ronda,
Indian Council of Agricultural Research, India

***Correspondence:**
Seetha Anitha
s.anitha@cgiar.org;
dr.anithaseetha@gmail.com

Specialty section:
This article was submitted to Nutrition and Sustainable Diets, a section of the journal Frontiers in Nutrition

Received: 29 March 2021
Accepted: 07 June 2021
Published: 28 July 2021

Citation:
Anitha S, Kane-Potaka J, Tsusaka TW, Botha R, Rajendran A, Givens DI, Parasannanavar DJ, Subramaniam K, Prasad KDV, Vetriventhan M and Bhandari RK (2021) A Systematic Review and Meta-Analysis of the Potential of Millets for Managing and Reducing the Risk of Developing Diabetes Mellitus. Front. Nutr. 8:687428. doi: 10.3389/fnut.2021.687428

Millets (including sorghum) are known to be highly nutritious besides having a low carbon footprint and the ability to survive in high temperatures with minimal water. Millets are widely recognised as having a low Glycaemic Index (GI) helping to manage diabetes. This systematic review and meta-analyzes across the different types of millets and different forms of processing/cooking collated all evidences. Of the 65 studies that were collected globally, 39 studies with 111 observations were used to analyze GI outcomes and 56 studies were used to analyze fasting, post-prandial glucose level, insulin index and HbA1c outcomes in a meta-analysis. It is evident from the descriptive statistics that the mean GI of millets is 52.7 ± 10.3, which is about 36% lower than in typical staples of milled rice (71.7 ± 14.4) and refined wheat (74.2 ± 14.9). The descriptive, meta and regression analyses revealed that Job's tears, fonio, foxtail, barnyard, and teff were the millets with low mean GI (<55) that are more effective (35–79%) in reducing dietary GI than the control samples. Millets with intermediate GI (55–69) are pearl millet, finger millet, kodo millet, little millet, and sorghum which have a 13–35% lower GI than the control with high GI (>69). A meta-analysis also showed that all millets had significantly ($p < 0.01$) lower GI than white rice, refined wheat, standard glucose or white wheat bread except little millet which had inconsistent data. Long term millet consumption lowered fasting and post-prandial blood glucose levels significantly ($p < 0.01$) by 12 and 15%, respectively, in diabetic subjects. There was a significant reduction in HbA1c level (from 6.65 ± 0.4 to 5.67 ± 0.4%) among pre-diabetic individuals ($p < 0.01$) who consumed millets for a long period. Minimally processed millets were 30% more effective in lowering GI of a meal compared to milled rice and refined wheat. In conclusion, millets can be beneficial in managing and reducing the risk of developing diabetes and could therefore be used to design appropriate meals for diabetic and pre-diabetic subjects as well as for non-diabetic people for a preventive approach.

Keywords: millets, sorghum, diabetes, glycaemic index, glycaemic response, meta-analaysis

INTRODUCTION

It is estimated that there will be a 51% surge in diabetics globally by 2045, from 463 million in 2019 to 700 million in 2045 (1) with type 2 diabetes accounting for about 90% of the total. Eighty-seven percent of diabetes-related deaths occur in low and middle income countries where there is less diversification of staple foods. It is important to note that apart from a sedentary lifestyle and obesity, the type of food consumed plays a key role in diabetes. Main staples such as refined rice, refined wheat and maize contribute up to 80% of the energy intake in developing countries (2). Diversifying food staples and mainstreaming traditional nutritious and less glucogenic staples in the majority of developing countries is very important to manage and prevent diabetes; millets and sorghum figure first in this list of staples.

The value of a Triple Bottom Line is well-recognised in businesses and has been the stimulus for the creation of new products and impactful investments. Customising it to the Food System is the Smart Food Triple Bottom Line, defining solutions (3) that in unison are good for you (nutritious and healthy), good for the planet (environmentally sustainable) and good for the farmer (resilient). It is an approach being used to analyze the value of millets and sorghum as staples. This is the first analyses focusing on how millets and sorghum are "good for you" in terms of reducing diabetes, and comparing them to rice, wheat and maize, the "Big 3" major staple foods in Asia and Africa. Of these, polished rice, which is inherently deficient in micronutrients, provides 80% of the energy intake (4) in high rice consuming countries. Growing lifestyle diseases like type 2 diabetes make it imperative to explore dietary solutions that include nutrition and tackle major health issues. Diversifying diets by diversifying staples with the right nutritious and healthy foods can play a major role in reducing multiple health related burdens.

There are 13 types of millets available globally (5) which include pearl millet, finger millet, sorghum, little millet, proso millet, kodo millet, barnyard millet, brown top millet, foxtail millet, Guinea millet, Job's tears, fonio, and teff. Except for Job's tears, fonio, and teff, the other millets are widely distributed in India. Finger millet is widely found in India, China and in some Eastern and Southern African countries, whereas fonio is widely distributed in Western Africa and Job's tears in northeast India, southern and eastern Asia and southern China. On the other hand, teff is mainly found in Ethiopia (5). Currently, these crops are mostly grown in Africa and Asia as well as in the USA, which is the largest producer of sorghum. Millets also occur in other parts of the world as feed and fodder or as a minor crop (www.smartfood.org/millets-sorghum-production-trends/).

A systematic review of 19 research articles showed that millets help manage diabetes due to their high fibre, polyphenol, and antioxidant content (6). Millets were traditionally consumed in African and Asian countries and were later largely replaced by rice, wheat and maize. Considering nutrient requirements, rising non-communicable health issues like diabetes and challenges posed by climate change, it is important to popularise smart foods, i.e., foods that fulfil all criteria of being good for you, the planet and the farmer.

Many studies have demonstrated the efficacy of millets in improving glycaemic control, decreasing fasting, and post-prandial rise in blood glucose concentration (7, 8), reducing insulin index and insulin resistance and lessening glycosylated haemoglobin (HbA1c) level (8–12). Glycaemic index (GI) is a measure of how much the carbohydrate present in the food affects the rate and extent of change in post-prandial blood glucose concentration. The general dietary strategy to enhance glycaemic control is to consume low GI food (13). Fasting blood glucose is generally measured following overnight fasting and post-prandial blood glucose is measured at regular intervals of up to 2 h after eating. Hyperinsulinemia is associated with insulin resistance that increases the risk of type 2 diabetes (14). Therefore, along with post-prandial glucose concentration, it is important to measure insulin concentration in order to evaluate a food's ability to reduce insulin resistance. In addition, long term glycaemic control can be measured by HbA1c marker (15).

Although there are several studies on millets related to these outcomes, their information is heterogeneous. Therefore, it is important to collate scientific evidence to determine whether the studies support the glycaemic controlling ability of millets or not, including all the types and forms of processing (including cooking) they undergo, in order to serve as a dietary guide on millets. Considering the growing prevalence of diabetes among high and low socioeconomic groups in both developed and developing countries, this paper for the first time aims to undertake an in-depth systematic review and meta-analysis, simple descriptive statistics, and regression analysis of all the studies conducted to test GI, fasting and post-prandial blood glucose concentrations, insulin response and HbA1c biomarker level in millet-based diets. This includes 11 types of millets, 1 mixed millet and many forms of processing that were tested. This information will form the scientific basis for any claims about millets vis-à-vis diabetes and be useful for the scientific community, dieticians, and nutritionists through to food processors and governments in setting policies and programs on health, nutrition and agriculture. Therefore, this study aims to address the following research question:

Does consuming millet(s)-based food help in managing and reducing the risk of developing type 2 diabetes compared to the consumption of typical staples?

METHODS

The systematic review was conducted by: (1) collating all the relevant studies on the glucogenic effect of millets relative to other staple foods; (2) reviewing the methods used to study this; (3) conducting a regression analysis to find the effect of millets in managing diabetes and (4) conducting a meta-analysis to assess the science-based evidence on millets' ability to reduce insulin concentration, HbA1c biomarker and fasting and post-prandial blood glucose concentration and their effect on managing individuals with type 2 diabetes mellitus and pre-diabetic individuals compared to non-millet-based regular diets or other staples.

The following sections describe the methods in detail.

Study Period and Protocol

The systematic review was conducted from October 2017 to February 2021. The study protocol is registered in the Research Registry (Unique Identification Number; reviewregistry1094) and a 27-item PRISMA checklist was used to conduct the systematic review and meta-analysis (16).

Search, Inclusion, and Exclusion Criteria

The search basically selected all the research studies in English conducted from the year 1950 to the last quarter of 2020. An initial scoping study was conducted using PubMed and MEDLINE to check for studies that overlapped with the research question of the systematic review as per the guidelines of Atkinson and Cipriani, 2018 (17). Later, a detailed search was conducted using search engines Google scholar, Scopus, Web of Science, PubMed (MEDLINE), CAB Abstracts ClinicalTrials.gov, grey literature, and other Clinical Trial Registries to find the studies relevant to the research question. The search was conducted using the search strategy and keywords indicated in **Table 1**, with further screening for study relevance, completeness of information and quality of research based on the inclusion and exclusion criteria.

Inclusion Criteria

1. Research studies conducted on humans with all types of millets including sorghum, finger millet, pearl millet, little millet, kodo millet, barnyard millet, foxtail millet, proso millet, teff, fonio, and Job's tears. 2. Where there were no or very few human studies on some millets (only for teff and fonio), *in-vitro* studies were included but these were considered separately. 3. Studies with information on any one or all of the outcomes including GI, fasting, post-prandial glucose level, insulin index and HbA1c of any millets were selected for the next level of screening. 4. A study conducted in any geographical location globally was selected. 5. Both randomised cross-over studies and self-controlled case studies were included. 6. Studies conducted on both normal healthy subjects, pre-diabetic, and type 2 diabetic subjects were included. 7. Only peer-reviewed research articles were selected.

Exclusion Criteria

These included review articles, animal studies and papers where the full information could not be accessed or if the methodologies were identified as weak. Papers representing glucose response values in figures without providing numeric values were excluded from the meta-analysis.

Data Collection Process

The PRISMA flow diagram (**Figure 1**) shows the study design and the criteria for including and excluding papers. Only relevant papers that addressed the research questions were downloaded. If only the abstract was suitable, then open access articles were downloaded, and the full paper was collected by contacting the authors, editors of the journals, universities that have library facilities and subscription to the journal. Some full papers were purchased. After collecting the full paper, if any information on GI and/or glucose response was missing, the authors were contacted and complete information was requested for use in the meta-analysis. A manual search was done in every article to find more related research articles. References in the selected articles were also searched and the full articles were acquired and included in the study, where appropriate.

The data were extracted from the articles and documented in an Excel sheet and then used to conduct the regression analysis, forest plots and publication bias plots.

Study Quality Assessment

Information such as the author's name, year of publication, geographical region of study, name of the study, gender of the subjects, age range, mean age, study type, sample size, dietary assessment methods used, outcomes, level of dietary exposure, and procedures and standards followed to estimate GI, etc., were extracted from the research articles. Using the eight-item Newcastle-Ottawa Scale (NOS), the quality (18, 19) of each study was assessed by two investigators, and any disagreements were resolved by discussing it with a third reviewer. The NOS allows the assessment of a study population and selection with comparable outcomes of interest. The scale ranged from 0 to 9, and studies with scores of <7 were assigned low quality and those >7 were assigned high quality. The researchers also applied the principle of Bell et al. (20) to further strengthen quality assessment.

Data Items and Extraction

Each study was labelled with details of the author and year. The numerical variables considered for the meta-analysis included mean GI with standard deviation (SD), mean fasting, and post-prandial blood glucose concentration with SD, the sample size in both intervention and control and mean insulin level with SD. The respective control samples for each study were identified and appropriate data were extracted. Control samples included those of wheat, refined wheat, rice (white and brown), roots, tubers, and legumes. When a food control was not used, then data for glucose or white bread were used as the control. The numerical variables corresponding to GI were extracted as mean GI with SD. If mean standard error (SEM or SE) values were provided in

TABLE 1 | Search strategy and keywords used to identify the relevant papers.

Number	Criteria and keywords used for the search
1	Boolean logic such as "AND," "OR," "NOT" were used
2	Finger millet glycaemic index. Repeat the search by replacing finger millet with other millets in the following list: "little millet," "foxtail millet," "barnyard millet," "proso millet," "kodo millet," "teff," "fonio," "job's tears," "pearl millet," "finger millet," and "sorghum"
3	Common name or local name of the millets. For example: adlay (job's tears), acha (fonio), samai (little millet), and navane (foxtail millet)
4	Glucose response of millets. Glycaemic Load (GL) of millets
5	Glucose response of finger millet. Repeated the search with all the millets in the list
6	Glucose lowering effect of finger millet. Repeated the search with all the millets in the list
7	Effect of finger millet on diabetes. Repeated the search with all the millets in the list
8	Effect of finger millet in managing diabetes. Repeated the search with all the millets in the list
9	Effect of millets on fasting blood glucose level. Repeated the search with all the millets in the list
10	Effect of millets on post-prandial blood glucose level. Repeated the search with all the millets in the list
11	Effect of millets on the insulin index. Repeated the search with all the millets in the list
12	Effect of millets on HbA1c or glycosylated haemoglobin
13	Search by using all the keywords mentioned above along with country and continent
14	A hand search was done using the reference list of one paper to find other papers

the study, then the SE values were converted into SD values. If the GI was not provided in the paper, it was either obtained from the author, or if the mean of all subjects Area Under the Curve (AUC) was available, then GI was calculated using the formula F/R × 100, where F is the mean of all subjects' AUC for the test food and R is the mean of all subjects' AUC for the control food (21). Fasting and post-prandial glucose concentrations were extracted into the Excel sheet in mg/dl units as per the guidelines provided by Harrer et al. (22). Where given as mmol/l, the values were converted into mg/dl to maintain uniformity of data. HbA1c was presented in percentage and taken as such. Categorical data was recorded on cooking method (baking/roasting, boiling, steam cooking), information on the cooked product (pancake, flatbread, porridge, cooked grain), the form of the samples used (grain, flour, batter) and the health condition (diabetic, pre-diabetic, and non-diabetic) of the study participants.

Summary Measures and Result Synthesis

A meta-analysis was conducted to estimate the standard mean difference (SMD) and associated heterogeneity (I^2) (23). The significance of the result was determined using a fixed effect model for a single source of information and random effect model for other studies. Subgroup analyses were conducted to ascertain the effect of different variables and conditions on fasting and post-prandial glucose levels. In addition, descriptive statistics such as mean, SD for GI, HbA1c, and glucose level were calculated for both intervention and control samples. A regression analysis was conducted to quantify the effect of millets and control samples on glycaemic control keeping GI as a dependent variable and food type, source, processing methods and participants' health (diabetic, pre-diabetic, and non-diabetic) as independent variables.

Data Analysis in Detail

In total, 80 studies were collected on the effect of millets on various outcomes in non-diabetic, pre-diabetic, and diabetic subjects. Of these, only 65 studies had complete information on either of the five key outcomes (GI, fasting, post-prandial blood glucose concentration, insulin concentration, and HbA1c level). The effects of millets and control samples were analysed by segregating them in several ways, such as by the effect of consuming millets on five key outcomes in diabetic, pre-diabetic, and non-diabetic subjects, and by comparing the effect of millets on five key outcomes with that on various staples segregated as rice (white and brown), wheat (whole and refined), roots and tubers, legumes and others, standard glucose and white bread. Descriptive statistics, regression and meta-analysis were conducted. Descriptive statistics calculated mean, standard deviation and percentage values of outcomes. A meta-analysis was conducted to generate evidence on the effects of millets' use on GI, fasting and post-prandial glucose levels compared to the pre intervention values (baseline) or control samples used in the studies which included rice (refined and brown), refined wheat and maize. Regression analysis was conducted to test the correlation between type of crop, cooking type, and GI. Both descriptive and regression analyses were conducted using STATA 16 (24). A meta-analysis was conducted using software R studio version 3.5.1 (2018) to obtain forest plots and estimates of heterogeneity (I^2) to evaluate the randomisation of the studies.

Meta-Analysis

Sixty-five human studies using various types and forms of millets were used for the meta-analysis to create forest plots for GI (112 observations) and glucose levels at 0 min (fasting blood glucose) and 120 min (post-prandial blood glucose) in normal, pre-diabetic, and diabetic subjects. The millets were compared with the corresponding control samples used in the study. The heterogeneity of the samples (I^2), and overall test results were obtained in forest plots along with p-values to test the significance of effect. Both the random effect model and fixed effect model were tested and used to interpret the results of each of the five outcomes. Wherever heterogeneity was low ($I^2 <$ 50%), a fixed effect model was used to interpret the result. In addition, where there was only a single source of information from the same population, a fixed effect model was used for the interpretation (25).

Subgroup Analysis

Three subgroup analyses were undertaken by identifying changes that possibly affect the five outcomes. This was done based on the type of control (glucose, refined wheat based, rice based, whole

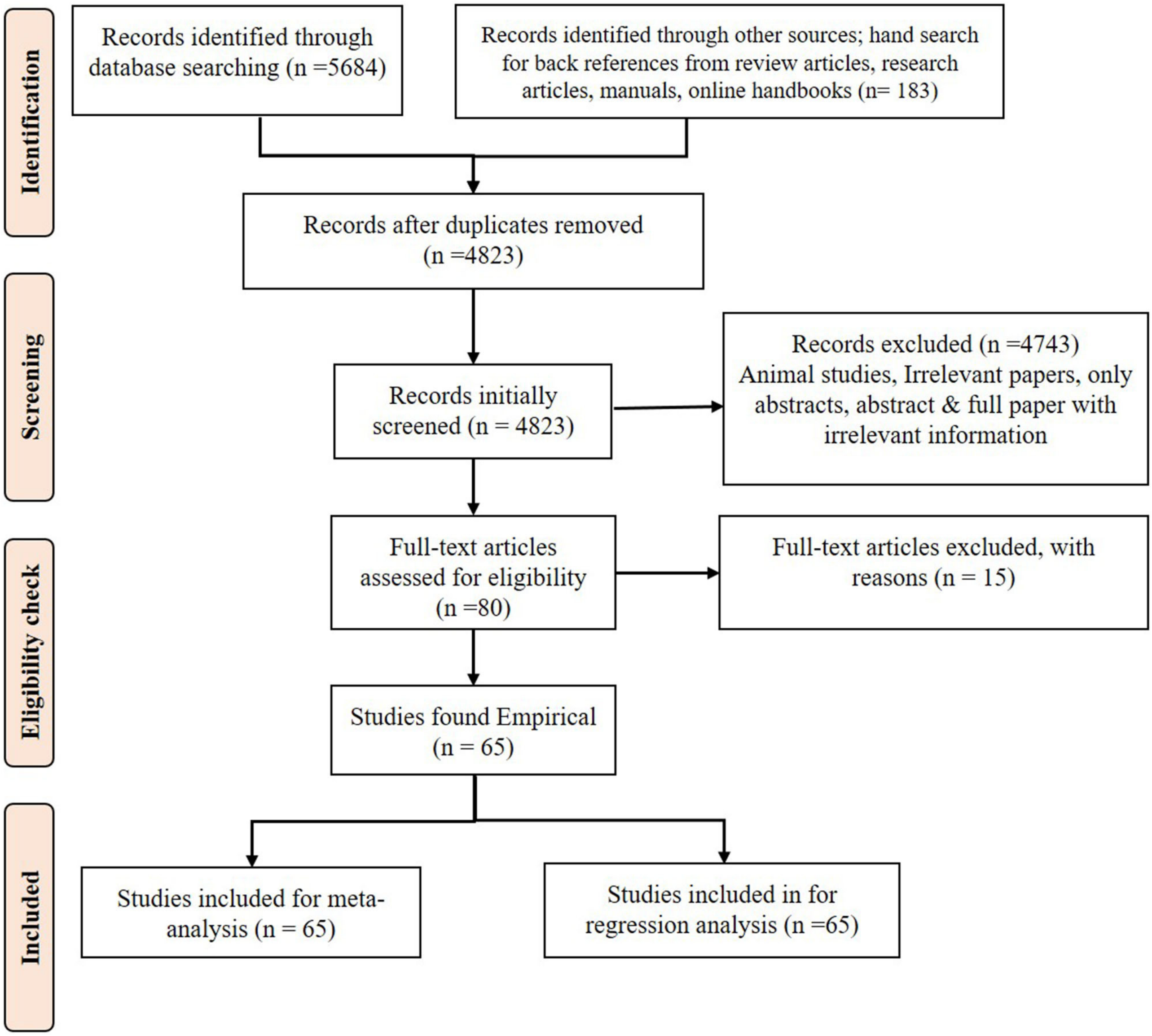

FIGURE 1 | A PRISMA flow diagram of the systematic review.

wheat based, pulses and legumes based, maize/corn based, other cereals based, and others) used in each study, participant's health condition (non-diabetic, pre-diabetic, and type 2 diabetic), and type of millet used in the studies. Note that the age group of the participants was given as the mean age in years in many studies. Hence, a subgroup analysis based on age was not conducted.

Risk of Bias

Funnel plots were generated to determine publication bias (23, 26). In addition, each study was scored for biases related to selection, performance, detection, attrition and reporting to generate a risk of bias plot.

Regression Analysis

Regression analysis is a statistical procedure for estimating the relationships between a dependent variable and independent variables. To quantify the effects of crop choice on GI (in all *in vivo* studies and only 2 *in vitro* studies), ordinary least squares (OLS) regression (27, 28) with cluster-robust standard errors (29) was performed using the metadata including 267 observations from 63 studies. OLS is the most common linear least square method of estimating the coefficient in a linear regression model. Here the dependent variable was the GI value, the main independent variables were a set of dummy variables representing different crops and the control variables were the grain processing and cooking methods, the Type 2 diabetes mellitus condition of the subjects and the method of GI measurement. More specifically, the OLS equation is expressed as follows:

$$y_i = \beta_0 + \sum_{j=1}^{17} \beta_{1j} x_{1ji} + \beta_2 x_{2i} + \sum_{l=1}^{3} \beta_{3l} x_{3li} + \beta_4 x_{4i} + \beta_5 x_{5i} + \sum_{n=1}^{63} \beta_{6n} x_{6ni} + \varepsilon_i$$

where y_i represents the GI value for the observation i ($i = 1, 2, 3, \ldots, 267$), β_0 is the intercept term, x_{1ji} is the set of 17 dummy variables representing 17 crops compared against maize being the base crop, x_{2i} is the dummy variable that takes the value of one when the food sample is made from a whole grain and zero otherwise, x_{3li} is the set of 3 dummies representing 3 cooking methods compared against raw consumption being the base method, x_{4i} is the dummy that takes one when the subject individual has the type 2 diabetic condition and zero otherwise, x_{5i} is the dummy that takes one when the GI value was estimated using the *in vitro* digestion rate and zero otherwise, x_{6ni} ($n = 1, 2, 3, \ldots, 63$) is the set of 63 dummies to control for any literature-specific fixed effects arising from any unobservable factor such as individual-specific food sample preparation practise, researcher-specific GI measurement practise, etc., and ε_i is the random error term. In addition, the interaction term between the type 2 diabetic condition and crop dummies was also examined.

The 17 crops compared with maize were Job's tears (adlay millet), barnyard millet, finger millet, fonio, foxtail millet, kodo millet, little millet, pearl millet, mixed millet (i.e., a mixture of millets and other crops), sorghum, teff, legume, roots and tubers, rice, refined wheat, wheat-based, and other (any other crops were regarded as one group). The three cooking methods analysed were boiling, steaming, and baking (and/or roasting) which were compared with no cooking. To account for literature-level clustering that results in downward bias in the standard errors stemming from any within-literature correlation, cluster-robust standard errors (29, 30) were adopted to correct for heteroscedasticity.

The most important feature of the multiple regression (there is more than one independent variable) is that the covariates are controlled for in the estimation of the coefficient of a certain variable. In our case, for instance, whether the food was made from whole grain or refined grain was controlled for when estimating the effect of a specific crop on GI. In other words, the estimation process incorporated both whole food and refined food, but only compared it with like variables (whole grain millet vs. whole grain maize, refined millet vs. refined maize, etc.,) where these values are either observed or estimated. Hence, the conclusion only reflects such fair comparisons.

RESULTS

For the meta-analysis, 65 human studies qualified for the five outcomes (GI, fasting blood glucose, post-prandial blood glucose, insulin level, and HbA1c). Some authors conducted studies on more than one type of millet; therefore, the same author contributed to more than one crop studied. This resulted in the identification of 99 studies from 65 authors, which included 19 studies on finger millet, 20 on foxtail millet, 10 each on sorghum and pearl millet, 7 on barnyard millet, 4 each on little and kodo millet, 3 each on teff, fonio and Job's tears, 1 on proso millet, and 15 on a mix of these millets. Apart from this, there were two *in vitro* studies that were included for teff and fonio, with 11 observations for GI (31, 32).

Descriptive Statistics

Table 2 shows the mean GI of each millet tested *in vivo* along with refined wheat and milled rice. The overall mean GI of millet, milled rice and refined wheat were 52.7 ± 10.3, 71.7 ± 14.4, and 74.2 ± 14.9, respectively. Except for proso millet, all other millets fell in the low to medium GI food category. **Table 2** also shows the *in vitro* GI of two types of millets.

Meta-Analysis

The effect of consuming millet-based food compared to the respective control samples or pre-intervention (baseline) values of participants of each study was determined through five outcomes, namely GI value (**Figures 2–4**), fasting, post-prandial, HbA1c, insulin level of blood in a meta-analysis and a forest plot was generated. The fixed effect model shows that except for little millet, the other 9 millets had a significantly low GI compared to control samples (**Table 3**). The fixed effect model was useful in explaining that fonio and teff samples were from a single source. Among 11 types of millets and one mixed millet tested, only little millet did not show a significantly lower GI compared with the control samples in both fixed effect and random effect models. There was no single study that determined GI of proso millet therefore it was not used in meta-analysis. All other studies generally showed a significantly lower GI than the control food tested, which included white refined wheat, rice, maize and glucose. Fonio showed low heterogeneity (0%) due to a single source sample and no randomisation with significantly low ($p < 0.01$) GI compared to standard glucose. Little millet had high heterogeneity (97%) with GI which was not significantly low ($p = 0.31$) compared to a rice-based diet. Teff showed moderate heterogeneity (75%) due to a single source sample and less randomisation with significantly low ($p < 0.01$) GI compared to corn injera (a white leavened Ethiopian flat bread with spongy texture) and white wheat bread. Barnyard millet exhibited high heterogeneity (95%) and significantly low GI ($p = 0.04$) with 95% confidence interval of −29.18; −0.99. Sorghum exhibited moderate heterogeneity (75%) and significantly low GI ($p = 0.03$) with 95% confidence interval of −2.59; −0.20 with Standardised Mean Difference (SMD) of −1.39. Pearl millet exhibited low heterogeneity (38%) and significantly low GI ($p < 0.01$) with 95% confidence interval of −2.11; −0.65. Kodo millet exhibited low heterogeneity (50%) and significantly low GI ($p < 0.01$) with 95% confidence interval of −1.76; −0.70. Foxtail millet exhibited high heterogeneity (89%) and significantly low GI ($p < 0.01$) with 95% confidence interval of −5.77; −1.44. Finger millet exhibited high heterogeneity (88%) and significantly low GI ($p < 0.01$) with 95% confidence interval of −5.35; −2.85. Mixed millets exhibited high

TABLE 2 | A comparison of millets' glycaemic index measured *in vivo* with control samples using different statistical analyses.

Type of millet	Mean glycaemic index	Regression coefficient (reduction in GI vs GI for maize) (%)	Meta-analysis (significant effect of millet-based diet on GI vs. control)		Glycaemic index food category
			Fixed effect model	Random effect model	
Barnyard millet	42.3	−27.2	$P < 0.01$	$P = 0.02$	Low
Fonio	42.0	−28.9	$P < 0.01$	$P = 0.07$	Low
Foxtail millet	54.5	−29.9	$P < 0.01$	$P < 0.01$	Low
Job's tears	54.9	−35.6	$P < 0.04$	$P = 0.4$	Low
Mixed millet	42.7	−26.4	$P < 0.01$	$P < 0.01$	Low
Teff	35.6	−27.1	$P < 0.01$	$P = 0.31$	Low
Finger millet	61.1	−26.0	$P < 0.01$	$P < 0.01$	Intermediate
Kodo millet	65.4	−20.1	$P < 0.01$	$P = 0.21$	Intermediate
Little millet	64.2	−13.3	$P = 0.98$	$P = 0.31$	Intermediate
Pearl millet	56.6	−18.1	$P < 0.01$	$P < 0.01$	Intermediate
Sorghum	61.2	−22.7	$P < 0.01$	$P < 0.01$	Intermediate
Control					
Milled rice	71.7	−11.4	NA	NA	High
Refined wheat	74.2	−15.9	NA	NA	High
***In vitro* studies**					
Teff	54.3	NA	$P < 0.01$	$P < 0.01$	Low
Fonio	56.3	NA	$P < 0.01$	$P < 0.17$	Low

$P < 0.01$, Significantly lower glycaemic index; NA, Not applicable. Fonio and teff data are from a single source; therefore, the results of a fixed effect model were more reliable than a random effect model.

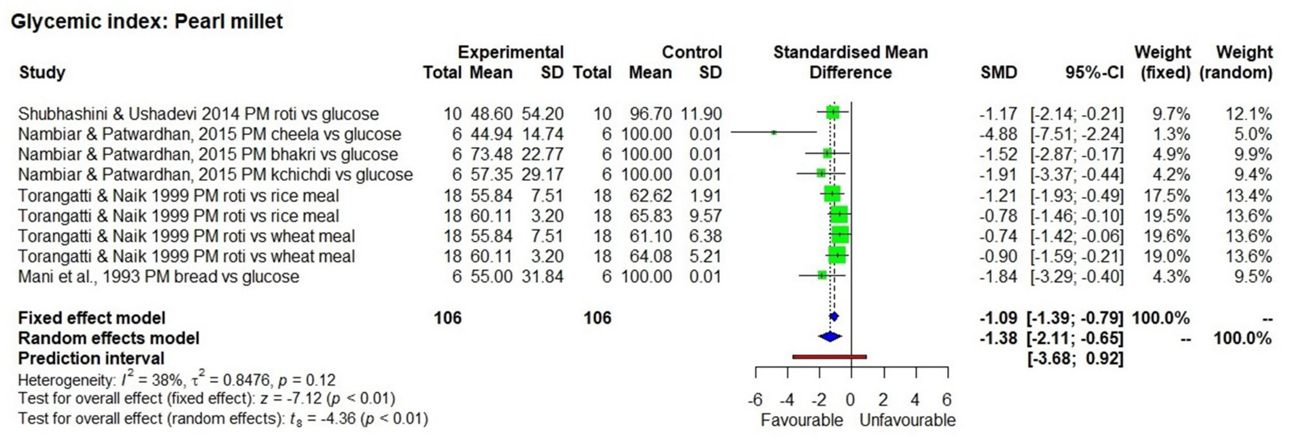

FIGURE 2 | The Glycaemic Index of a pearl millet diet compared to control samples. Abbreviations given in **Figures 2–8:** PM, Pearl millet; FM, Finger millet; DFM, Decorticated finger millet; ER, Expanded rice; WFM, Whole finger millet; GFM, Germinated finger millet; A vs. B, After vs. Before; SMY, Soya milk yoghurt; FMMY, Finger millet milk yoghurt; P, Probiotic; FTM, Foxtail millet; WB, White bread; KM, Kodo millet; KM-SU, Kodo millet sewai upma; RW-SU, Refined wheat sewai upma; SGG, Split green gram; WGG, Whole green gram; MM, Mixed millet; NM, Non-millet; FMFU, Finger millet flakes upma; FMVU, Finger millet vermicelli upma; RSR, Raw small roasted; RSU, Raw small unroasted; RLR, Raw large roasted; RLU, Raw large unroasted; RPSR, Raw parboiled small roasted; RPSU, Raw parboiled small unroasted; RPLR, Raw parboiled large roasted; RPLU, Raw parboiled large unroasted; FME, Finger millet extruded; FMB, Finger millet ball; SGF, Stone ground flour; KM, Kodo millet; MM, Mixed millet; FTM, Foxtail millet; BM, Barnyard millet. Description of food items in **Figures 2–8:** Dosa, Indian pan cake; Roti, a flat round bread cooked on a griddle; Chapatti, a thin flat bread of unleavened wholemeal bread cooked on a griddle; Pittu, Portioned steam cooked cake; Khichdi, Pulse, millet, spices mixed, and cooked together; Laddu, an Indian sweet made from a mixture of flour, sugar, and shortening, which is shaped into a ball; Baati, Hard unleavened bread; Burfi, Indian milk based sweet (here prepared with millet); Upma, a breakfast dish made by simmering roasted grain in tampered and spiced boiling water; Cheela, a savoury pan cake; Bhakri, a round flat unleavened bread.

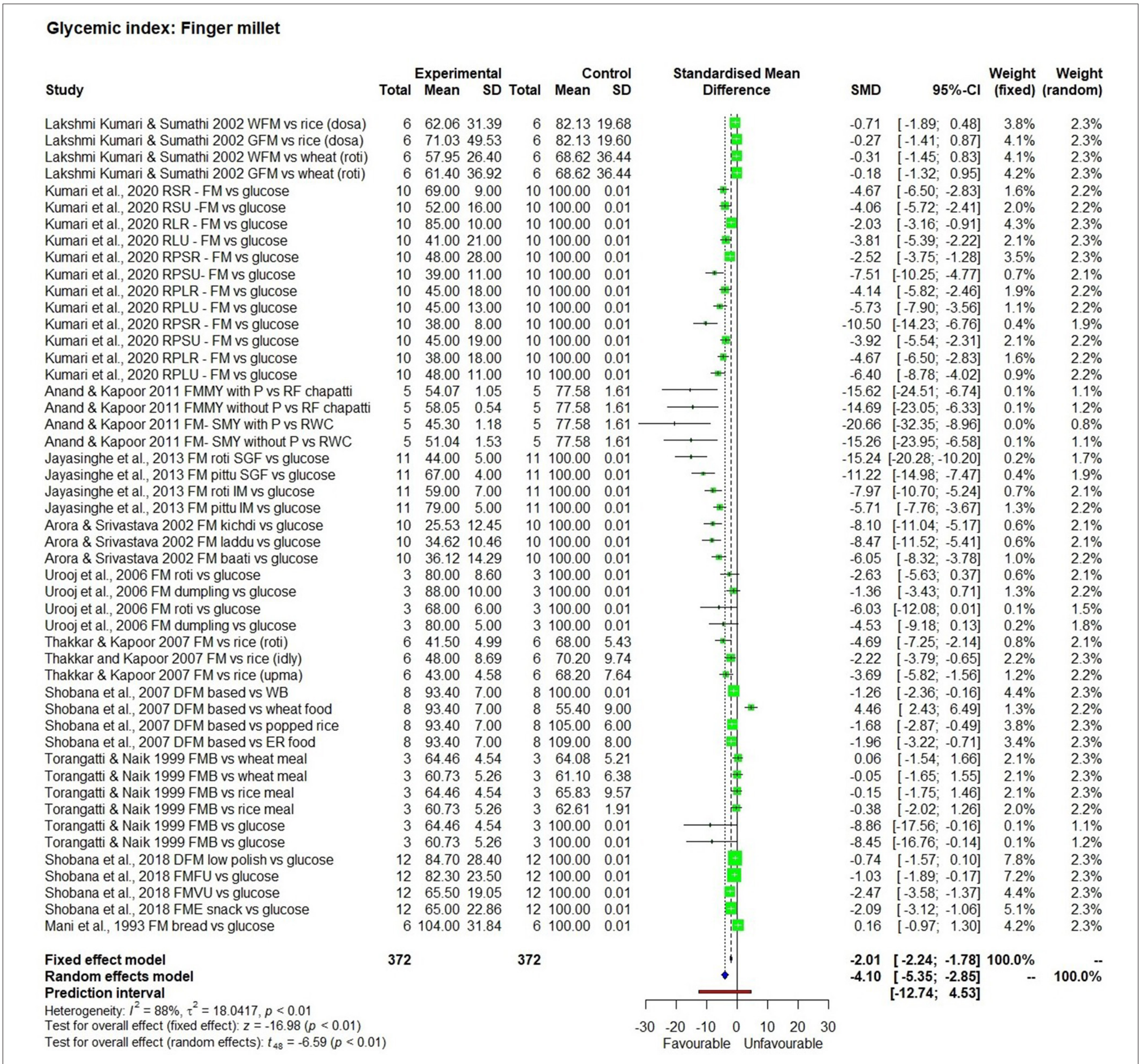

FIGURE 3 | The Glycaemic Index of a finger millet diet compared to control samples.

heterogeneity (93%) and significantly low GI ($p < 0.01$) with 95% confidence interval of −10.15; −3.73.

Fasting and Post-prandial Blood Glucose Level

In short term studies, all the 9 millets tested for post-prandial blood glucose significantly (**Table 3**) reduced blood glucose concentration compared to the control sample ($p < 0.01$). However, short term studies with overnight fasting didn't have a significant effect on fasting blood glucose level. In contrast, **Figures 5**, **6** show the significant effect ($p < 0.01$) being fed on millets for a long time (one study for 7 days and others were for 4 weeks to several weeks) had on reducing fasting (SMD −0.89 with 95% confidence interval of −1.11; −0.67) and post-prandial (SMD −0.95 with 95% confidence interval of −1.46; −0.44) blood glucose levels. While using a random effect model, kodo millet, little millet, and barnyard millet did not have a significant effect on post-prandial blood glucose levels compared to control samples. However, fonio and proso millet came from a single source of reference and the samples were the same; so only a fixed effect model was used in the interpretation which

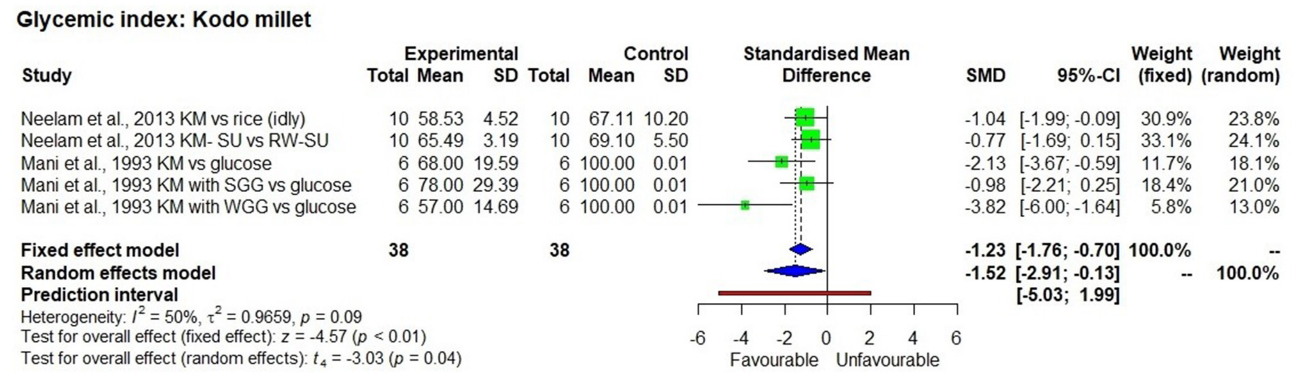

FIGURE 4 | The Glycaemic Index of a kodo millet diet compared to control samples.

demonstrated a significant effect in reducing post-prandial blood glucose levels.

HbA1c

There were six long term studies conducted to determine the effect of a millet diet on HbA1c level (**Figure 7**). All of them showed a reduction in HbA1c levels as a result of long term millet consumption; this reduction was significantly lower compared to when consuming a control rice-based diet or pre-intervention (baseline) HbA1c levels ($p < 0.01$).

Insulin Level

Albeit not shown, there were five studies that determined the insulin index (1 study with 5 observations), fasting insulin level (2 studies) and Area Under the Curve of Insulin (AUC) (2 studies) as a co-effect of reduction in GI, and the result showed significant reduction in fasting insulin level ($p < 0.01$) and insulin index in fixed effect model with no significant effect on AUC insulin ($p = 0.24$).

Subgroup Analysis

Results of the subgroup analysis (**Figure 8**) showed that consuming a millet-based diet for a long time (>3 months) had a significant effect on reducing fasting blood glucose levels in all participants regardless of the group (non-diabetic, pre-diabetic, and diabetic) compared to a regular rice or wheat-based diet ($p < 0.01$). There was no significant difference among groups ($p < 0.13$). However, when looking at post-prandial blood glucose level, a significant reduction in blood glucose was observed among type 2 diabetic subjects compared to non-diabetic ones and the subgroup effect was significant ($p < 0.01$). It was not possible to see this difference between diabetic and pre-diabetic subjects due to the small number of studies on the latter. The subgroup effect was not significant ($p = 0.69$) based on the type of millet in reducing both fasting and post-prandial blood glucose levels; this goes to show that regardless of the type of millet, its long term consumption has the potential to reduce both fasting and post-prandial blood glucose levels.

Regression Analysis

GI levels varied among various millets with the average GI of low to intermediate. Various millets and food forms tested in 63 studies with 267 observations on millets and sorghum and 267 observations on maize, wheat, rice, or reference food (glucose or wheat bread). Millets had low GI (≤55%), lower than milled rice, refined wheat flour, white wheat bread, maize, or glucose (reference food).

Table 4 presents the frequency distribution of all the categorical independent factors included in the regression analysis. Finger millet and foxtail millet were the most frequently observed crops, followed by rice, wheat and sorghum. Most cases used food made from a refined grain, while a few cases used food originating from whole grain. Boiling was the most common cooking method, followed closely by baking (and/or roasting). About 14% of the cases used the *in vitro* estimation of GI values while the rest tested on normal subjects (59%) and type 2 diabetic subjects (27%).

Table 5 shows the results of the Cluster-Robust OLS analysis of the effects of different crops on GI values. To keep the table succinct, the 63 control variables included controlling for literature fixed effects were dispensed with. The result indicates that 14 out of the 18 analysed crops had negative and statistically significantly lower ($p < 0.10$) GI values compared to maize flour-based control food. In descending order, the marginal effects were −35.6 for Job's tears, −28.9 for fonio, −29.9 for foxtail millet, −27.1 for teff, −27.2 for barnyard millet, −26.4 for mixed millet, −26.0 for finger millet, −22.7 for sorghum, and −20.1 for kodo millet.

DISCUSSION

Most of the studies showed a glucose-lowering effect of various types of millets that were served in various forms compared to the control foods. A variety of processed products and cooking methods were tested and often compared to milled rice, refined wheat and maize-based foods. The regression analysis clearly shows that millets have a lower GI compared to other cereals such as maize, milled rice and refined wheat flour. This means,

TABLE 3 | Heterogeneity and p values from fixed and random effect models from forest plots on glycaemic index, fasting and post-prandial blood glucose levels.

Millet	Heterogeneity (I^2) (%)	Fixed effect model (*p*)	Random effect model (*p*)	95%–confidence interval	Author details
Glycaemic index(GI)					
Fonio	0	<0.01	0.07	−6,655.5; −3,803.9	(33)
Little millet	97	0.98	0.31	−52.02; 27.43	(8, 34, 35)
Teff	75	<0.01	0.31	−1.98; −0.55	(36)
Job's tears	97	0.04	0.40	0.08; 2.46	(14, 37)
Barnyard millet	95	0.01	0.04	−29.18; −0.99	(8, 38–42)
Sorghum	75	<0.01	<0.01	−2.59; −0.20	(43–46)
Kodo millet	50	<0.01	0.04	−2.91; −0.13	(8, 46, 47)
Mixed millet	93	<0.01	<0.01	−10.15; −3.73	(48–51)
Finger millet	88	<0.01	<0.01	−5.35; −2.85	(41, 44–46, 52–58)
Pearl millet	38	<0.01	<0.01	−2.11; −0.65	(44, 46, 59, 60)
Foxtail millet	89	<0.01	<0.01	−5.77; −1.44	(8, 35, 39, 44, 48, 61–63)
0 min/fasting blood glucose level					
Fonio	93	0.80	0.70	22.77; 21.01	(33)
Little millet	0	0.83	0.71	−1.53; 1.42	(8, 64)
Job's tears	87	<0.01	0.77	10.20; 9.64	(37, 65)
Proso millet	51	0.03	0.20	−1.19; 0.34	(66)
Barnyard millet	40	0.04	0.13	−1.19; 0.22	(8, 38, 42, 67)
Pearl millet	0	0.97	0.99	−0.30; 0.31	(8, 44, 46, 60, 68–70)
Sorghum	0	0.49	0.25	−0.31; 0.09	(44–46, 64, 71, 72)
Kodo millet	86	<0.01	0.21	−0.14; 0.32	(8, 46, 68)
Mixed millet	86	<0.01	0.03	−2.48; −0.13	(11, 12, 49, 50, 73–78)
Finger millet	55	<0.01	0.05	−0.52; 0.00	(7, 44, 45, 52–54, 56, 79–81)
Foxtail millet	33	<0.01	0.09	−56; 0.04	(8, 10, 13, 35, 39, 44, 48, 61–63, 68, 82–85)
120 min/post-prandial blood glucose level					
Fonio	28	<0.01	0.17	−9.09; 4.98	(33)
Little millet	99	<0.01	0.48	84.88; 88.11	(8, 64)
Proso millet	87	<0.01	0.19	−2.54; 0.70	(66)
Barnyard millet	97	<0.01	0.33	−28.09; 120.33	(8, 38)
Pearl millet	86	<0.01	0.07	−2.89; 0.14	(8, 44, 46, 60, 68–70, 86)
Sorghum	0	<0.01	0.01	−0.82; −0.12	(44–46, 64, 71, 72, 87)
Mixed millet	90	<0.01	0.02	−1.97; −0.27	(49, 50, 73–76)
Finger millet	79	<0.01	<0.01	−3.51; −0.94	(7, 44, 45, 52–54, 56, 64, 80, 81)
Foxtail millet	91	<0.01	0.02	−3.68; −0.29	(8, 9, 13, 44, 61–63, 68, 82)
Area under the curve glucose					
Finger and foxtail millet	11	<0.01	0.03	−3.24; −0.23	(88)
Proso millet	37	0.98	0.98	−0.65; 0.66	(66)

for instance, that when Job's tears-based food was consumed, the GI value was significantly lower by 36 units on average than when maize-based food was consumed, taking into account that all the other conditions (i.e., processing, cooking methods, type 2 diabetes condition, and GI estimation methods) were equal. Similarly, when foxtail millet-based food was consumed, the GI value was significantly lower by 30% on average than maize-based food (**Table 5**). It may be noted that Job's tears-based food is comparable with whole wheat-based food and legumes as these two foods lower GI by 37.8 units and 37.0 units, respectively on average than the consumption of maize-based food. Major crops such as milled rice and refined wheat did not show a GI advantage against maize, indicating that they tend to have relatively high GI values. On the other hand, among the broad group of millet crops (millets, sorghum, and teff), all of them showed lower GI values except little and pearl millet, for which the coefficient was negative (−13.3 and −18.1) but not statistically significant ($p = 0.445$ and 0.127).

All the cooking methods raised GI values. In particular, steaming, baking (including flat bread cooked in a pan) and

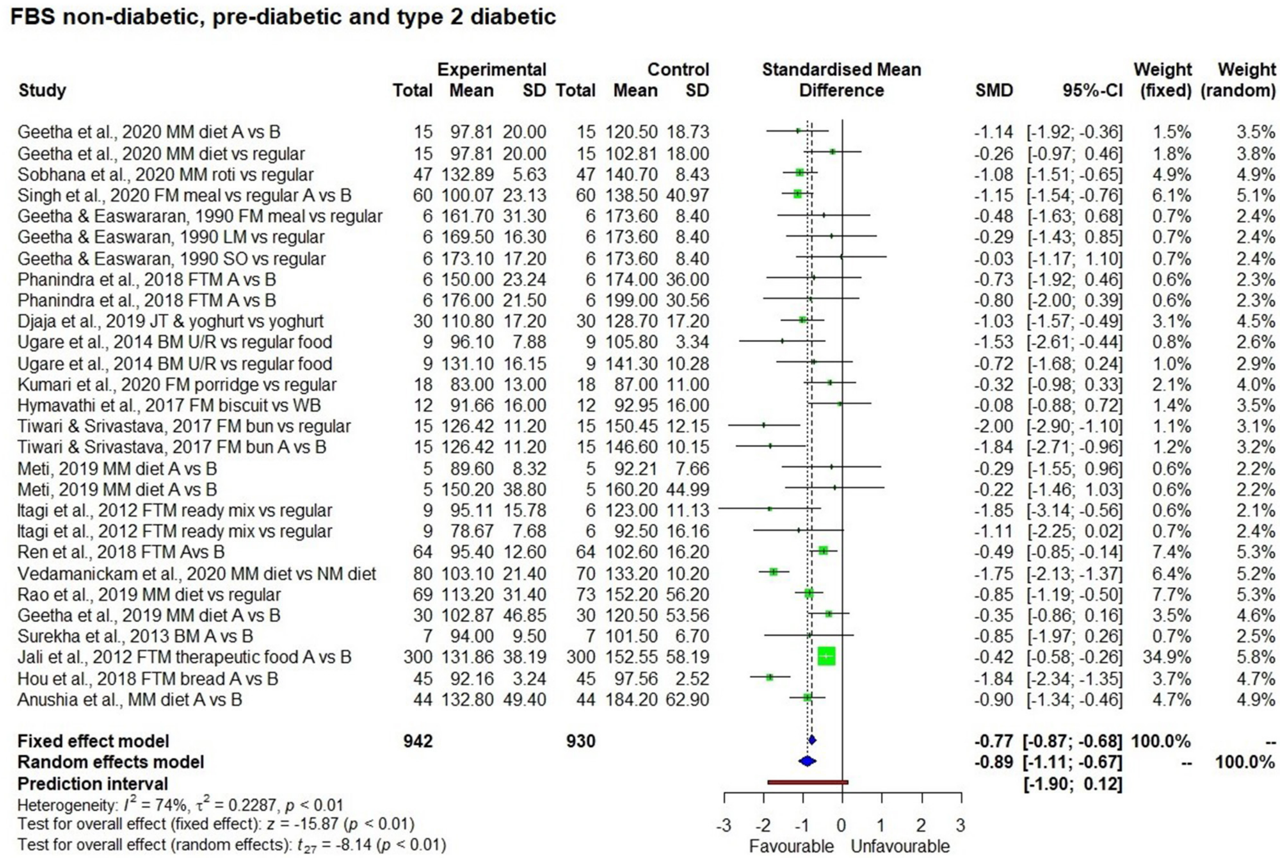

FIGURE 5 | Effect of long term consumption of millet on fasting blood glucose levels in non-diabetic, pre-diabetic, and diabetic subjects compared to the control group consuming a regular diet or pre vs post intervention comparison.

boiling increased the GI of the food by up to 18.4 units, 16.3 units and 11.3 units, respectively. Despite this, the overall GI of millets was 52.7. This could be due to the addition of other ingredients such as fats and oils in different types of cooking. Somewhat unexpectedly, the use of whole grain millets did not affect GI values significantly compared to decorticated millets. This could be because of the fewer sample numbers that used whole grain.

The coefficient of type 2 diabetes showed that subjects with type 2 diabetes tend to exhibit higher GI (+5.3, $p = 0.002$) values after a meal compared to those without diabetes. The coefficient of the *in vitro* estimation was not significant, implying that on an average the GI values were not different when *in vitro* estimation was used instead of human testing on non-diabetic subjects, which supports the validity of the GI values estimated with *in vitro* experiments. Although not included in the table, the additional analysis using the interaction terms between the type 2 diabetes condition and crop variables showed that the GI benefits from millets such as barnyard millet, finger millet, fonio, foxtail millet, kodo millet, pearl millet, and sorghum did not differ between type 2 diabetic subjects and non-diabetic subjects. This indicated that these millets may be more effective in lowering GI values compared to major cereals irrespective of whether the subjects were diabetic or not. These findings demonstrate that the consumption of food items made from various millets contribute to keeping the blood glucose level low compared to the food based on maize and milled rice. Moreover, barnyard millet, fonio, foxtail millet, kodo millet, pearl millet, and sorghum were equally beneficial for type 2 diabetes and non-diabetes individuals.

The regression analysis' results were generally supported by the meta-analysis conducted for the data on GI which showed that all the studies except those on little millet had no significant effect on reducing blood glucose levels.

Two forest plots constructed during the meta-analysis were repeated in different ways to determine the effect of removing one study that was identified as an outlier or having an odd Standard Mean Difference (SMD) value. In finger millet, the study conducted by Ruhembe et al. (89) showed highest SMD of 230 while the overall SMD of the study was −1.84. Removing this particular study changed the overall effect with an SMD of −3.38. Similarly in sorghum, the same study showed highest SMD of 311.16 vs. an overall SMD of 14.49; removing the study changed the SMD to −1.2 and the *p*-value became more significant. These two studies were masking the effect of other studies, and this could be because of the lack of non-random sample selection and allocation, no blinding test (both participants and the testing person) and eventually scored highly

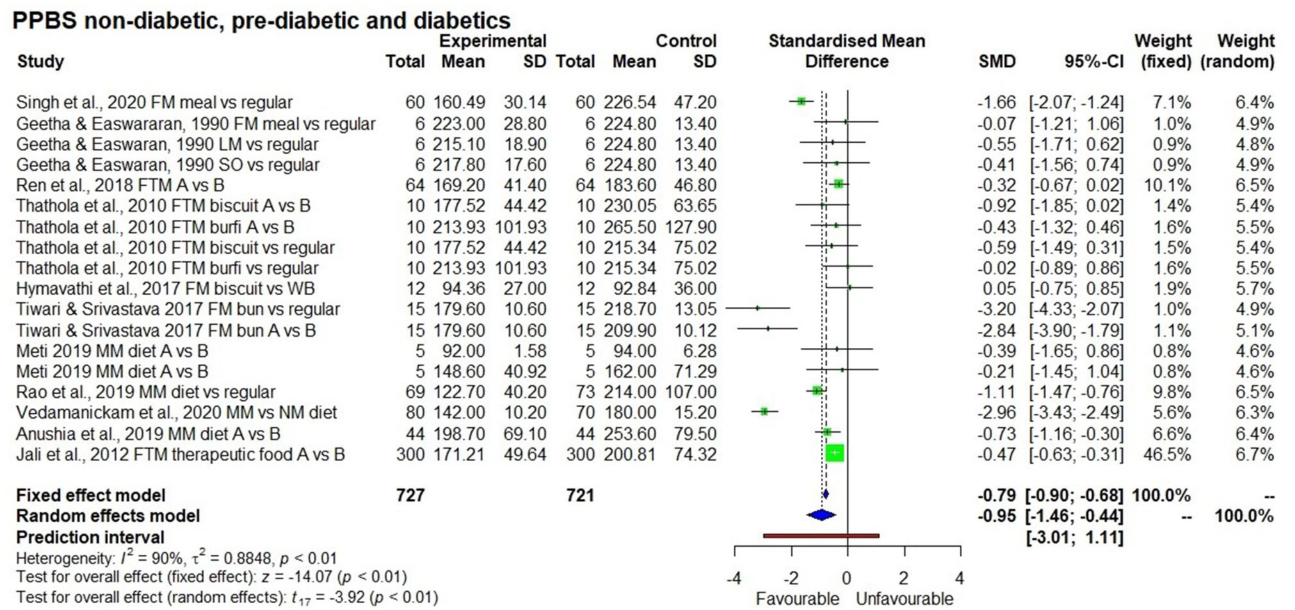

FIGURE 6 | Effect of long term consumption of millet on post-prandial blood glucose levels in non-diabetic, pre-diabetic, and diabetic subjects compared to the control group consuming a regular diet or pre vs post intervention comparison.

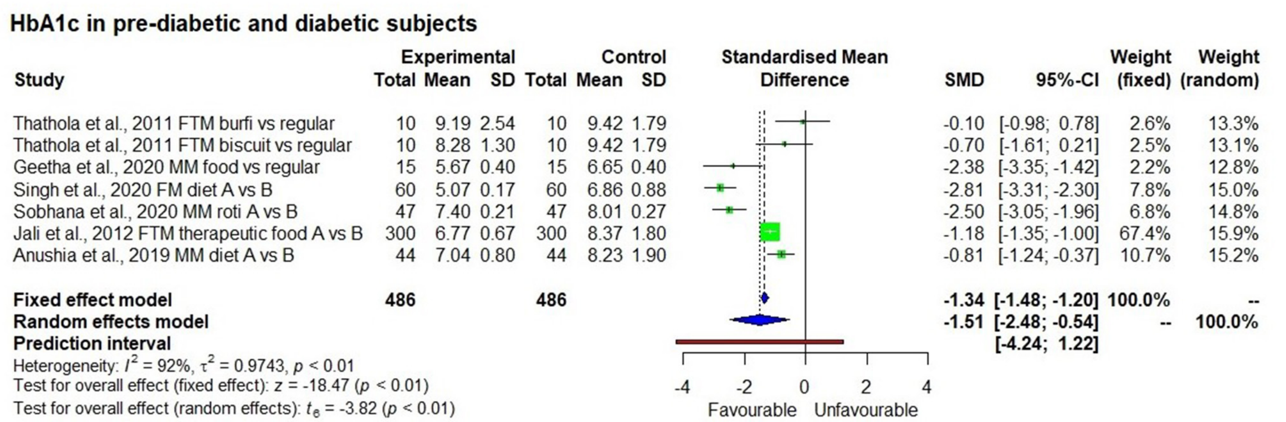

FIGURE 7 | Effect of long term consumption of millet on HbA1c levels in pre-diabetic and diabetic subjects compared to the control group consuming a regular diet or pre vs post intervention comparison.

critical rank in risk assessment. Therefore, the risk of bias could be reflected in getting small standard deviation between sample and high SMD in the meta-analysis. There were only 5 studies available on little millet, of which 2 didn't have complete data on SD and hence were not used in the meta-analysis. Of the 5 studies, only 1 reported that little millet has high GI (35). The SMD value reported by Malavika et al. (35) deviated highly from all other studies. If that one study was removed, then little millet showed a significant effect on reducing GI in the fixed effect model ($p < 0.01$). The high GI in little millet was attributed to polishing millets. However, this study needs a detailed evaluation to generate more evidence on little millet given the limited number of studies available. Proso millet was studied by only one author (66) who didn't calculate GI but studied the change in blood glucose level for a period of 2 h after the consumption of proso millet products which showed significant reduction in blood glucose level ($p < 0.05$).

It may be noted that consuming a millet-based diet for long periods (more than 3 months) was also associated significantly with reduced HbA1c marker levels in both pre-diabetic and diabetic subjects ($p < 0.01$) compared to consuming a regular rice or wheat-based diet or pre-intervention HbA1c level. HbA1c is a glycated haemoglobin, i.e., it is bound to glucose and is

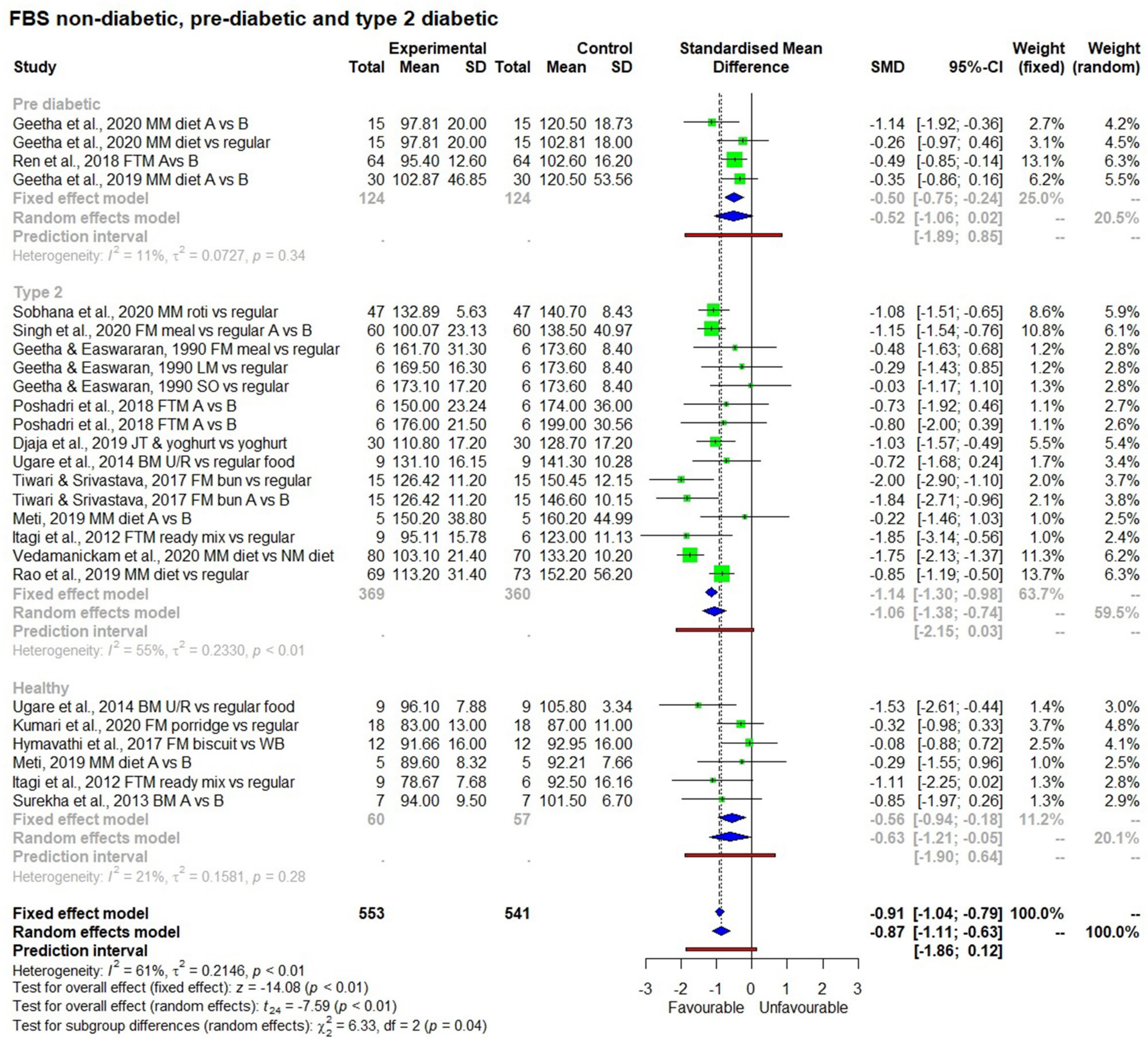

FIGURE 8 | The effect of long term consumption of millet on non-diabetic, pre-diabetic and diabetic subjects consuming millet compared to a control group consuming a regular diet.

different from free unbound glucose in blood. Unlike fasting blood glucose level which reflects the blood glucose level at a particular point of testing time, HbA1c reflects the average blood glucose level typically over a period of 8 to 12 weeks and is therefore an indicator of long-term glycaemic control. Overall, there was a 15% reduction in HbA1c level (from 8.1 ± 1.0 to 7.0 ± 1.4%). Especially in pre-diabetic subjects, HbA1c levels fell to the normal reference level (from 6.65 ± 0.4 to 5.67 ± 0.4%) (12). The reduction is attributed to the high fibre content and low glycaemic index of the millet-based diet (11) which reduces the availability of glucose to form HbA1c and thereby regulates the HbA1c glycation process. It is evident that a millet- based diet has a positive effect on managing diabetes.

Another study conducted on pre-diabetic subjects (those with impaired glucose tolerance) fed on foxtail millet (82) for a long period (12 weeks) showed that the fasting blood glucose level reduced to normal levels (from 102.6 ± 16.2 to 95.4 ± 12.6 mg/dl) in 64 study subjects ($p < 0.001$). This is evidence of millets' effect on averting rising blood glucose levels and preventing pre-diabetic individuals from entering the diabetic stage. However, more studies are needed to reconfirm this.

It is important to note that most of the studies were conducted after overnight fasting and the introduction of the test food or control food as breakfast. This was followed by the measurement of fasting and post-prandial blood glucose levels. This method does not give information on how the glycaemic response might

TABLE 4 | Frequency distribution of the independent variables in the regression analysis: crop, cooking method, and method used to determine GI ($n = 267$).

Crop	Number of observation	%
○ Job's tears	2.0	0.7
○ Barnyard millet	16.0	6.0
○ Finger millet	46.0	16.9
○ Fonio	7.0	2.6
○ Foxtail millet	33.0	12.4
○ Kodo millet	10.0	3.8
○ Little millet	3.0	1.1
○ Pearl millet	11.0	4.1
○ Mixed millet	21.0	7.9
○ Sorghum	18.0	6.7
○ Teff	3.0	1.1
○ Maize	11.0	4.0
○ Rice	32.0	12.0
○ Refined wheat	26.0	9.7
○ Others	15.0	5.6
○ Roots and tubers	3	1.1
○ Legumes	3	1.1
Cooking method		
○ Bake and/or roast	119.0	44.6
○ Boil	119.0	44.6
○ Steam	17.0	6.4
○ Raw	12.0	4.5
Method used to determine GI		
○ *In vitro*	38.0	14.0
○ Human testing on normal subjects	157.0	58.8
○ Human testing on type 2 diabetes subjects	72.0	27.0

TABLE 5 | The effects of crop choice on GI values compared with maize, using ordinary least squares with cluster-robust standard errors.

	Coef.	Robust SE	*p*-value
Crop			
Job's tears	−35.580***	12.620	0.006
Barnyard millet	−27.168**	11.633	0.023
Finger millet	−26.012*	13.186	0.053
Fonio	−28.900**	10.933	0.010
Foxtail millet	−29.858**	11.662	0.013
Kodo millet	−20.068*	11.235	0.079
Little millet	−13.336	17.356	0.445
Pearl millet	−18.064	11.696	0.127
Mixed millet	−26.426**	10.941	0.019
Sorghum	−22.657*	12.267	0.069
Teff	−27.096**	10.534	0.012
Rice	−11.448	12.439	0.361
Refined wheat	−15.882	11.265	0.164
Wheat based	−37.826***	10.941	0.001
Legumes	−37.006*	21.804	0.095
Others	−21.719**	10.008	0.034
Cooking method			
Baked and/or roasted	16.361***	3.382	0.000
Boiled	11.329***	2.491	0.000
Steamed	18.405***	4.537	0.000
GI estimation methods (base: human testing on normal subjects)			
Human testing on type 2 diabetes subjects	5.275***	1.598	0.002
In vitro	−24.928	6.397	0.644
Constant	71.663***	6.778	0.000

*Dependent variable = Glycaemic Index (GI) value: $n = 267$, $R^2 = 0.660$, Adj. $R^2 = 0.416$. NB, The estimation included 63 literature dummies which were not included in the table. ***$p < 0.01$, **$p < 0.05$, and *$p < 0.10$, respectively.*

change after acclimatisation to millet-based food. However, 21 studies conducted using millet as a test food for long periods of time ranging from 7 days to several weeks after which fasting and post-prandial blood glucose levels were measured, provided information on changes in both levels after acclimatisation to millet-based food. The results show that consuming millet for a long duration has a positive effect of reducing both fasting blood glucose level ($p < 0.05$) by 12%, with a mean reduction of 16 mg/dl (from 134 mg/dl to 117.9 mg/dl) and post-prandial blood glucose level by 15%, with a mean reduction of 30 mg/dl (from 202 to 172 mg/dl) which is near normal levels for diabetic subjects. While testing after overnight fasting (short term studies) had no significant effect on fasting blood glucose level, there was a significant reduction ($p < 0.05$) in post-prandial blood glucose level.

There were only two studies (14, 62) that determined insulin index and GI. It may be noted that although Job's tears' GI was low (55), its insulin index was slightly higher (67). The insulin index in Job's tears was less compared to brown rice (81%) and Taro or colacasia esculenta, a root vegetable (73%). The author of these studies ascribed the insulin response of the food increase to the co-injection of protein or fat through the meal. This clearly suggests the need for extreme caution while preparing food for diabetic individuals to ensure it has not just low GI but also a low insulin index to avoid raising insulin levels in the blood; high insulin concentration is associated with insulin resistance and cardiac risk (14). Consuming millet based diet for three months was shown to increase in mean insulin sensitivity from 68.1 ± 4.7 to 88.2 ± 6.0 (11). Ren et al. (62) demonstrated that when foxtail millet was cooked with only water, the insulin index was very low (49.8) compared to processed food, and the ratio of insulin index and GI was <1 compared to the processed products. Hence, it was reported as a suitable product for managing diabetes.

Several studies have shown that resistance starch formation in millets and high fibre in millet retard starch hydrolysis, thereby exhibiting low GI (45) and its potential to reduce blood glucose level. The high presence of a non-starch polysaccharide such as dietary fibre in millets compared to wheat and rice (90) decreases enzymes' activities in the gut and results in incomplete hydrolysis of carbohydrates, protein and fats present in millet-based diets. This delays the absorption of starchy polysaccharides and lowers the rate of absorption of mono and disaccharides (46), thereby exhibiting low glycaemic response. High resistant starch formation in millets is due to the presence of amylose which tends to retrogradation of starch (set back viscosity) which forms resistant starch and thereby is difficult to hydrolyze by

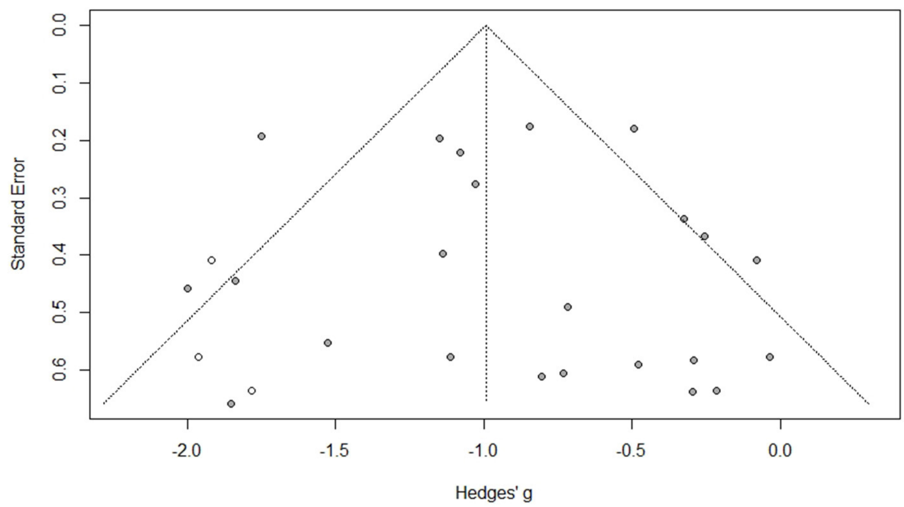

FIGURE 9 | Publication bias assessment of the long term studies used for fasting blood glucose level as an effect of consuming millet-based meal (after applying trim and fill method) ($p < 0.0001$).

digestive enzymes (61), leading to low glycaemic response. Also, fat and protein content in any food slow down the rate of gastric emptying, thereby slowing down the digestion of food in the intestine. Millets are known to have high protein and fat compared to milled rice (90) and thereby contributing to low GI (61), this is because, protein and fat combined with other factors slows down the digestion in small intestine which leads to incomplete digestion and thereby contributes to low GI. Protein content in millet increases insulin sensitivity thereby helping to maintain better glycaemic response.

Lakshmi Kumari and Sumathi (52) and Abdelgadir et al. (87) reported that high fibre content in finger millet gives rise to slower gastric emptying or the formation of non-absorbable complexes with carbohydrates in the gut lumen. Itagi et al. (10), Thilakavathy and Muthuselvi (68), Pathak et al. (48) and Narayanan et al. (13) have also reported the glucose lowering effect of finger millet due to high-soluble dietary fibre in food which reduces gastric emptying, the absorption of glucose after a meal and decreases the activity of digestive enzymes. This results in incomplete hydrolysis of carbohydrates, protein and fats, thereby delaying absorption. Jayasinghe et al. (55) reported that when two different processing methods such as stone milling and industrial milling were used to make flour, the large particles of flour produced make starch gelatinization relatively difficult and slow down enzyme attack. This slows down the release of glucose from food, causing a significant decrease in glycaemic response. Nambiar and Patwardhan (60) reported both high GI of some foods and low GI of others which they attribute to processes like boiling and pressure (steam) cooking that result in faster rates of digestion compared to roasting. This could be the reason for the high GI in *khichadi* (a mix of pulse, millet, spices) compared to *cheela* (savoury pancake), *thalipeeth* (savoury multi-grain flat bread), sorghum *bhakri* (round flat unleavened bread), and wheat roti. It is further confirmed in current systematic review, that boiling of millet in whole or decorticated form either unprocessed or minimally processed by milling into coarse grain or flour produced average GI of 52.1 ± 3.9 (low GI) compared to milled rice (63.1 ± 10.7) or maize (58.8 ± 18.9). In addition, Ren et al. (82) clearly demonstrates that including foxtail millet in the diet can reduce fasting blood glucose level provided the consumer is restricted to the specified diet, which is important contributing factor in achieving impact.

A risk of bias assessment conducted on all the 65 studies revealed that more than 50% of them had low risk of bias. High risk of bias in the overall effect is contributed by blinding of samples tested. Some studies indicated that blinding was not possible with millet-based foods due to their unique texture, flavour and appearance (66, 82). However, participants were blinded for the proportion of millet in any food tested and the name of the millet (70, 86). The asymmetrical funnel plot obtained was due to the small sample size which created publication bias. This effect on the funnel plot was adjusted and accounted for using trim and fill method until the plot became symmetrical ($p < 0.0001$; **Figure 9**).

Limitations of the Study

Most of the *in vivo* studies included in the systematic review did not have the standard number of 8–12 subjects to determine GI, as recommended by FAO/WHO (91). Some studies had as few as three subjects, which is a major limitation; but they were not excluded considering the limited number of studies available for some of the millets and the importance of this information. There were only two studies conducted on pre-diabetic subjects to establish the link between millet-based food and its diabetes preventing effect through the reduction of HbA1c and fasting blood glucose levels from higher to normal range. The great variability in using control food further reduced the sample size corresponding to each control. The age group effect was not analysed as most of the studies presented the age group as mean age group rather than the range, which was another limitation.

Recommendation on Methodology for Future Research

The study captured evidence from the 1990s to 2020. There was no uniformity in method used; only a few studies mentioned having followed 2010 ISO standards. Using this standard to determine GI improves the accuracy of results and uniformity among different studies (regardless of geography and laboratory) as the standard deviation obtained from different laboratories using ISO 2010 is much lower (21).

It may perhaps be helpful to conduct interventions of longer duration by using the continuous glucose monitoring system (92). This system with a sensor can analyze interstitial fluid glucose levels at 15-min intervals for 24 h for 14 consecutive days. It can calculate the mean 24 h interstitial glucose values and incremental area under the curve (iAUC) over the 14 days for an intervention diet and the control diet and iAUC for an individual meal. The use of this system is recommended in future interventions to enhance the accuracy of results in order to generate robust and better evidence on glucose management using millets.

CONCLUSIONS

This systematic review and meta-analysis confirm that the millets evaluated have strong potential in dietary management and the prevention of diabetes. Apart from policy implications, it has implications in terms of nutrition sensitive agriculutre interventions with millets and sorghum and on the dissemination of the beneficial effect of millets and sorghum for glycaemic control.

DATA AVAILABILITY STATEMENT

The original contributions generated for the study are included in the article/**Supplementary Material**, further inquiries can be directed to the corresponding author/s.

AUTHOR CONTRIBUTIONS

SA and JK-P: conceptualisation. SA, JK-P, and KDVP: review and selection of papers. SA, DIG, RKB, AR, TWT, and MV: writing. JK-P: resource. SA, KS, DJP, KDVP, AR, and MV: data collection, screening, and extraction. SA, RB, and TWT: data extraction, meta-analysis, regression analysis, and risk assessment. SA, JK-P, KDVP, DIG, AR, DJP, KS, RKB, and MV: review and writing. All authors contributed to the article and approved the submitted version.

FUNDING

This systematic review was supported by the Smart Food Endowment fund.

ACKNOWLEDGEMENTS

The authors would like to acknowledge the following authors and editors of journals who sent in missing data or their full paper to support this meta-analysis: Lily Arsanti Lestari, Bernatal Saragih, Chithra Muthu, Raju Nagaraju, Matthew Mcsweeney, Sun-Ok Lee, Damayanti Korrapati, Neelam Yadav, Radhai Sree, Jali M.V, and Alegbejo Janet. We also thank author Ram B. Singh who clarified having used finger millet in his study. The support rendered in data collection by the staff and library of the Avinashilingam University for Women, Coimbatore, is gratefully acknowledged. Thanks are also due to M. Azim Aijaz, Associate Managing Editor, Journal of Pure and Applied Microbiology (JPAM) for providing the article. Our special thanks goes to Joycelyn M. Boiteau, Division of Nutritional Sciences, Cornell University, Tata-Cornell Institute for Agriculture and Nutrition, United States, for reviewing this paper. The authors acknowledge Ms. Smitha Sitaraman, ICRISAT, for editing the manuscript.

SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fnut.2021.687428/full#supplementary-material

REFERENCES

1. International Diabetes Federation. *Global Diabetes Data Report 2010–2045*. Available online at: diabetesatlas.org
2. Anitha S, Kane-Potaka J, Tsusaka TW, Tripathi D, Upadhyay S, Kavishwar A, et al. Acceptance and impact of millet-based mid-day meal on the nutritional status of adolescent school going children in a peri urban region of Karnataka State in India. *Nutrients*. (2019) 11:2077. doi: 10.3390/nu11092077
3. Poole N, Kane-Potaka J. *The Smart Food Triple Bottom Line – Starting with Diversifying Staples Including Summary of latest Smart Food studies at ICRISAT, Agriculture for Development journal, No. 41.* Tropical Agriculture Association (2020). p. 21–3. Available online at: https://taa.org.uk/wp-content/uploads/2021/01/Ag4Dev41_Winter_2020_WEB.pdf
4. Awika JM. Major cereal grains production and use around the world. In: Awika JM, Piironen V, Bean S, editors. *Implications to Food Processing and Health Promotion, Advances in Cereal Science*. Washington, DC: American Chemical Society (2011). p. 1–13. doi: 10.1021/bk-2011-1089.ch001
5. Vetriventhan M, Vania C, Azevedo R, Upadhyaya HD, Nirmalakumari A, Kane-Potaka J, et al. Genetic and genomic resources, and breeding for accelerating improvement of small millets: current status and future interventions. *Nucleus.* (2020) 63:217–39. doi: 10.1007/s13237-020-00322-3
6. Almaski A, Shelly COE, Lightowler H, Thondre S. Millet intake and risk factors of type II diabetes: a systematic review. *J Food Nutr Disord.* (2019) 8:2.
7. Singh RM, Fedacko J, Mojto V, Isaza A, Dewim M, Watanabe S, et al. Effects of millet based functional foods rich diet on coronary risk factors among subjects with diabetes mellitus: a single arm real world observation from hospital registry. *MOJ Public Health.* (2020) 9:18–25. doi: 10.15406/mojph.2020.09.00318
8. Palanisamy T, Sree R. Efficacy of millets (foxtail, kodo, small, barnyard and pearl millet) varieties on post prandial glycaemic response in patients with type 2 diabetes. *Eur J Biomedpharm Sci.* (2020) 7:443–9.
9. Thathola A, Srivastava S, Singh G. Effect of foxtail millet (*Setaria Italica*) supplementation on serum glucose, serum lipids and glycosylated haemoglobin in type 2 diabetics. *Diabet Croat.* (2011) 40:23–9.

10. Itagi S, Naik R, Bharati P, Sharma P. Readymade foxtail millet mix for diabetics. *Int J Sci Nat.* (2012) 3:47–50.
11. Sobhana PP, Kandlakunta B, Nagaraju R, Thappatla D, Epparapalli S, Vemula SR, et al. Human clinical trial to assess the effect of consumption of multigrain Indian bread on glycaemic regulation in type diabetic participants. *J Food Biochem.* (2020) 44:e13465. doi: 10.1111/jfbc.13465
12. Geetha K, Geetha MY, Hulamani S, Hiremath N. Glycaemic index of millet based food mix and its effect on pre diabetic subjects. *J Food Sci Technol.* (2020) 57:2732–8. doi: 10.1007/s13197-020-04309-5
13. Narayanan J, Sanjeevi V, Rohini U, Trueman P, Viswanathan V. Postprandial glycaemic response of foxtail millet dosa in comparison to a rice dosa in patients with type 2 diabetes. *Indian J Med Res.* (2016) 144:712–7. doi: 10.4103/ijmr.IJMR_551_15
14. Lin MA, Wu MC, Lu S, Lin J. Glycaemic index, glycaemic load and insulinemic index of Chinese starchy foods. *World J Gastroenterol.* (2010) 16:4973–9. doi: 10.3748/wjg.v16.i39.4973
15. Selvin E, Marinopoulos S, Berkenblit G, Rami T, Brancati FL, Powe NR, et al. Meta-Analysis: glycosylated hemoglobin and cardiovascular disease in diabetes mellitus. *Ann Intern Med.* (2004) 141:421–31. doi: 10.7326/0003-4819-141-6-200409210-00007
16. Atkinson LZ, Cipriani A. How to carry out a literature search for a systematic review: a practical guide. *BJPsych Adv.* (2018) 24:74–82. doi: 10.1192/bja.2017.3
17. Mohar D, Liberati A, Tetzlaff J, Altman DG. Preferred reporting items for systematic reviews and meta-analysis: the PRISMA statement. *Open Med.* (2009) 3:e1000097. doi: 10.1371/journal.pmed.1000097
18. Luchini C, Stubbs B, Solmi M, Veronese N. Assessing the quality of studies in meta-analyses: advantages and limitations of the Newcastle Ottawa Scale. *World J Meta-Anal.* (2017) 5:80–4. doi: 10.13105/wjma.v5.i4.80
19. Peterson J, Welch V, Losos M, Tugwell P. *The Newcastle-Ottawa Scale (NOS) for Assessing the Quality of Non-Randomised Studies in Meta-Analyses*. Ottawa: Ottawa Hospital Research Institute (2011).
20. Bell A, Fairbrother M, Jones K. Fixed and random effects models: making an informed choice. *Qual Quant.* (2019) 53:1051–74. doi: 10.1007/s11135-018-0802-x
21. Wolever TMS, Meynier A, Jenkins AL, Brand-Miller JC, Atkinson FS, Gendre D, et al. Glycaemic index and insulinemic index of foods: an interlaboratory study using the ISO 2010 method. *Nutrients.* (2019) 11:2218. doi: 10.3390/nu11092218
22. Harrer M, Cuijpers P, Furukawa TA, Ebert DD. *Doing META-analysis in R: A Hands-on Guide*. (2019). Available online at: https://bookdown.org/MathiasHarrer/Doing_Meta_Analysis_in_R/
23. Zlowodzki M, Poolman RW, Kerkhoffs GM, Tornetta P, Bhandari M. How to interpret a meta-analysis and judge its value as a guide for clinical practice? *Acta Orthopaedica.* (2007) 78:598–609. doi: 10.1080/17453670710014284
24. *Stata* Corp (2017). *Stata Statistical Software: Release 16.* College Station, TX: StataCorp LLC.
25. Isreal H, Richter RP. A guide to understanding meta-analysis. *J Orthop Sports Phys Ther.* (2011) 41:496–504. doi: 10.2519/jospt.2011.3333
26. Balduzzi S, Rücker G, Schwarzer G. How to perform a meta-analysis with R: a practical tutorial? *Evid Based Ment Health.* (2019) 22:153–60. doi: 10.1136/ebmental-2019-300117
27. Anitha S, Munthali W, Msere HW, Swai E, Muzanila Y, Sichone E, et al. Occurrence of aflatoxins and its management in diverse cropping systems of central Tanzania. *Mycotoxin Res.* (2017) 33:323–31. doi: 10.1007/s12550-017-0286-x
28. Hutcheson GD, Sofroniou N. *The Multivariate Social Scientist: Introductory Statistics Using Generalized Linear Models*. Sage Publications (1999).
29. Abadie A, Athey S, Imbens GW, Jeffrey W. *When Should You Adjust Standard Errors for Clustering?* NBER working paper series. (2017). Available online at: http://www.nber.org/system/files/working_papers/w24003/w24003.pdf
30. Seetha A, Tsusaka TW, Munthali TW, Musukwa M, Mwangwela A, Kalumikiza Z, et al. How immediate and significant is the outcome of training on diversified diets, hygiene and food safety? An effort to mitigate child undernutrition in rural Malawi. *Public Health Nutr.* (2018) 21:1156–66. doi: 10.1017/S1368980017003652
31. Shumoy H, Bockstaele FV, Devecioglu D, Raes, K. Effect of sourdough addition and storage time on in vitro starch digestibility and estimated glycaemic index of tef bread. *Food Chem.* (2018) 264:34–40. doi: 10.1016/j.foodchem.2018.05.019
32. Olagunju AL, Omoba OS, Enujiugha VN, Aluko RE. Development of value-Added nutritious crackers with high antidiabetic properties from blends of Acha (*Digitaria exilis*) and blanched pigeon pea (*Cajanus cajan*). *Food Sci Nutr.* (2018) 6:1791–1802. doi: 10.1002/fsn3.748
33. Alegbejo J, Ameh DA, Ogala W, Salisu I. Glycaemic index and load of acha (fonio) in healthy and diabetic subjects. *J Pure Applied Microbiol.* (2011) 5:117–22.
34. Patil KB, Bharati CV, Sunanda I. Glycaemic index and quality evaluation of little millet (*Panicum miliare*) flakes with enhanced shelf life. *J Food Sci Technol.* (2015) 52:6078–6082. doi: 10.1007/s13197-014-1663-5
35. Malavika M, Shobana S, Vijayalakshmi P, Ganeshjeevan R, Gayathri R, Kavitha V, et al. Assessment of quality of minor millets available in the south Indian market & glycaemic index of cooked unpolished little & foxtail millet. *Indian J Med Res.* (2020) 152:401–9. doi: 10.4103/ijmr.IJMR_2309_18
36. Dereje N, Bekele G, Nigatu Y, Worku Y, Holland RP. Glycaemic index and load of selected ethiopian foods: an experimental study. *J Diabetes Res.* (2019) 2019:1–5. doi: 10.1155/2019/8564879
37. Saragih B. Glycaemic index and functional properties of jelai (*Coix lacryma-*Jobi L). In: *International Conference on Tropical Agrifood, Feed and Fuel (ICTAFF).* Samarinda (2018).
38. Joshi S, Srivastava S. Barnyard millet as a substitute of rice in preparation of khichdi for diabetics. *Int J Sci Res.* (2016) 5:1798–802. doi: 10.21275/v5i6.NOV164628
39. Anju T, Sarita S. Suitability of Foxtail millet and barnyard millet for development of low GI biscuits 361 suitability of foxtail millet (*Setaria italica*) and barnyard millet (*Echinochloa frumentacea*) for development of low glycaemic index biscuits. *Malays J Nutr.* (2010) 16:361–8.
40. Bisht AT, Srivastava S. Efficacy of millets in the development of low glycaemic index sweets for diabetics. *Malays J Nutr.* (2013) 19:215–22.
41. Arora S, Srivastava S. Suitability of millet-based food products for diabetics. *J Food Sci Technol.* (2002) 39:423–6.
42. Ugare R, Chimmad B, Naik R, Bharati P, Itagi S. Glycaemic index and significance of barnyard millet (*Echinochloa frumentacae*) in type II diabetics. *J Food Sci Technol.* (2014) 51:392–5. doi: 10.1007/s13197-011-0516-8
43. Prasad MPR, Dayakar Rao B, Kalpana K, Vishuvardhana Rao M, Patil JV. Glycaemic index and glycaemic load of sorghum products. *J Sci Food Agri.* (2014) 95:1626–30. doi: 10.1002/jsfa.6861
44. Torangatti G, Naik R. Glycaemic and lipemic response of composite foods in normal and NIDDM subjects. *Int J Diabetic Dev Countries.* (1996) 19:161–71.
45. Urooj A, Rupashri K, Puttraraj S. Glycaemic responses to finger millet based indian preparations in non-insulin dependent diabetic and healthy subjects. *J Food Sci technol.* (2006) 43:620–5.
46. Mani UV, Prabhu BM, Damle SS, Mani I. Glycaemic index of some commonly consumed foods in western India. *Asia Pac J Clin Nutr.* (1993) 2:111–4.
47. Neelam Y, Kanchan C, Alka S, Alka G. Evaluation of hypoglycaemic properties of kodo millet based food products in healthy subjects. *J Pharm.* (2013) 3:14–20. doi: 10.9790/3013-32201420
48. Pathak P, Srivastava S, Grover S. Development of food products based on millets, legumes and fenugreek seeds and their suitability in the diabetic diet. *Int J Food Sci Nutr.* (2000) 51:409–14. doi: 10.1080/096374800427019
49. Gowri Manohari R, Poongodi Vijayakumar T. Characterization and glycaemic load of ready to cook (RTC) prebiotic flour mixture. *Int J Sci Nat.* (2013) 4:591–7.
50. Nagaraju R, Sobhana PP, Thappatla D, Epparapalli S, Kandlakunta B, Korrapati D. glycaemic index and sensory evaluation of whole grain based multigrain Indian breads (rotis). *Prevent Nutr Food Sci.* (2020) 25:194–202. doi: 10.3746/pnf.2020.25.2.194
51. Nidhi K, Alka G, Sarita S. Development of low glycaemic foods with the use of pearl millet and finger millet. *Int J Sci Res.* (2014) 3:193–5. doi: 10.15373/22778179/August2014/55
52. Lakshmi Kumari P, Sumathi S. Effect of consumption of finger millet on hyperglycemia in non-insulin dependent diabetes mellitus (NIDDM) subjects. *Plant Foods Human Nutr.* (2002) 57:205–13. doi: 10.1023/A:1021805028738
53. Kumari D, Chandrasekara A, Athukorale P, Shahidi F. Finger millet porridges subjected to different processing conditions showed low glycaemic index

and variable efficacy on plasma antioxidant capacity of healthy adults. *Food Product Proc Nutr.* (2020) 2:13. doi: 10.1186/s43014-020-00027-9
54. Anand D, Kapoor R. Attenuation of post prandial glycaemic response of refined flour chapatti with probiotic containing soya based yoghurt. *J Dairy Food Home Sci*. (2011) 30:252–6.
55. Jayasinghe MA, Ekanayake S, Nugegoda DB. Effect of different milling methods on glycaemic response of foods made with finger millet (*Eucenea coracana*) flour. *Ceylon Med J.* (2013) 58:148–52. doi: 10.4038/cmj.v58i4.6305
56. Thakkar R, Kapoor R. Enrichment of rice and finger millet based preparations with gum acacia and their effects on glycaemic response in non-insulin dependent diabetic subjects. *J Food Sci Technol.* (2007) 44:183–5.
57. Shobana S, Singh R, Kumari U, Malleshi NG, Ali SZ. Glycaemic response of rice, wheat, and finger millet based diabetic food formulations in normoglycaemic subjects. *Int J Food Sci Nutr.* (2007) 58:363–72. doi: 10.1080/09637480701252229
58. Shobana S, Selvi RP, Kavitha V, Gayathri N, Geetha G, Gayathri R, et al. Development and evaluation of nutritional, sensory and glycaemic properties of finger millet (*Eleusine coracana* L.) based food products. *Asia Pac J Clin Nutr.* (2018) 27:84–91. doi: 10.6133/apjcn.032017.18
59. Shubhashini V, Ushadevi C. Estimation of glycaemic index of pearl millet. *Int J Adv Res.* (2014) 2:922–9.
60. Nambiar VS, Patwardhan T. Assessment of glycaemic index (GI) and glycaemic load (GL) for selected traditional millet-based Indian recipes. *Int J Diabetes Dev Countries.* (2015) 35:S157–62. doi: 10.1007/s13410-014-0275-y
61. Wahlang B, Joshi N, Ravindra U. Glycaemic index lowering effect of different edible coatings in foxtail millet. *J Nutr Health Food Eng.* (2018) 8:404–8. doi: 10.15406/jnhfe.2018.08.00303
62. Ren X, Chen J, Molla MM, Wang C, Diaob X, Shen Q. *In vitro* starch digestibility and *in vivo* glycaemic response of foxtail millet and its products. *Food Funct.* (2015) 7:372–9. doi: 10.1039/C5FO01074H
63. Lestari LA, Huriyati E, Marsono Y. The development of low glycaemic index cookie bars from foxtail millet (*Setaria italica*), arrowroot (*Maranta arundinacea*) flour, and kidney beans (*Phaseolus vulgaris*). *J Food Sci Technol.* (2017) 54:1406–13. doi: 10.1007/s13197-017-2552-5
64. Geetha C, Eswaran P. Hypoglycaemic effect of millet incorporated breakfast items; on selected non-insulin dependent diabetic patients. *Ind J Nutr Dietet.* (1990) 27:316–20.
65. Djaja N, Permadi I, Witjaksono F, Soewondo P, Abdullah M, Agustina R, et al. The effect of Job's tears-enriched yoghurt on GLP-1, calprotectin, blood glucose levels and weight of patients with type 2 diabetes mellitus. *Med J Nutrition Metab.* (2019) 12:163–71. doi: 10.3233/MNM-180258
66. McSweeney MB, Ferenc A, Smolkova K, Lazier A, Tucker A, Seetharaman K, et al. Glycaemic response of proso millet-based (*Panicum miliaceum*) products, *Int J Food Sci Nutr.* (2017) 68:873–80. doi: 10.1080/09637486.2017.1301890
67. Surekha N, Devi R, Naik RS. Development of value added low glycaemic index barnyard millet (*Echinochloa frumentacea* Link) noodles. *Int J Food Nutr Sci.* (2013) 2:20–4.
68. Thilakavathy S, Muthuselvi S. Development and evaluation of millets incorporated chappathi on glycaemic response in type II diabetics. *Indian J Nutr Dietet.* (2010) 2:42–50.
69. Aliyu L, Tijjani SA, Ameh DA, Babagana A. Glycaemic index of traditional meal (dambu) from pearl millet and maize maimuna. *Sci J Anal Chem.* (2020) 8:29–32. doi: 10.11648/j.sjac.20200801.15
70. Alyami J, Whitehouse E, Yakubov GE, Pritchard SE, Hoad CL, Blackshaw E, et al. lycaemic gastrointestinal hormonal and appetitive responses to pearl millet or oats porridge breakfasts: a randomized crossover trial in healthy humans. *Br J Nutr.* (2019) 122:1142–54. doi: 10.1017/S0007114519001880
71. Poquette NM, Gu X, Lee SO. Grain sorghum muffin reduces glucose and insulin responses in men. *Food Funct.* (2014) 5:894–9. doi: 10.1039/C3FO60432B
72. Lakshmi KB, Vimala V. Hypoglycaemic effect of selected sorghum recipes. *Nutr Res.* (1996) 16:1651–8. doi: 10.1016/0271-5317(96)00184-4
73. Rao A, Prabhu D, Krishnamurthy V, Thimmaiah AK. Millets in metabolic syndrome- time to tap the potential. *J Evid Based Med Healthc.* (2019) 6:1284–7. doi: 10.18410/jebmh/2019/266
74. Vedamanickam R, Anandan P, Bupesh G, Vasanth S. Study of millet and non-millet diet on diabetics and associated metabolic syndrome. *Biomedicine.* (2020) 40:55–8.
75. Meti R. Role of multi-millet mix in reducing blood glucose levels in type II diabetic patients. *IJMER.* (2019) 4:27.
76. Unnikrishnan V, Chithra R, Sujitha S. Formulation of millets incorporated non-vegetarian foods and study of glycaemic index and glycaemic load. *Int J Pure App Biosci.* (2018) 6:291–95. doi: 10.18782/2320-7051.6786
77. Geetha K, Geetha Yankanchi M, Hiremath N. Effect of high fibre food mix on lipid profile and body weight in obese subjects. *Biomed Res.* (2019) 30:655–8. doi: 10.35841/biomedicalresearch.30-19-257
78. Anushia K, Uma Mageshwari J, Trueman P, Viswanathan V. The effect of millet supplementation on weight and lipid profile. *TAPI J.* (2018) 13:1–7.
79. Hymavathi TV, Rao SK, Boini P, Rajesh P, Rodrigues L, Sampathi S, et al. Profiling of *Eleusine coracana* biscuits for glycaemic index, stress and lipid. *MOJ Drug Design Dev Ther.* (2017) 1:52–8. doi: 10.15406/mojddt.2017.01.00010
80. Tiwari N, Srivastava S. Effect of finger millet (*Eleusine coracana*) buns supplementation on serum glucose and serum lipids level in type 2 diabetics. *Asian. J Dairy Food Res.* (2017) 36:337–40. doi: 10.18805/ajdfr.DR-933
81. Kavita MS, Prema L. Glycaemic response to selected cereal-based south indian meals in non-insulin-dependent diabetics. *J NutrEnviron Med.* (1997) 7:287–93. doi: 10.1080/13590849762402
82. Ren X, Yin R, Hou D, Xue Y, Zhang M, Diao X, et al. The glucose-lowering effect of foxtail millet in subjects with impaired glucose tolerance: a self-controlled clinical trial. *Nutrients.* (2018) 10:1509. doi: 10.3390/nu10101509
83. Phanindra P, Poshadri A, Ramesh P, Naik SS. Efficacy of therapeutic extruded snack food consumption on glycaemic response in type 2 diabetic patients. *Int J Sci Eng Res.* (2018) 9:1730–40.
84. Jali MV, Kamatar MY, Jali SM, Hiremath MB, Naik RK. Efficacy of value added foxtail millet therapeutic food in the management of diabetes and dyslipidamea in type 2 diabetic patients. *Recent Res Sci Technol.* (2012) 4:3–4.
85. Hou D, Chen J, Ren X, Wang C, Diao X, Hu X, et al. A whole foxtail millet diet reduces blood pressure in subjects with mild hypertension. *J Cereal Sci.* (2018) 84:13–9. doi: 10.1016/j.jcs.2018.09.003
86. Alyami J, Ladd N, Pritchard SE, Hoad CL, Sultan AA, Spiller RC, et al. Glycaemic gastrointestinal and appetite responses to breakfast porridge from ancient cereal grains: a MRI pilot study in healthy human. *Food Res Int.* (2017) 118:49–57. doi: 10.1016/j.foodres.2017.11.071
87. Abdelgadir M, Abbas M, Järvi A, Elbagir M, Eltom M, Berne C. Glycaemic and insulin responses of six traditional sudanese carbohydrate-rich meals in subjects with type 2 diabetes mellitus. *Diabetic Med.* (2004) 22:213–7. doi: 10.1111/j.1464-5491.2004.01385.x
88. Chhavi A, Sarita S. Evaluation of composite millet breads for sensory and nutritional qualities and glycaemic response. *Malays J Nutr.* (2012) 18:89–101.
89. Ruhembe CC, Nyaruhucha CNM, Mosha TCE. Glycaemic index of selected staple foods used in the management of type 2 diabetes mellitus in tanzania. *Int J Sci Basic Applied Res.* (2014) 13:48–62.
90. Longvah T, Ananthan R, Bhaskarachary K, Venkaiah K. *Indian Food Composition Table*. Hyderabad: National Institute of Nutrition (2017).
91. Wolever TMS, Vorster HH, Bjorck I, Brand-Miller J, Brighenti F, Mann JI, et al. Determination of the glycaemic index of foods: interlaboratory study. *Eur J Clin Nutr.* (2003) 57:475–82. doi: 10.1038/sj.ejcn.1601551
92. Hirsch IB, Wright EE. Using flash continuous glucose monitoring in primary practice. *Clin Diabetes J.* (2019) 37:150–61. doi: 10.2337/cd18-0054

Conflict of Interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

frontiers in Sustainable Food Systems

ORIGINAL RESEARCH
published: 06 August 2021
doi: 10.3389/fsufs.2021.694160

Drivers of Millet Consumption Among School Aged Children in Central Tanzania

*Monica Chande[1], Happiness Muhimbula[1], Ruth Mremi[1], Yasinta C. Muzanila[2], Nelson C. Kumwenda[3], John Msuya[1], Harry Msere[3], Mateete Bekunda[4], Patrick Okori[3] and Wanjiku N. Gichohi-Wainaina[3]**

[1] Department of Food Technology, Nutrition and Consumer Studies, Sokoine University of Agriculture, Morogoro, Tanzania, [2] Department of Biosciences, Solomon Mahlangu College of Science and Education, Sokoine University of Agriculture, Morogoro, Tanzania, [3] International Crops Research Institute for the semi-arid Tropics, Lilongwe, Malawi, [4] Africa Rising Program, International Institute for Tropical Agriculture, Arusha, Tanzania

Background: Iron and zinc deficiency are common public health problems in low-income countries largely due to poor consumption of iron and zinc rich foods. It has previously been observed that 57% of school aged children (SAC) in Tanzania suffer from anemia. In addition, estimates indicate that over 25% of the population have inadequate zinc intake. Pearl millet is an example of a nutrient dense, resilient cereal crop, that can be promoted to diversify diets and combat iron and zinc deficiency. This study overall aim was to increase pearl millet consumption among school aged (5 – 12 years) children. As part of the study, we investigated, the drivers of food choice relating to pearl millet consumption.

Methods: The study was a cross-sectional study of randomly selected households in Kongwa district, Dodoma region of Tanzania. In total, 128 women of reproductive age (20 – 49 years) were randomly selected for the study. A study questionnaire consisting of 66 items, was developed and validated. The constructs in the questionnaire were categorized in two groups: internal and external factors. Respondents were asked to indicate their level of agreement or disagreement with statements read to them by interviewers. The scores on intention and behavior constructs were based on the number of times caregivers intended to, or had fed their school going children with pearl millet in the referent month. Intention was considered high if it was higher than the median intention score of the group, and low if it was equal to or lower than the median scores. Correlations and multiple linear regressions were performed to measure association between constructs and to identify predictive constructs. The Mann-Whitney U test was used for score comparison.

Results: There was a significant difference between intention and behavior among those who did not consume pearl millet ($P = 0.003$), and those who consumed pearl millet two or more times a week, in the same month ($P = 0.01$). Knowledge was significantly correlated with behavior identity ($\rho = 0.58$, $P = 0.001$), while health behavior identity was significantly correlated with intention ($\rho = 0.31$, $P = 0.001$). Intention of caregivers was significantly and positively correlated ($\rho = 0.44$, $P = 0.001$) with and predicted consumption of pearl millet ($\rho = 0.87$, $P = 0.067$).

OPEN ACCESS

Edited by:
Taku Tsusaka, Ostrom, Thailand

Reviewed by:
Daniela Spina, University of Catania, Italy
Giulia Maesano, University of Catania, Italy

***Correspondence:**
Wanjiku N. Gichohi-Wainaina
wngichohi@gmail.com; w.gichohi@cgiar.org

Specialty section:
This article was submitted to Nutrition and Sustainable Diets, a section of the journal Frontiers in Sustainable Food Systems

Received: *12 April 2021*
Accepted: *28 June 2021*
Published: *06 August 2021*

Citation:
Chande M, Muhimbula H, Mremi R, Muzanila YC, Kumwenda NC, Msuya J, Msere H, Bekunda M, Okori P and Gichohi-Wainaina WN (2021) Drivers of Millet Consumption Among School Aged Children in Central Tanzania. Front. Sustain. Food Syst. 5:694160. doi: 10.3389/fsufs.2021.694160

Conclusion: Increasing knowledge or awareness on nutritional benefits of pearl millet among caregivers may increase consumption of pearl millet by children of school going age.

Keywords: pearl millet, theory of planned behavior, health belief model, drivers of food choice, school aged children and Tanzania

INTRODUCTION

Iron and zinc deficiency are common public health problems in low-income countries largely due to poor consumption of iron and zinc rich foods (Bouis, 2002; Brown et al., 2004; Nestel et al., 2006). Anemia is one of the many consequences of iron deficiency (Lopez et al., 2016). Among school aged children (SAC) specifically, a study conducted in 2001, indicated that 57% of SAC in Tanzania suffered from anemia (Hall et al., 2001). Anemic school-children have decreased motor activity, social inattention, and decreased school performance (Grantham-McGregor and Ani, 2001). In Tanzania, estimates indicate that over 25% of the population have inadequate zinc intake (Wessells et al., 2012). In terms of consequences of zinc deficiency, observational studies among school-age children, have shown hair zinc to be associated with reading ability, suggesting that zinc deficiency interfered with academic performance (Butrimovitz and Purdy, 1978; Cavan et al., 1993). Addressing these deficiencies is therefore crucial not only for the aforementioned consequences on growth and health but also for economic development (Zimmermann and Hurrell, 2007). Food based strategies to combat micronutrient deficiencies present a sustainable and accessible solution (FAO, 2011). Pearl millet (PM) is an example of a nutrient dense, resilient cereal crop, that can be promoted to diversify diets and combat iron and zinc deficiency (Rao et al., 2006; Kanatti et al., 2014). It is grown mostly in marginal environments in the arid and semi-arid tropical regions of Asia and Africa (Jukanti et al., 2016). In terms of dietary contribution, it is a major contributor of dietary protein, iron, and zinc intake in a variety of rural populations in sub-Saharan Africa (Agte et al., 1999; Kodkany et al., 2013).

This study was embedded within a wider program that aimed to link agriculture production to nutrition to address malnutrition in the semi-arid agro ecologies of Kongwa district, Dodoma Region, Tanzania, Although pearl millet is already consumed in Dodoma region of Tanzania (Rohrbach and Kiriwaggulu, 2007), we aimed to increase its consumption to a wider group beyond pre-school children. The results presented herein elucidate via a combination of two psychosocial theories-the Theory of Planned Behavior (TPB) and Health Belief Model (HBM); the factors that drive pearl millet consumption among their school going children (5 – 12 years).

METHODS

Ethical Approvals and Consent

This study did not seek for ethical approval from an ethical review board because it did not involve blood collection, any invasive procedure or anthropometry. Approvals from district administrative officials as well as traditional authorities were obtained as part of the study preparation. Prior to questionnaire administration, all respondents had the study explained to them in the local language Kiswahili, assured of confidentiality and offered the opportunity to ask questions. Respondents were also informed that they were free to decline participation at any point during the questionnaire administration. Study participants then indicated their approval by giving written informed consent or thumb print.

Study Site

This cross-sectional study of randomly selected households was conducted in Kongwa district, Dodoma region of Tanzania. Kongwa district is one of the action districts of the Africa Research in Sustainable Intensification for the Next Generation (Africa RISING) program. The program aims to create opportunities for smallholder farm households to move out of hunger and poverty through sustainably intensified farming systems that improve food, nutrition, and income security, particularly for women and children (Africa RISING, 2021). In Kongwa the study was conducted in, Laikala, the driest village in the district and largest producer of pearl millet. Laikala village receives about 357 mm of rainfall annually, with annual ambient temperatures ranging between 18 and 34°C. The main economic activity in the area is integrated crop and livestock farming (NBS, 2013).

Sampling and Study Participants

In total, 128 caregivers who were women of reproductive age (20 – 49 years) were randomly selected for the study. According to Francis et al. (2004) it is reasonable to assume at least a moderate effect size with a sample size of 80 – 160 individuals for Theory of Planned Behavior studies using a multiple regression. Households with school aged children (5 – 12 years) were intentionally selected using the random walk sampling method (Wei et al., 2004), and all caregivers from selected households were listed. From this list, one caregiver was randomly selected in each household for questionnaire administration. The main selection criteria were willingness to participate, presence of a school aged child (SAC) within the household and previous or current consumption of pearl millet. When a selected woman did not fulfill the selection criteria, she was replaced with another woman in the same household that met the inclusion/exclusion criteria. All selected women met the selection criteria.

Questionnaire Development and Validation

The study questionnaire consisted of 66 items, identified from literature. The items were characterized into 12 constructs based on the combined model of TPB and HBM as was undertaken in several studies (Sun et al., 2006; Fanou-Fogny et al., 2011; Macharia-Mutie et al., 2011; Abizari et al., 2013; Talsma et al., 2013) (**Table 1**). The following constructs were used: knowledge of iron and zinc deficiency and pearl millet, perceived susceptibility to iron and zinc deficiency, perceived severity of iron and zinc deficiency, health value attached to anemia or zinc deficiency in the SAC, health behavior identity attached to giving pearl millet to address iron deficiency, anemia or zinc deficiency, perceived barriers to give pearl millet, attitudes toward pearl millet consumption, subjective norms, external control beliefs and cues to action influencing (lack of) consumption of pearl millet by school aged children, intention to give pearl millet to the SAC, and behavior or pearl millet consumption among school aged children. In detail the constructs assessed the following (**Table 1**):

1. Knowledge assessed the caregivers understanding on the relationship between pearl millet and health, and specifically its' relationship to iron deficiency or anemia and zinc deficiency
2. Perceived susceptibility assessed caregiver's feeling about her school aged child being exposed to iron deficiency or anemia and zinc deficiency
3. Perceived severity assessed caregiver's feeling that her school aged child being exposed to iron deficiency or anemia and zinc deficiency is serious
4. Health value assessed the importance caregiver places on the outcome of her school aged child being anemic or zinc deficient
5. Health behavior identity assessed caregiver's evaluation of the effectiveness of giving pearl millet or iron rich food in reducing the perceived threat-that is iron deficiency anemia and zinc deficiency
6. Perceived barriers assessed caregiver's evaluation of various complications that hinder her in giving pearl millet to the school aged child
7. Attitude toward behavior assessed caregiver's evaluation of sensory and nutrient content attributes of pearl millet and how these influenced giving this to the SAC
8. Cues to action assessed surrounding situation that cause a caregiver to change her health behavior in feeding pearl millet to her school aged child
9. Control belief assessed a caregiver's perceived ability to make a decision on feeding pearl millet to their school aged child
10. Subjective norms assessed a caregiver's perceived social influencers to give, or not to give, pearl millet to their school aged child. In detail, this assessed who is important for the giving pearl millet to the SAC and the value of that persons opinion
11. Behavioral intention indicated how much effort a caregiver is planning to make, in order to give pearl millet to school aged child
12. Behavior assessed the giving pearl millet to SAC by caregivers

Examples of item statements are provided in **Table 5**.

To assess nutrition related behavior of individuals, constructs were further categorized in two groups that is, internal and external factors. The internal factors were further grouped into "background and perception," "beliefs and attitudes" and "intention." "Background and perception" consisted constructs such as knowledge, perceived susceptibility, perceived severity and health value while "beliefs and attitudes" contained health behavior identity, attitude toward behavior and perceived barriers. External factors were subjective norms, cues to action and control belief.

Respondents were asked to indicate their level of agreement or disagreement with statements read to them by interviewers. Prior to administration, the questionnaire was verified in focus group discussions and pre-tested among caregivers in a site similar to Laikala village. Changes to the questionnaire were them made after pretesting and validation. The questionnaire was subsequently translated into the local language (Swahili) and correctness checked with back translation into English.

Scale Measurements and Analysis

Knowledge, perceived susceptibility, perceived severity, health value, health behavior identity, perceived barriers, cues to action and control belief constructs were rated using a five-point likert scale that ranged from strongly disagree, disagree, neutral, agree and strongly agree. The reason that odd numbered scales were chosen was so that a central neutral response and an equal number of positive and negative responses above and below the neutral middle response were provided (Emerson, 2017). The score for each construct was computed as the sum of individual item scores. The scores for the constructs *"Attitudes toward behavior"* and *"Subjective norms"* were sums of products of paired items; *attitudes* × *evaluation of attitudes*, and *normative beliefs* × *motivation to comply*, respectively. To show negative, neutral or positive influences, item scores of *attitudes* and *normative beliefs* ranged from −2 to 2 and the scores of the *evaluation of attitudes* and *motivation to comply* ranged from +1 to +5. This resulted in a paired-item score range of −10 to 10. For intention and behavior, the rating scale ranged from not consumed, four or less times per month and two or more times per month. The scores on intention and behavior constructs were based on the number of times caregivers intended to, or had fed their school going pearl millet in the refereed month, respectively. Intention was considered high if it was higher than the median intention score of the group, and low if it was equal to or lower than the median scores as it was in previous studies (Sun et al., 2006; Fanou-Fogny et al., 2011; Macharia-Mutie et al., 2011; Abizari et al., 2013; Talsma et al., 2013).

Statistical Analyses

Descriptive statistics were performed to describe the caregivers and children. Multiple sentence constructs were tested for reliability of the questionnaire and internal consistency using Cronbach alpha and sentence-total correlation. The items within a construct were regarded as consistent when Cronbach alpha was ∼0.75 or higher and the corrected sentence-total correlation of all sentences in a construct were higher than 0.30 (Field, 2005). The Mann-Whitney U test was used to examine differences in construct items between high and low intenders as the variables were on the ordinal scale (Karadimitriou et al., 2018). Spearman's rank correlation was used to test for bivariate

TABLE 1 | Functioning explanation of constructs used to examine internal and external factors predicting intention of caregivers to give pearl millet to school aged children.

Construct	Operational definition
Knowledge	The caregiver's understanding on the relationship between pearl millet and health, and specifically its relationship to iron deficiency anemia and zinc deficiency
Perceived susceptibility	Caregiver's feeling about her school aged child being exposed to iron deficiency anemia and zinc deficiency
Perceived severity	Caregiver's feeling that her school aged child being exposed to iron deficiency anemia and zinc deficiency is serious
Health value	The importance caregiver places on the outcome of her school aged child being anemic or zinc deficient
Health behavior identity	Caregiver's evaluation of the effectiveness of health behavior in reducing the perceived threat-that is iron deficiency anemia and zinc deficiency
Perceived barriers	Caregiver's evaluation of various complications that hinder her in giving pearl millet to school aged child
Attitude toward behavior	Caregiver's evaluation of giving pearl millet to school aged child
Cues to action	Surrounding situation that cause a caregiver to change her health behavior in feeding pearl millet to her school aged child.
Control belief	Shows a caregiver's perceived ability to make decision on feeding pearl millet to their school aged child
Subjective norms	Reveals a caregiver's perceived social influencers to give, or not to give, pearl millet to their school aged child (who is important for the behavior and is the opinion of that person important?)
Behavioral intention	Sign of how much effort a caregiver is planning to make, in order to give pearl millet to school aged child
Behavior	Giving of pearl millet to school going children by caregivers

associations within the combined model of TPB and HBM as the variables were on the ordinal scale (Schober et al., 2018). Three multiple linear regression models were used to examine the contribution of constructs to health behavior identity, intention and behavior. The first model was designed to identify constructs within background and perception that were associated with health behavior identity (Model 1). To identify constructs associated with intention, the second model combined internal factors (Perceived barrier, Health behavior identity, and Attitudes toward behavior) and external factors (Subjective norms, Control beliefs, and Cues to action) as predictor variables (Model 2). Finally, to identify constructs associated with pearl millet consumption among SAC, we included constructs that were significantly associated with intention (Health behavior identity, Attitudes toward behavior, Subjective norms, Cues to action) as well as intention. We included perceived barriers despite the fact that it was not a construct significantly associated with intention because of the importance of considering the role barriers to consumption may play. An interaction term between perceived barriers and intention was also included in this model to investigate how perceived barriers modulated the association between intention and behavior. All models were corrected for age of the child, education and interviewer effect.

The three models were therefore constructed as follows:

Model 1: Health behavior identity = f (Knowledge, Perceived susceptibility, Perceived severity, Health value, Age of child, Education and Interview effect).

Model 2: Intention = f (Perceived barrier, Health behavior identity, Attitudes toward behavior, Subjective norms, Control beliefs, Cues to action, Age of child, Education and Interview effect).

Model 3: Behavior = f (Health behavior identity, Attitudes toward behavior, Subjective norms, Cues to action, Perceived barrier, Intention, Perceived barriers * behavioral intention, Age of child, Education and Interview effect).

Overall, statistical tests were 2-tailed, and p-values < 0.10 were considered statistically significant. We used this P-value cut off due to the finite sample size of our study and because this study was prone to random errors (Thiese et al., 2016). All analyses were performed using IBM SPSS Statistics for Windows (Version 20.0. IBM Corp, 2011, Armonk, NY).

RESULTS

Characteristics of the Study Participants

A total of 128 caregivers with a similar number of children participated in the study (**Table 2**). Majority of the school aged children included in the study were female (59.4%). The age of the children ranged from 6 to 12 years, with more than 60% in the age groups 7–8 years and 9–10 years. The majority of the respondents were married (82.0%). Only 40.7% of the caregivers had attained primary or secondary school education. The respondents were majorly from the Kaguru ethnic group (96.9%), the predominant tribe in Laikala. Agriculture and farming in particular were the predominant income generating occupation for most of the caregivers (89.1%). We then conducted preliminary analyses that investigated the associations between intention and behavior related to pearl millet consumption among the school aged children.

There Was a Significant Difference Between Intention and Behavior Among Those Who Did Not Consume Pearl Millet and Those Who Consumed Pearl Millet Two or More Times a Week in the Referent Month

There was significant difference between intention and behavior when consumption was two or more times per week (53.1 vs. 32.8; $P = 0.001$) or where there was no intention to or consumption taking place (30.5 vs. 13.3%; $P = 0.003$). No significant difference was observed between intention and

TABLE 2 | Socio-demographic characteristics of school aged children and their caregivers in Kongwa ($n = 128$).

Variable	*n* (Percentage)
Schoolchild characteristics	
Sex of children	
Male	52 (40.6)
Female	76 (59.4)
Age of children	
5–6	22 (17.2)
7–8	54 (42.2)
9–10	33 (25.8)
11–12	19 (14.8)
Caregiver characteristics	
Relationship with a child	
Mother	121 (94.5)
Guardian	7 (5.5)
Education	
None	76 (59.3)
Primary school*	50 (39.1)
Secondary school*	2 (1.6)
Ethnic group	
Kaguru	124 (96.8)
Other tribes (Gogo, Hehe, Nyamwezi)	4 (3.2)
Marital status	
Married	105 (82.0)
Divorced	16 (12.5)
Single	7 (5.5)
Caregivers' occupation	
Agriculture	114 (89.0)
Civil servant	7 (5.5)
Artisans	7 (5.5)
Household size	
3–6	74 (57.8)
7–10	49 (38.3)
≥11	5 (3.9)

**Some of the caregivers did not complete the level of schooling indicated.*

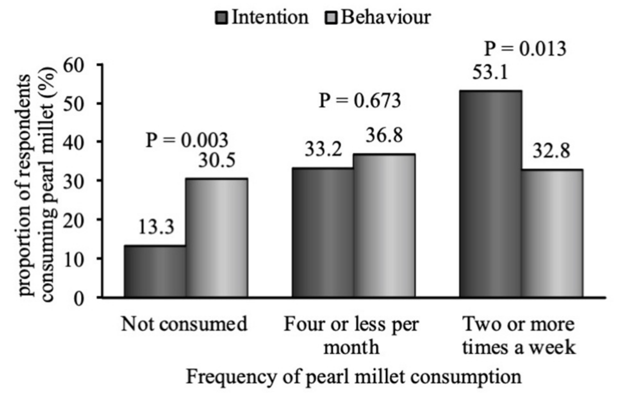

FIGURE 1 | Comparison of pearl millet consumption between behavioral intention and behavior ($n = 128$). *P*-value obtained using chi-square test comparisons of per cent of intention and behavior. Significant difference between intention and behavior $P \leq 0.10$.

behavior among respondents who were classified as consuming or intending to consume pearl millet four or less times per month (**Figure 1**). We further investigated knowledge, attitudes and perceptions of caregivers related to feeding pearl millet to SAC to elucidate internal factors influencing pearl millet consumption.

Caregivers Have Low Knowledge on the Nutritional Content and Benefits of Pearl Millet Consumption

In assessing knowledge of the nutrient composition of pearl millet, 22.7 and 29.0% of caregivers were aware that pearl millet contained zinc and iron, respectively. In terms of their importance of zinc and iron in supporting growth, 25.8 and 34.3% of caregivers, respectively, were aware of this role (**Table 3**). Majority of respondents however perceived pearl millet as tasty (92.3%) and nutritious (82.9%) with significantly higher positive perception among high intenders compared to low intenders for taste and nutritive value ($P = 0.044$ and $P = 0.01$, respectively; **Tables 3**, **4**). Interestingly, respondents attributed positive health outcomes to consumption of pearl millet despite not being aware of its micronutrient composition. In detail, majority agreed that pearl millet can improve intelligence (73.2%), health (82.9%) and survival (80.6%) of their children. When comparisons for high and low intenders were made, high intenders had significantly higher scores and therefore agreement on these benefits compared to low intenders (intelligence; $P = 0.041$, health; $P = 0.005$ and survival; $P = 0.015$).

Majority of Caregivers View Their Child's Health as Important but a Lower Proportion Connect Specific Health Outcomes of Their SAC to Consumption of Pearl Millet

Over 90% caregivers reported the health, growth, intelligence, school performance, strength and survival of their SAC as important to them, that is they placed a high health value on these attributes (**Table 3**). Interestingly, when we assessed the caregiver's evaluation of the effectiveness of giving pearl millet or iron rich food to the various health value attributes, the proportion of caregivers that agreed with the statements reduced. In fact when we particularly considered the relationship between consuming iron rich foods and cognition, the proportion of caregivers that agreed with this association was 50.2%. There was a higher appreciation for pearl millet consumption ($P = 0.0001$) and the role of iron rich foods in cognition (0.076), intelligence ($P = 0.041$), health ($P = 0.005$), and survival ($P = 0.015$) among SAC when high and low intenders were compared.

Various barriers limiting pearl millet consumption were identified though investigating attitudes toward pearl millet consumption by SAC as well as other perceived barriers by respondents.

TABLE 3 | Internal and external factors influencing pearl millet consumption.

Internal and external factors	Proportion of respondents that agree with statement
Internal factors influencing pearl millet consumption	
Knowledge	
Pearl millet contains zinc	22.7
Pearl millet contains iron	29.0
Zinc is important for the growth	25.8
Iron is important for the growth	34.3
Perceived severity of anemia	
Shortage of blood leads to shortness of breath	50.4
Shortage of blood makes a child weak and tied all the time	59.3
Health value caregiver places on child's health	
The health of my school child is very important to me	95.5
The growth of my school child is important to me	95.5
The intelligence of my school child is important to me	90.6
The performance of my school child is important to me	90.6
It is important that my school child is strong	94.6
The survival of my school child is important to me	93.7
Health behavior identity related to health benefits of pearl millet consumption	
Pearl millet is best thing for my school child	83.0
Pearl millet is the best thing for my family members	89.1
Food that contains iron is one of the best things I can give to my school child to improve his/her cognitive development	50.2
Feeding my school child pearl millet is one of the best things I can do to improve her/his intelligence	73.2
Feeding my school child pearl millet is one of the best things I can do to the improve her/his health	82.9
Feeding my school child pearl millet is one of the best things I can do for her/his survival	80.6
Perceived barriers limiting pearl millet consumption	
Pearl millet being contaminated with stones	59.5
Pearl millet is expensive during a rain season	71.9
Availability of fuel required to cook pearl millet	58.6
Quantity of fuel required to cook pearl millet	62.6
Pearl millet flour has a short shelf life	65.0
Attitude toward pearl millet consumption	
Pearl millet has a good taste.	93.5
My school child prefers foods that taste good	92.3
Pearl millet is a nutritious grain.	82.9
It is important for me to feed my school child with foods that are nutritious	89.7
External factors influencing pearl millet consumption	
Control beliefs	
I am the one who decides my school child should consumes pearl millet.	85.1
Cues promoting pearl millet consumption	
I comply with doctors, clinicians or health worker advice to feed pearl millet-based foods to my child of school going age	56.4
Subjective norms influencing pearl millet consumption	
My mother-in-law advices me to feed pearl millet to my child	71.1
My mother advises me to feed pearl millet to my child	51.7
My child's teacher advices me to feed my child pearl millet	79.0
My nurse advices me to feed pearl millet to my child	51.7

(Continued)

TABLE 3 | Continued

Attitudes Toward Consumption of Pearl Millet Are Positive Though Price, Seasonality, Availability, and Quantity of Fuel Required for Pearl Millet Preparation Limit Consumption

Over 90% of respondents indicated that pearl millet had good taste (93.5%), is nutritious (82.9%) and that they valued feeding their children with nutritious foods (89.7%). High intenders had a more positive attitude toward the taste of pearl millet grain ($P = 0.006$) and it's nutrient value ($P = 0.044$) compared to low intenders. Respondents indicated several barriers to pearl millet consumption among school aged children. Majority of respondents (71.9%) indicated price increases during rainy seasons as a barrier to consumption. When analyses were stratified according to high and low intention groups, high intention groups worried more about the high price of pearl millet in general ($P = 0.021$) especially during the rainy season ($P = 0.043$; **Table 4**). Other barriers identified by majority of the respondents include: contamination with stones (59.5% quantity of fuel required for preparation (62.6%), short shelf life of pearl millet flour (65.0%; **Table 3**).

The Role of Mothers, Teachers, and Nurses Is Crucial in Promoting Increased Pearl Millet Consumption Among School Aged Children

Caregivers indicated that mothers-in-law (71.1%), mothers (51.6%), teachers (78.9%) and nurses (51.6%) were influential in their decision on whether to give pearl millet to their school aged children (**Table 3**). In addition, community trainings were viewed as important in positively influencing consumption of pearl millet in the high intention group compared to the low intention group ($P = 0.014$; **Tables 3**, **4**).

To elucidate associations of individual constructs with intention, we assessed various constructs reliability as well as their correlations with each other and eventually with intention.

There Was High Reliability of the Multiple Constructs and Correlations Between Various Constructs Were Observed

Cronbach-α scores ranged from 0.7 to 0.8, which demonstrating a medium reliability for most of the constructs, median scores of the constructs ranged from 3 to 30 (**Table 5**). Median scores of

TABLE 4 | Comparison between high and low intenders of pearl millet consumption.

Constructs/statements	All (%)	Mean scores		*P*-value
		Low intention[a]	High intention[b]	
Knowledge				
Pearl millet contain iron	22.7	3.1	3.2	0.061
Iron is important for the health of my schoolchild	33.6	3.1	3.3	0.100
Perceived susceptibility				
My schoolchild is disinterested with the environment easily	22.7	2.6	2.1	0.037
Health behavior identity				
Giving pearl millet is one of the best thing I can do for my schoolchild	83.0	3.6	4.1	0.001
Food that contain zinc is one of the best thing I can give to my schoolchild to improve his/her cognitive development	50.2	3.4	3.6	0.076
Giving pearl millet is one of the best things I can do for my schoolchild to improve her/his intelligence	73.2	3.1	3.6	0.041
Giving pearl millet is one of the best thing I can do for my schoolchild to the improve her/his health	82.9	3.3	4.0	0.005
Giving pearl millet is one of the best thing I can do for my schoolchild for her/his survival	80.6	3.3	3.8	0.015
Barriers				
I worry about the price of pearl millet on the market	48.5	2.5	3.0	0.021
Pearl millet is expensive in the rainy season compared to dry season.	71.8	3.0	3.7	0.043
Attitude toward behavior				
My child prefers foods that taste good	92.3	3.0	3.6	0.006
Pearl millet is a nutritious grain.	82.9	2.1	2.4	0.044
Cues to action				
Special guest(s) at home make my school child want to eat pearl millet	36.7	2.1	2.6	0.051
The media makes me want to use pearl millet	21.1	2.1	2.4	0.080
Trainings in the community makes me want to feed pearl millet to my school child	26.7	2.0	2.5	0.014
Subjective norms				
My husband gives me the advice to feed pearl millet to my child.	46.2	1.0	1.1	0.007
My mother advises me to feed pearl millet to my child	51.6	1.1	1.1	0.059
My mother-in-law advices me to feed pearl millet to my child	39.9	1.1	1.1	0.019
My Childs' teacher(s) gives me the advice to feed my child with pearl millet	28.9	1.1	1.1	0.022
The advice of my child's teacher(s) is important to me	78.9	3.3	3.8	0.068
Doctors give me the advice to feed my child with pearl millet	47.0	1.1	1.1	0.009

aLow intention (n = 53) = intention to consume pearl millet less than once a week.
[b]High intention (n = 75) = intention to consume pearl millet once a week or more.
P-value obtained using Mann Whitney test comparisons of mean scores of high and low intenders.
Significant difference between intenders P ≤ 0.10.

health value, attitude toward behavior, control belief, subjective norms and intention constructs were high compared to the range values. This showed that caregivers tended to agree with the statements in those constructs. Low medians values were found for knowledge, susceptibility, severity, health behavior identity, barriers, cues to action and behavior construct when compared to their range scores. This indicated that most caregivers tended to disagree with the statements. Control belief, behavioral intention, and behavior constructs consisted of only one item each, and therefore their reliability analyses were not carried out.

In investigating associations between constructs, knowledge was significantly and positively associated with health behavior identity ($\rho = 0.58$, $P = 0.001$). Within Beliefs and attitudes constructs, health behavior identity was significantly and positively correlated with attitude toward behavior ($\rho = 0.46$, $P = 0.001$) and behavioral intention ($\rho = 0.31$, $P = 0.001$). Finally, health behavior identity was significantly and positively correlated with intention ($\rho = 0.31$, $P = 0.001$, respectively). In investigating external factors associated with intention, subjective norms ($\rho = 0.27$, $P = 0.002$) and cues to action ($\rho = 0.30$, $P = 0.001$) were significantly and positively correlated with intention. A statistically significant positive correlation between intention and consumption of pearl millet among school aged children was observed ($\rho = 0.44$, $P = 0.001$, **Figure 2** and **Supplementary Table 1**).

To identify a combination of factors that are associated with intention and behavior, regression analyses were utilized.

Intention Is a Significant Predictor of Pearl Millet Consumption Among School Aged Children

Model 1 had predictors as knowledge, perceived susceptibility, perceived severity, health value and the outcome as health

TABLE 5 | Internal consistency and median scores of the constructs.

Construct	Example of item statement	Cronbach α	Items	Median (IQR)	Range values[a]
Knowledge[b]	Pearl millet contains iron	0.8	7	21 (21, 25)	7–35
Perceived susceptibility[b]	My child of school going age suffers easily from shortage of blood	0.7	5	11.5 (10, 15)	5–25
Perceived severity[b]	Iron deficiency leads to shortage of blood	0.7	3	10 (9, 12)	3–15
Health value[b]	The intelligence of my child of school is important to me	0.8	6	26 (24, 28)	6–30
Health behavior identity[b]	Feeding pearl millet is one of the best things I can do for my child of school	0.8	7	26.5 (25, 28)	7–35
Perceived barriers[b]	I worry about the price of pearl millet on the market	0.8	9	30 (25, 34)	9–45
Attitude toward behavior[c]	(Pearl millet is a nutritious grain) * (It is important for me to feed my child of school with foods that are nutritious)	0.7	4	10 (10, 12)	−40 to 40
Cues to action[b]	Illness of my child of school makes me want to use pearl millet.	0.8	11	25 (22, 31)	11–55
Control belief[b]	I am the one who decides my child of school should consume pearl millet	–	1	4 (4, 5)	1–5
Subjective norms[d]	(My child's teacher(s) gives me the advice to feed my child with pearl millet) * (The advice of my child's teacher(s) is important to me)	0.8	11	6 (1, 10)	−110 to 110
Behavioral intention[e]	How often do you think you will feed pearl millet to your child in the coming month?	–	1	5 (3, 5)	1–5
Behavior[e]	How often did you feed pearl millet to your child in the last month?	–	1	3 (1, 5)	1–5

[a] *Range refers to the minimum and maximum possible scores from complete set of questions within a construct before consistency evaluation, except c and d, whose scores were from paired questions.*
[b] *Scores ranged from 1 = strongly disagree to 5 = strongly agree.*
[c] *(behavioral beliefs) items ranged from 1 = strongly disagree to 5 = strongly agree * (outcome evaluation) items which ranged from −2 = strongly disagree to 2 = strongly agree.*
[d] *Scores ranged from 1 = very unlikely to 5 = very likely * Mc (motivation to comply) items which ranged from −2 = strongly disagree to 2 = strongly agree.*
[e] *Items ranged from 1 = none to 5 = more than 2 times a week.*

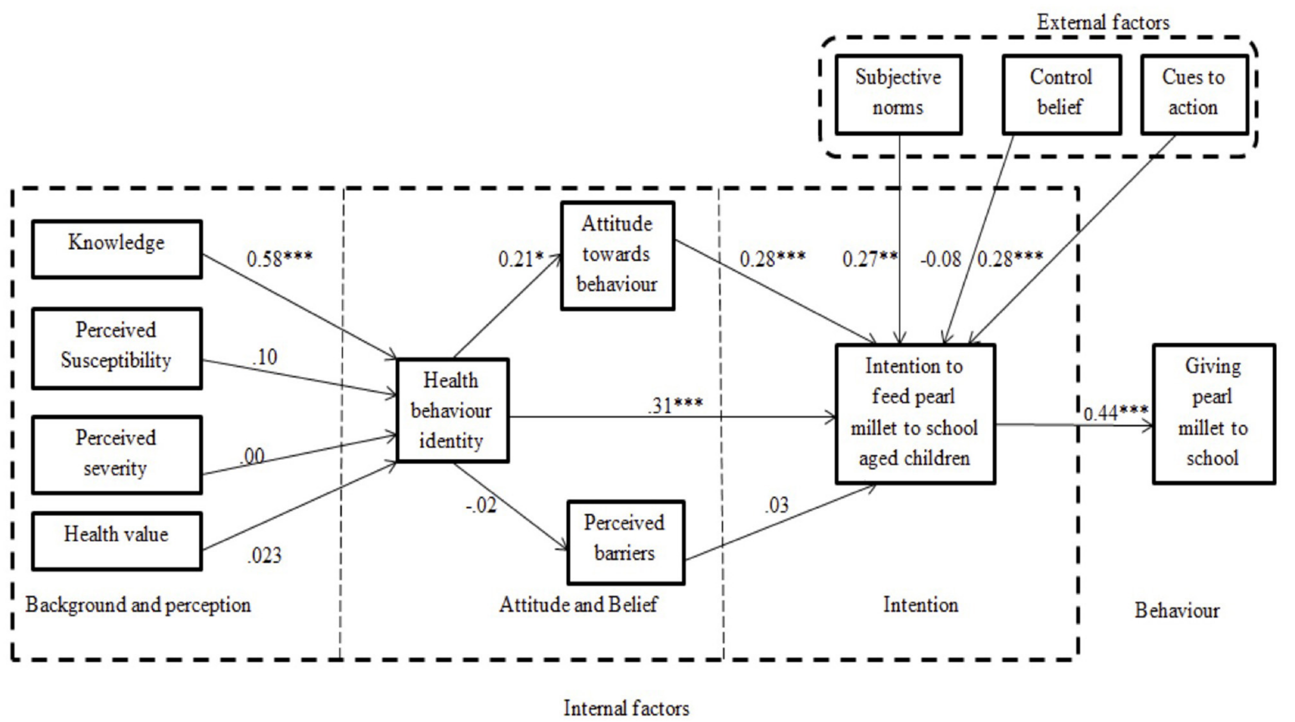

FIGURE 2 | A combined model of the theory of planned behavior and health belief model with Spearman correlation coefficients between related constructs $^{**}P \leq 0.01$ and $^{***}P \leq 0.001$ (2-tailed).

behavior identity. The control variables included were age of child, interviewer effect and caregivers' education. Knowledge was a significant predictor of health behavior identity ($\beta = 0.53$, $P = 0.001$) with an increase in knowledge resulting in a more positive evaluation of the effectiveness of giving pearl millet or iron rich foods to reduce iron deficiency or anemia and zinc deficiency. Perceived susceptibility ($P = 0.271$), perceived severity ($P = 0.342$), health value ($P = 0.291$), age of child

TABLE 6 | Constructs associated with health behavior identity, intention to feed pearl millet to children of school going age and feeding pearl millet to school aged children.

Model description	Predictors	Unstandardized β	*P*	R^2	Adjusted R^2
Model 1: Y = Identity				0.34	0.30
	Knowledge	0.60	0.001*		
	Perceived susceptibility	0.08	0.271		
	Perceived severity	−0.13	0.342		
	Health value	0.09	0.291		
	Age of a child	0.14	0.624		
	Interviewer effect	−0.13	0.693		
	Caregiver's education	0.97	0.532		
Model 2: Y = Intention				0.27	0.21
	Perceived barriers	−0.01	0.664		
	Health behavior identity	0.09	0.021*		
	Attitude toward behavior	0.17	0.010*		
	Subjective norms	0.03	0.243		
	Control belief	−0.30	0.041*		
	Cues to action	0.03	0.133		
	Age of a child	0.39	0.005*		
	Interviewer effect	−0.03	0.869		
	Caregiver's Education	0.48	0.048*		
Model 3: Y = Behavior				0.28	0.22
	Health behavior identity	0.08	0.051*		
	Attitude toward behavior	−0.01	0.878		
	Control belief	−0.13	0.407		
	Perceived barriers	0.03	0.654		
	Intention	0.87	0.067*		
	Barriers*intention	−0.01	0.409		
	Age of a child	0.09	0.545		
	Interviewer effect	0.05	0.769		
	Caregiver's education	0.74	0.008*		

All models were adjusted for interviewer effect, education of caregivers and age of a child.
**Significant predictor in the model ($p < 0.10$).*

($P = 0.624$), interviewer effect ($P = 0.693$) and caregivers' education ($P = 0.532$) were not significant predictors. This model explained 34% of the variance in health behavior identity.

Model 2 had both internal factors (perceived barriers, health behavior identity and attitudes toward behavior) and external factors (subjective norms, control beliefs and cues to action) as predictors of intention. Age of child, interviewer effect and caregivers' education were included as control factors. We observed that intention to give pearl millet to the SAC increased as the caregiver's evaluation of the effectiveness of giving pearl millet or iron rich food to reduce iron deficiency anemia and zinc deficiencies increased (Health behavior identity, $\beta = 0.09$, $P = 0.021$). Similarly, caregivers' attitude toward pearl millet consumption was also positive (Attitude toward behavior $\beta = 0.17$, $P = 0.010$) their intention to give pearl millet to the SAC increased. When considering the control variables, as the age of the SAC child ($\beta = 0.39$, $P = 0.005$) and caregivers' education ($\beta = 0.48$, $P = 0.048$) increased, so did the intention to give pearl millet. Interestingly, as the mother asserted more control on what the school aged child was fed (Control belief, $\beta = -0.30$, $P = 0.041$), the effort she was intending to make toward giving her child pearl millet (intention) decreased, indicating she was less likely to feed pearl millet to the SAC. This model explained 27% of the variation in intention to give pearl millet to the SAC.

Model 3 investigated predictors of pearl millet consumption among SAC. We observed that as the caregiver's evaluation of the effectiveness of giving pearl millet or iron rich food to reduce iron deficiency or anemia and zinc deficiencies became more positive (Health behavior identity, $\beta = 0.08$, $P = 0.051$) or the effort that the caregiver planned to make in. order to give pearl millet to their SAC increased (Intention, $\beta = 0.87$, $P = 0.067$) or the caregivers' education increased (Caregivers' education $\beta = 0.74$, $P = 0.008$), the SAC was more likely to be fed pearl millet. Attitude toward behavior, Control belief, Perceived barriers, age of child and interviewer effect were not significantly associated with giving pearl millet to the SAC ($P > 0.10$). The interaction term investigating how perceived barriers influence the association between intention and behavior was also not significant (Barriers*intention, $\beta = -0.01$, $P = 0.409$). This model explained 28% of the variation in pearl millet consumption among school aged children. Details of all models are provided in **Table 6**.

DISCUSSION

The study herein presents an investigation aimed at identifying factors influencing consumption of the nutrient dense cereal crop-pearl millet among school aged children. We observed that there was a significant difference between intention and behavior among the children who did not consume pearl millet ($P = 0.003$) and those who consumed millet two or more times a week ($P = 0.013$). Additionally, caregivers did not have adequate knowledge on the nutritional content and benefits of pearl millet consumption although the health of their school aged child was important to them. The barriers identified as limiting pearl millet consumption were price, seasonality, availability and quantity of fuel required for preparation. In terms of external factors, influencing consumption, the role of mothers, teachers and nurses was observed as crucial in promoting increased pearl millet consumption among school aged children. Using the combined TPB and HBM model we observed that knowledge significantly predicted health behavior identity ($\beta = 0.60$, $P = 0.001$) while health behavior identity significantly predicted intention ($\beta = 0.09$, $P = 0.021$). Intention of caregivers was significantly correlated ($\rho = 0.44$, $P < 0.001$) and predicted consumption of pearl millet ($\beta = 0.87$, $P = 0.067$).

Our current study is in concordance with several studies that have observed a positive relationship between caregiver knowledge, nutritional behavior and nutritional status (Zeng et al., 2012; Christian et al., 2016; Oly-Alawuba and Ihedioha, 2018; Oduor et al., 2019). Caregivers with improved knowledge and skills are more likely to ensure proper composition of foods. However, when passing on knowledge, subjective norms that are the social pressures the individual experiences to adopt or avoid the desired behavior should be considered. This could explain why we observed that when the caregivers assertion of giving pearl millet increased, the likelihood that the SAC consumed pearl millet decreased. It may be possible that other reinforcing actors are crucial. For example, we observed that the role of female grandparents, teachers and nurses is crucial in promoting increased pearl millet consumption among school aged children. The impact of grandparental caregiving on child feeding becomes more direct and influential as their caregiving role with grandchildren increases. Previous research has shown that parent attitudes, beliefs and feeding practices have a significant influence on child dietary intake and weight status (Contento et al., 1993; Appoh and Krekling, 2005; Burchi, 2010; Fadare et al., 2019a,b). As grandparents take responsible roles in the lives of their grandchildren, it can be assumed that their attitudes, beliefs and feeding practices may have a similarly significant impact on child dietary intake and weight status as those of parents. This is additionally important in cultures where the role of grandparents in the family is valued and respected. While a large number of studies have examined the influence of various familial factors on child dietary intake or weight status, the vast majority of these have focused primarily on parents and on pre-school children. There is however now growing evidence to suggest that grandparents play an increasingly important role in influencing their grandchildren's feeding practices (Mukuria et al., 2016; Negin et al., 2016; Karmacharya et al., 2017; Young et al., 2018). Many of these studies observe that grandparents are important in influencing the diets of pre-school children. More studies are however required to investigate whether the grandparents influence on diets and nutrition statue extends beyond early childhood. That notwithstanding, our results buttress the observation that for a successful nutrition behavior change program in Kongwa, the role of female grandparents would have to be factored into interventions. Such an approach could involve nurses, who were also observed to be influential in our study. Indeed, a study on the role of nurses in Sub Saharan Africa has observed that they are often required to provide health education to the communities in which they serve (Ugochukwu et al., 2013). Expanding actors involved in disseminating nutrition knowledge is crucial especially because it has previously been observed that there is low coverage of nutrition education in Central Tanzania (Bundala et al., 2020). For nurses' involvement to be effective, they need to be trained to give in-depth nutrition education, a skill they often lack (DiMaria-Ghalili et al., 2014). For example, a study by Davis et al. (2017) among Ghanian nurses reported a lack of in-depth nutrition knowledge and young child feeding (YCF) education as a barrier to effective nutrition education. Perhaps incorporating other influential actors may also make up for this gap in nutrition education. Teachers also have an important role as health promoters (Pickett et al., 2015). It is important that it is however noted that a successful nutrition education program involving teachers also depends on their training and following of a standardized protocol to ensure fidelity (Murimi et al., 2018). Other factors that have been observed to be vital for success of a nutrition education program include an intervention period of at least 6 months and use of age-appropriate activities (Murimi et al., 2018). Based on these observations, Agriculture for nutrition programs that seek to promote pearl millet consumption therefore need to have a multi actor approach that are age appropriate, be of adequate duration and ensure fidelity and proper alignment between the stated objectives, the intervention, and the desired outcomes to ensure success. In addition, such program should include the use of appropriate media channels to encourage utilization. In our study, we did observe that respondents mentioned that the media had a positive effect on their consumption of pigeon pea.

It is important to take into account that though nutrition knowledge is necessary, it may not be a sufficient factor for changes in food consumption. Education interventions, which are expected to be effective in influencing dietary behaviors or choice, need to consider external influencing factors as well. For example, respondents in our study identified price, seasonality, availability and quantity of fuel required for pearl millet preparation as barriers to consumption, which interestingly has been observed in India too (Amarender Reddy et al., 2013; Singh et al., 2018). A previous study conducted in Tanzania indicates that virtually all of the pearl millet production is carried out on a subsistence basis (Rohrbach and Kiriwaggulu, 2001). In fact, <2% of the harvest is available in the formal market; with the bulk of the harvest consumed by farm households. These limited quantities of grain traded may explain the issues of seasonality, availability and price experienced by our respondents. As various programs begin to promote consumption of pearl millet, the production and functionality of the entire pearl millet grain value chain will need to be simultaneously improved to meet the growing demand and ensure affordability.

Our results confirm the mediating role of health behavior identity between background and perception constructs and intention in the combined TPB and HBM model. This indicates that the knowledge of caregivers about pearl millet together with the health values they hold for pearl millet consumption by their children resulted in a positive health behavior identity. This positive health behavior identity influenced attitude to feeding pearl millet to school aged children positively that subsequently predicted intention of caregivers to give pearl millet to their children. For programs and efforts to promote pearl millet consumption, it is imperative that pearl millet should be promoted as a nutrient dense crop to combat iron and zinc deficiency among school aged children since this what caregivers value.

Intention was utilized in our study as a predictor of previous behavior, which in turn is a surrogate for future behavior. We observed that though the internal reliability of our constructs was good, our model had low predictive ability. Three other studies in Africa that have utilized these predictive models have shown similar trends in low predictive abilities of models on intention (Fanou-Fogny et al., 2011; Macharia-Mutie et al., 2011; Abizari et al., 2013). The fact that pearl millet is consumed in combination with maize flour may explain the low contribution of the constructs to the prediction of intention in this study. Since the TPB requires participants to describe their cognitions, this requirement is based on the assumption that the answers given will reveal pre-existing states of mind (Armitage and Conner, 2001). Thus, if the behavior investigated is uncommon, in this case consumption of pearl millet alone, it is possible that the cognitions may be created simply by completing a questionnaire (Ogden, 2003). We sought to reduce the effect of unfamiliarity by setting certain requirements for participation such as, knowing and having consumed pearl millet. However, though majority of the respondents had consumed pearl millet in the past, they had not consumed it regularly and had consumed it as a blend with maize flour. Predictive ability may have been improved if reference to a specific food was made.

In summary, though pearl millet consumption presents a viable option to combat iron and zinc deficiency several considerations need to be made. Firstly, increasing awareness about iron and zinc deficiencies and nutritional benefits of pearl millet as well as health consequences of consuming pearl millet (health behavior identity) such as pearl millet improves cognitive development, intelligence, health and survival should be targeted. In addition, value chain factors such as price, seasonality and availability that present barriers should be addressed. For example, programs aiming to increase pearl millet consumption should not only invest in its increased production but also on new technologies, including processing machines, packaging and storage to influence growth of local markets. These strategies may not only promote pearl millet consumption among school aged children at household level but also at scale in school feeding programs.

DATA AVAILABILITY STATEMENT

The raw data supporting the conclusions of this article will be made available upon approval by the funder.

ETHICS STATEMENT

Ethical review and approval was not required for the study on human participants in accordance with the local legislation and institutional requirements. The patients/participants provided their written informed consent to participate in this study.

AUTHOR CONTRIBUTIONS

MC: questionnaire validation and administration, data analyses, interpretation of results, visualization, writing—original draft, and writing final draft—review and editing. HMu: data analyses, interpretation of results, visualization, writing—original draft, and writing final draft—review and editing. RM, YM, NK, JM, and HMs: questionnaire development and validation, administration, and review of final draft. MB and PO: supervision and review of final draft. WG-W: project administration, conceptualization, methodology, interpretation of results, visualization, writing—original draft, writing—review and editing, and supervision. All authors contributed to the article and approved the submitted version.

FUNDING

This research publication was made possible with support from the United States Agency for International Development (USAID) through the Africa Research in Sustainable Intensification for the Next Generation (Africa RISING) program as part of the US Government's Feed the Future Initiative. The funders had no role in the design and/or implementation of the study or the preparation of the manuscript thereof.

ACKNOWLEDGMENTS

The authors wish to thank the administrative and local authorities as well as all caregivers/respondents from Laikala village that participated in the study. The support of Mr. Peter Ngowi in identifying potential respondents for the survey is appreciated. Additionally, we thank the interviewers for their work in the field. Furthermore, we are grateful to Tanzania Agricultural Research Institute (TARI) Hombolo for all the logistical support. The Authors also acknowledge various contributions by the International Institute for Tropical Agriculture (IITA).

SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fsufs.2021.694160/full#supplementary-material

REFERENCES

Abizari, A. R., Pilime, N., Armar-Klemesu, M., and Brouwer, I. D. (2013). Brouwer. Cowpeas in Northern Ghana and the factors that predict caregivers' intention to give them to schoolchildren. *PLoS ONE* 8:e72087. doi: 10.1371/journal.pone.0072087

Africa RISING. (2021). *Africa Research in Sustainable Intensification for the Next Generation*. Africa RISING. Available online at: https://africa-rising.net/ (accessed January 4, 2021).

Agte, V. V., Khot, S., Girigosavi, S. T., Paknikar, K. M., and Chiplonkar, S. A. (1999). Comparative performance of pearl millet- and sorghum- based diets vs. wheat- and rice-based diets for trace metal bioavailability. *J. Trace Elements Med. Biol*. 13, 215–219. doi: 10.1016/S0946-672X(99)80038-8

Amarender Reddy, A., Dharm Pal Malik, O. Y., Singh, I. P., Ardeshna, N. J., Kundu K. K., Gupta, S. K., et al. (2013). Utilization pattern, demand and supply of pearl millet grain and fodder in Western India. *Working Paper Series no*. 37. Patancheruvu: International Crops Research Institute for the Semi Arid Tropics, 24. Available online at: http://oar.icrisat.org/6694/1/WPS_37_MIP.pdf

Appoh, L. Y., and Krekling, S. (2005). Maternal nutritional knowledge and child nutritional status in the Volta region of Ghana. *Matern. Child Nutr*. 1, 100–110. doi: 10.1111/j.1740-8709.2005.00016.x

Armitage, C. J., and Conner, M. (2001). Efficacy of the theory of planned behaviour: a meta-analytic review. *Br. J. Soc. Psychol*. 40, 471–499. doi: 10.1348/014466601164939

Bouis, H. E. (2002). Plant breeding: a new tool for fighting micronutrient malnutrition. *J. Nutr*. 132, 491S−4S. doi: 10.1093/jn/132.3.491S

Brown, K. H., Rivera, J. A., Bhutta, Z., Gibson, R. S., King, J. C., Lönnerdal, B., et al. (2004). International Zinc Nutrition Consultative Group (IZiNCG) technical document #1. Assessment of the risk of zinc deficiency in populations and options for its control. *Food Nutr. Bull*. 25, S99–203.

Bundala, N., Kinabo, J., Jumbe, T., Bonatti, M., Rybak, C., and Sieber, S. (2020). Gaps in knowledge and practice on dietary consumption among rural farming households; a call for nutrition education training in Tanzania. *Int. J. Food Sci. Nutr*. 71, 341–351. doi: 10.1080/09637486.2019.1655533

Burchi, F. (2010). Child nutrition in Mozambique in 2003: the role of mother's schooling and nutrition knowledge. *Econ. Hum. Biol*. 8, 331–345. doi: 10.1016/j.ehb.2010.05.010

Butrimovitz, G. P., and Purdy, W. C. (1978). Zinc nutrition and growth in a childhood population. *Am. J. Clin. Nutr*. 31, 1409–1412. doi: 10.1093/ajcn/31.8.1409

Cavan, K. R., Gibson, R. S., Grazioso, C. F., Isalgue, A. M., Ruz, M., and Solomons, N. W. (1993). Growth and body composition of periurban Guatemalan children in relation to zinc status: a longitudinal zinc intervention trial. *Am. J. Clin. Nutr*. 57, 344–352. doi: 10.1093/ajcn/57.3.344

Christian, A. K., Marquis, G. S., Colecraft, E. K., Lartey, A., Sakyi-Dawson, O., Ahunu, B. K., et al. (2016). Caregivers' nutrition knowledge and attitudes are associated with household food diversity and children's animal source food intake across different agro-ecological zones in Ghana. *Br. J. Nutr*. 115, 351–360. doi: 10.1017/S0007114515004468

Contento, I. R., Basch, C., Shea, S., Gutin, B., Zybert, P., Michela, J. L., et al. (1993). Relationship of mothers' food choice criteria to food intake of preschool children: identification of family subgroups. *Health Educ. Q*. 20, 243–259. doi: 10.1177/109019819302000215

Davis, J. N., Brown, H., and Ramsay, S. A. (2017). Gaps in international nutrition and child feeding guidelines: a look at the nutrition and young child feeding education of Ghanaian nurses. *Public Health Nutr*. 20, 2215–2224. doi: 10.1017/S1368980017000908

DiMaria-Ghalili, R. A., Mirtallo, J. M., Tobin, B. W., Hark, L., Van Horn, L., and Palmer, C. A. (2014). Challenges and opportunities for nutrition education and training in the health care professions: intraprofessional and interprofessional call to action. *Am. J. Clin. Nutr*. 99, 1184S−1193S. doi: 10.3945/ajcn.113.073536

Emerson, R. W. (2017). Likert scales. *J. Vis. Impair. Blindness* 111:488. doi: 10.1177/0145482X1711100511

Fadare, O., Amare, M., Mavrotas, G., Akerele, D., and Ogunniyi, A. (2019a). Correction: mother's nutrition-related knowledge and child nutrition outcomes: empirical evidence from Nigeria. *PLoS ONE* 14:e0215110. doi: 10.1371/journal.pone.0215110

Fadare, O., Amare, M., Mavrotas, G., Akerele, D., and Ogunniyi, A. (2019b). Mother's nutrition-related knowledge and child nutrition outcomes: empirical evidence from Nigeria. *PLoS ONE* 14:e0212775. doi: 10.1371/journal.pone.0212775

Fanou-Fogny, N., van Dam, B., Koreissi, Y., Dossa, R. A., and Brouwer, I. D. (2011). Factors predicting consumption of fonio grain (Digitaria exilis) among urban Malian women of reproductive age. *J. Nutr. Educ. Behav*. 43, 219–228. doi: 10.1016/j.jneb.2010.03.008

FAO (2011). Rome: *Combating Micronutrient Deficiencies: Food-Based Approaches*.

Field, A. (2005). *Discovering Statistics Using SPSS*. 2nd ed. London, United Kingdom: SAGE Publications.

Francis, J., Eccles, M. P., Johnston, M., Walker, A. E., Grimshaw, J. M., Foy, R., et al. (2004). *Constructing Questionnaires Based on the Theory of Planned Behaviour: A Manual for Health Services Researchers*. N: Newcastle: Centre for Health Services Research, University of Newcastle, UK.

Grantham-McGregor, S., and Ani, C. (2001). A review of studies on the effect of iron deficiency on cognitive development in children. *J. Nutr*. 131, 649S−666S. doi: 10.1093/jn/131.2.649S

Hall, A., Bobrow, E., Brooker, S., Jukes, M., Nokes, K., Lambo, J., et al. (2001). Anaemia in schoolchildren in eight countries in Africa and Asia. *Public Health Nutr*. 4, 749–756. doi: 10.1079/PHN2000111

Jukanti, A. K., Gowda, C. L., Rai, K. N., Manga, V. K., and Bhatt, R. K. (2016). Bhatt Crops that feed the world 11. Pearl Millet (*Pennisetum glaucum* L.): an important source of food security, nutrition and health in the arid and semi-arid tropics. *Food Secur*. 8, 307–329. doi: 10.1007/s12571-016-0557-y

Kanatti, A., Rai, K. N., Radhika, K., Govindaraj, M., Sahrawat, K. L., and Rao, A. S. (2014). Grain iron and zinc density in pearl millet: combining ability, heterosis and association with grain yield and grain size. *SpringerPlus* 3:763. doi: 10.1186/2193-1801-3-763

Karadimitriou, S. M., Marshall, E., and Knox, C. (2018). *Mann Whitney U-test*. Sheffield: Sheffield Hallam University.

Karmacharya, C., Cunningham, K., Choufani, J., and Kadiyala, S. (2017). Grandmothers' knowledge positively influences maternal knowledge and infant and young child feeding practices. *Public Health Nutr*. 20, 2114–2123. doi: 10.1017/S1368980017000969

Kodkany, B. S., Bellad, R. M., Mahantshetti, N. S., Westcott, J. E., Krebs, N. F., Kemp, J. F., et al. (2013). Biofortification of pearl millet with iron and zinc in a randomized controlled trial increases absorption of these minerals above physiologic requirements in young children. *J. Nutr*. 143, 1489–1493. doi: 10.3945/jn.113.176677

Lopez, A., Cacoub, P., Macdougall, I. C., and Peyrin-Biroulet, L. (2016). Iron deficiency anaemia. *Lancet* 387, 907–916. doi: 10.1016/S0140-6736(15)60865-0

Macharia-Mutie, C. W., Van de Wiel, A. M., Moreno-Londono, A. M., Mwangi, A. M., and Brouwer, I. D. (2011). Sensory acceptability and factors predicting the consumption of grain amaranth in Kenya. *Ecol. Food Nutr*. 50, 375–392. doi: 10.1080/03670244.2011.604584

Mukuria, A. G., Martin, S. L., Egondi, T., Bingham, A., and Thuita, F. M. (2016). Role of social support in improving infant feeding practices in Western Kenya: a quasi-experimental study. *Glob. Health Sci. Pract*. 4, 55–72. doi: 10.9745/GHSP-D-15-00197

Murimi, M. W., Moyeda-Carabaza, A. F., Nguyen, B., Saha, S., Amin, R., and Njike, V. (2018). Factors that contribute to effective nutrition education interventions in children: a systematic review. *Nutr. Rev*. 76, 553–580. doi: 10.1093/nutrit/nuy020

NBS (2013). *2012 Population and Housing Census: Population Distribution by Administrative Areas*. Dare es Salaam and Office of Chief Government Statistician President's Office, Zanzibar, United Republic of Tanzania.

Negin, J., Coffman, J., Vizintin, P., and Raynes-Greenow, C. (2016). The influence of grandmothers on breastfeeding rates: a systematic review. *BMC Pregnancy Childbirth* 16:91. doi: 10.1186/s12884-016-0880-5

Nestel, P., Bouis, H. E., Meenakshi, J. V., and Pfeiffer, W. (2006). Biofortification of staple food crops. *J. Nutr*. 136, 1064–1067. doi: 10.1093/jn/136.4.1064

Oduor, F. O., Boedecker, J., Kennedy, G., Mituki-Mungiria, D., and Termote, C. (2019). Caregivers' nutritional knowledge and attitudes mediate seasonal shifts in children's diets. *Matern. Child Nutr*. 15:e12633. doi: 10.1111/mcn.12633

Ogden, J. (2003). Some problems with social cognition models: a pragmatic and conceptual analysis. *Health Psychol*. 22, 424–428. doi: 10.1037/0278-6133.22.4.424

Oly-Alawuba, N. M. A., and Ihedioha, S. (2018). Nutritional knowledge of mothers/caregivers in relation to the anthropometric indices of children(2–5 years) in Obowu Local Government Area, Imo State, Nigeria. *FASEB J*. 31, 957–913. doi: 10.1096/fasebj.31.1_supplement.957.13

Pickett, K., Byrne, J., Rietdijk, W., Shepherd, J., Roderick, P., and Grace, M. (2015). Teachers as health promoters: a longitudinal study of the effect of a health education curriculum for trainee teachers on their practice in schools. *Lancet*. 386:S63. doi: 10.1016/S0140-6736(15)00901-0

Rao, P. P., Birthal, P. S., Reddy, B. V. S., Rai, K. N., and Ramesh, S. (2006). Diagnostics of sorghum and pearl millet grains-based nutrition in India. *Int. Sorghum Millets Newslett*. 47, 93–96.

Rohrbach, D. D., and Kiriwaggulu, J. A. B. (2001). Commercialization prospects for Sorghum and Pearl Millet in Tanzania. *SAT e J*. 3, 1–28.

Rohrbach, D. D., and Kiriwaggulu, J. A. B. (2007). Commercialization prospects for Sorghum and Pearl Millet in Tanzania. *ICRISAT e J*. 3, 1–28.

Schober, P., Boer, C., and Schwarte, L. A. (2018). Correlation coefficients: appropriate use and interpretation. *Anesth. Analg*. 126, 1763–1768. doi: 10.1213/ANE.0000000000002864

Singh, N., Singh, S. P., Kumar, M., Kanhiya, K., and Kumar, A. (2018). Nutri cereal pearlmillet: way forward. *Int. J. Curr. Microbiol. Appl. Sci*. 7, 578–581. doi: 10.20546/ijcmas.2018.706.066

Sun, X., Guo, Y., Wang, S., and Sun, J. (2006). Predicting iron-fortified soy sauce consumption intention: application of the theory of planned behavior and health belief model. *J. Nutr. Educ. Behav*. 38, 276–285. doi: 10.1016/j.jneb.2006.04.144

Talsma, E. F., Melse-Boonstra, A., de Kok, B. P., Mbera, G. N., Mwangi, A. M., and Brouwer, I. D. (2013). Biofortified cassava with pro-vitamin A is sensory and culturally acceptable for consumption by primary school children in Kenya. *PLoS ONE* 8:e73433. doi: 10.1371/journal.pone.0073433

Thiese, M. S., Ronna, B., and Ott, U. (2016). P value interpretations and considerations. *J. Thorac. Dis*. 8, E928–E931. doi: 10.21037/jtd.2016.08.16

Ugochukwu, C. G., Uys, L. R., Karani, A. K., Okoronkwo, I. L., and Diop, B. N. (2013). Roles of nurses in Sub-Saharan African region. *Int. J. Nurs. Midwifery* 5, 117–131. doi: 10.5897/IJNM2013.0104

Wei, W., Erenrich, J., and Selman, B. (2004). *Towards Efficient Sampling: Exploiting Random Walk Strategies*. Ithaca, NY: Department of Computer Science, Cornell University.

Wessells, K. R., Singh, G. M., and Brown, K. H. (2012). Estimating the global prevalence of inadequate zinc intake from national food balance sheets: effects of methodological assumptions. *PLoS ONE* 7:e50565. doi: 10.1371/journal.pone.0050565

Young, K. G., Duncanson, K., and Burrows, T. (2018). Tracy burrows influence of grandparents on the dietary intake of their 2-12-year-old grandchildren: a systematic review. *Nutr. Dietetics* 75, 291–306. doi: 10.1111/1747-0080.12411

Zeng, R., Luo, J. Y., Tan, C., Du, Q. Y., Zhang, W. M., and Li, Y. (2012). Relationship between caregivers' nutritional knowledge and children's dietary behavior in Chinese rural areas. *J. Cent. South Univ*. 37, 1097–1103. doi: 10.3969/j.issn.1672-7347.2012.11.003

Zimmermann, M. B., and Hurrell, R. F. (2007). Nutritional iron deficiency. *Lancet* 370, 511–520. doi: 10.1016/S0140-6736(07)61235-5

Conflict of Interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's Note: All claims expressed in this article are solely those of the authors and do not necessarily represent those of their affiliated organizations, or those of the publisher, the editors and the reviewers. Any product that may be evaluated in this article, or claim that may be made by its manufacturer, is not guaranteed or endorsed by the publisher.

frontiers in Sustainable Food Systems

ORIGINAL RESEARCH
published: 13 August 2021
doi: 10.3389/fsufs.2021.680777

Assessing Millets and Sorghum Consumption Behavior in Urban India: A Large-Scale Survey

Joanna Kane-Potaka[1], Seetha Anitha[1*], Takuji W. Tsusaka[2], Rosemary Botha[3], Muralidhar Budumuru[1], Shweta Upadhyay[4], Parkavi Kumar[1], Karuna Mallesh[5], Raghavendra Hunasgi[6], Ashok Kumar Jalagam[1] and Swamikannu Nedumaran[1,7]

[1] Smart Food Initiative, International Crops Research Institute for the Semi-Arid Tropics, Hyderabad, India, [2] Organization for Advanced and Integrated Research, Kobe University, Kobe, Japan, [3] Development Strategy and Governance Division, International Food Policy Research Institute, Lilongwe, Malawi, [4] United Nations International Children's Emergency Fund, Lilongwe, Malawi, [5] Marketing Leadership Institute, SR Global Media and Research Pvt. Ltd, Hyderabad, India, [6] Thought Folks Digital, Hyderabad, India, [7] Innovation Systems for the Dry Land, International Crops Research Institute for the Semi-Arid Tropics, Hyderabad, India

There is growing attention by governments and industry in regard to the role played by millets (including sorghum) to help build resilience for farmers and cope with climate change, malnutrition, diabetes, and some other major issues. To understand public knowledge and practices of consuming millets in urban areas, a survey was conducted with 15,522 individuals from seven major cities of India using a structured questionnaire, and after data cleaning 15,139 observations were subjected to analysis using descriptive and inferential statistics. It was found that the largest group among early adopters of millets were people with health problems (28%), it being the single largest reason for consuming millets, followed by those wanting to lose weight (15%) and those selecting millets for its taste (14%). There was a significant gap between people who were health conscious (91%) and those who were sure millets were healthy (40%). The major reason the respondents did not eat more millets was that it was not eaten at home (40%), followed by reactions such as not liking the taste (22%). Reaching the urban consumers through social media is recommended, given that it is their main source of information. There was no statistically significant relationship between state-wise per capita production and frequency of consumption of millets in the urban areas ($p = 0.236$). In conclusion, three key actions are recommended to enhance the consumption of millets: developing delicious products to satisfy the taste, providing knowledge on nutritional and health facts on millets, and improving accessibility of millets in urban markets.

Keywords: millets, sorghum, health benefit, knowledge and practice, consumer behavior

OPEN ACCESS

Edited by:
*Giuseppe Di Vita,
University of Turin, Italy*

Reviewed by:
*Aida Turrini,
Independent Researcher, Rome, Italy
Gabriele Carullo,
University of Siena, Italy*

***Correspondence:**
*Seetha Anitha
s.anitha@cgiar.org;
dr.anithaseetha@gmail.com*

Specialty section:
This article was submitted to Nutrition and Sustainable Diets, a section of the journal Frontiers in Sustainable Food Systems

Received: 15 March 2021
Accepted: 29 June 2021
Published: 13 August 2021

Citation:
Kane-Potaka J, Anitha S, Tsusaka TW, Botha R, Budumuru M, Upadhyay S, Kumar P, Mallesh K, Hunasgi R, Jalagam AK and Nedumaran S (2021) Assessing Millets and Sorghum Consumption Behavior in Urban India: A Large-Scale Survey. Front. Sustain. Food Syst. 5:680777. doi: 10.3389/fsufs.2021.680777

INTRODUCTION

The recent paper from The Lancet Commissions (Willett et al., 2021) recognizes the need for identifying healthy and environmentally sustainable diets and enhanced usage of underused plant species, such as quinoa, millets, sorghum, or teff grains, due to their climate resilience and dense nutritional content. It also clearly captured that among 14,000 edible plants, only three crops, namely, rice, maize, and wheat, contribute 60% of caloric intake. On the other hand, the SDGs 2030 have its ambitious goal of eliminating all forms of malnutrition by 2030. To achieve this, interventions are required to replace the major portion of the diet currently occupied by rice, wheat, and maize with highly nutritious grains, such as millets.

In India and other Asian and African countries, millets commonly include sorghum, pearl millet, and a range of small millets (Vetriventhan et al., 2020). The term "millets" in this paper refers to all of these crops. India is the leading producer and consumer of different types of millets, such as finger millet, pearl millet, kodo millet, foxtail millet, barnyard millet, proso millet, and little millet (www.smartfood.org[1],[2]). India is the sixth largest producer of sorghum globally (www.smartfood.org). Traditionally, many kinds of foods and beverages were made from these grains in different regions, which played an important role as a staple food in the local food culture. However, their presence in the Indian food basket has been declining over the years largely due to government policies favoring the production and consumption of fine cereals, such as rice and wheat (Kane-Potaka and Kumar, 2019), as well as rising incomes and urbanization. Between 1960 and 2015 in India, wheat production more than trebled, and rice production increased by ∼800%; on the contrary, millet production was stagnant at low levels (Kane-Potaka and Kumar, 2019). Between 1962 and 2010, India's per capita consumption of millets fell drastically from 32.9 to 4.2 kg, while that of wheat almost doubled from 27 to 52 kg (www.indiaspend.com)[3]. Another study reported in particular on pearl millet that per capita consumption trend declined between 1972/1973 and 2004/2005 in both rural and urban regions of India from 11.4 to 4.7 and from 4.1 to 1.4 kg per year, respectively (Basavaraj et al., 2010). A similar declining trend in per capita consumption was reported for sorghum in both rural and urban India from 19.1 to 5.2 and from 8.5 to 2.7 kg per year, respectively, representing 68 and 70% reduction (Parthasarathy Rao et al., 2010).

Millets are often referred to as smart food (www.smartfood.org), which is "good for the individual" (nutritious and healthy), "good for the planet" (environmentally sustainable), and "good for the farmer" (resilient). Millets are recognized for their resilience, ability to survive under high temperatures and in degraded soils, and minimum requirements of water, pesticides, and fertilizers (Saleh et al., 2013). Their farming methods leave a lower carbon footprint than the major staples that are grown with greater use of fertilizers and pesticides. Millets complement commonly used legumes in India, such as pigeon pea and chickpea, for amino acid content to form complete protein with improved digestibility upon cooking (Anitha et al., 2019a). Apart from protein, depending on the variety and species, millets are also rich in minerals, such as iron, zinc, and calcium, which deliver health benefits to all age groups and genders.

The high nutritional content of millets compares well with other foods with similar nutritional value; it is especially high compared with polished rice, maize, and refined wheat flour, the post-green revolution major staples (Longvah et al., 2017). Innovative millet processing technologies that provide safe, easy-to-handle, ready-to-cook, and ready-to-eat products and meals at a commercial scale are mainly available in urban areas (Ushakumari et al., 2004), where rice and refined wheat flour dominate and are much more accessible and affordable. The consumption of refined grains, namely, refined white rice, is shown to be associated with non-communicable diseases, such as type II diabetes mellitus (Radhika et al., 2009) and obesity. This has led to an increasing emphasis globally on consuming whole grains (Edge et al., 2005), underlining the importance of mainstreaming nutritious smart food crops and promoting them as a staple. This is one of the major objectives of the Smart Food initiative (Singh and Raghuvanshi, 2012; Kane-Potaka, 2018). Providing more nutritious and healthier traditional whole grain and multigrain substitutes for refined carbohydrates can be an important aspect of therapeutic dietary modification and diversity.

There is a growing interest in reviving millets in India (Kane-Potaka and Kumar, 2019) and also globally, owing to their nutrition content and ability to grow in harsh climatic conditions due to their climate smart traits. The Government of India declared 2018 as a National Year of Millets and followed on with preparing a national Millet Mission, as well as a proposition to the Food and Agricultural Organization of the United Nations (UN) for a UN International Year of Millets. Several state governments in India also followed suit to establish state millet missions. These initiatives all recognized the need and built-in components to engage with consumers to drive demand and not only to invest in agricultural production and productivity. The demand for value added (processed) millet-based food products (being promoted as health foods) is increasing steadily in global markets (Rooney, 2010). This transition phase during which perceptions of millets are changing and there is greater health consciousness (Umanath et al., 2018) is the right stage to assess current knowledge, perceptions, and practices related to millets, which will lay the foundation for a plan to promote millets as a staple effectively.

Understanding current knowledge and practices is important for researchers, nutrition volunteers, community health workers, and food manufacturers in planning millet-based products, interventions, and promotional activities to improve the nutritional status and general health of the population and for companies to make nutritious foods a viable business. A limited number of studies have investigated the perceptions, awareness, and knowledge on millets. For instance, the study conducted on pearl millet, finger millet, and sorghum in Maharashtra state involved a relatively small sample of 111 participants who were all diabetic (Nambiar and Patwardhan, 2014). Another study on finger millet and oats in South India recognized the widespread lack of knowledge about the nutritional benefits of finger millet and the need to promote it through a survey with 260 women (Sreedhar and Shaji, 2017). To our knowledge, no formal study has focused on understanding consumers' knowledge, perceptions, and attitude on millets using a large sample survey covering various states of India. Therefore, this study aimed to assess the knowledge, perceptions, and practices of millets consumption in seven major cities of India.

[1] Learning from the Web. Available online at: http://www.smartfood.org/

[2] Learning from the Web. Available online at: https://www.smartfood.org/millets-sorghum-%20production-trends/

[3] Learning from the Web. Available online at: (https://archive.indiaspend.com/cover-story/a-millets-revival-could-solve-indias-malnutrition-problem-benefit-farmers-31717)

METHODS

A large sample survey was conducted to collect primary data on trends, attitudes, and opinions on millets consumption behavior of the target population who were urban consumers in India. The data were statistically analyzed to examine the relationship between variables (Creswell and Creswell, 2017). Surveys on beliefs, reasons, and barriers in purchase and consumption are widely used to evaluate food choice behavior (Roche et al., 2012; Irianto, 2015).

Survey Participants

Convenience sampling (Jager et al., 2017) was adopted where respondents were approached in shopping malls. This provided access to large numbers of men and women in cities in a short time at locations they commonly frequent for purchasing groceries. It also accessed shopping areas where targeted individuals purchased food ingredients for use at home. Participation in the survey was voluntary and anonymous. Participants were informed upfront of the purpose of the study and the use of the data, emphasizing that the information requested would be exclusively used for statistical analysis, guaranteeing confidentiality. Informed consent was obtained before the interview. To ensure confidentiality, the collected data were managed anonymously by providing sample identity based on institutional data management policy.

The optimal size of the survey sample was identified considering both the budget and the statistical power. As a result, a total of 15,522 individuals from seven cities in seven states of South, West, North, and East India participated in the survey. Approximately 300 survey personnel were deployed to conduct the interviews. The participants were provided with an information flyer and a link to an App about millets at the end of the survey.

Questionnaire

A structured questionnaire was developed with questions about millets including Food Frequency Questions (FFQ) on millet by custom designing to understand the millet consumption patterns [Usual Dietary Intakes: NHANES Food Frequency Questionnaire (FFQ) | EGRP/DCCPS/NCI/NIH (cancer.gov)], followed by socioeconomic information of the respondents (**Supplementary Materials**). An expert panel was consulted to ensure the validity and clarity of the questions. To ensure reliable results, the questionnaire was pre-tested with 20 subjects before the survey to validate and identify problems with the content and comprehensiveness of the questions, as well as other causes of (dis)satisfaction, which were added to the options sheet used by the interviewers. Based on the satisfaction or dissatisfaction, annotations, and comments from the pre-testing, the questionnaire was improved and finalized, which helped ensure that the questions were understood by both interviewers and respondents and minimize subsequent measurement errors.

The survey was administered to visitors to shopping malls over the course of August 2017. Participants were asked the questions in person about their knowledge, perceptions, and consumption patterns, as well as the reasons for their practices and sources of information, on millets.

All the qualitative and quantitative questions were asked without prompts or options to select. The interviewees had options on the question sheet to assist with faster recording, and additional answers were written. These options were collated from pilot testing of the questions, and the options for demographics were based on common standards. A picture of three popular smart food grains, namely, finger millet, pearl millet, and sorghum, was used as a visual aid at the beginning of the survey along with a structured questionnaire. The picture included the crop and the grain with their names in English, Hindi, and the state language. The income of a participant's household was self-reported and included a variety of questions designed based on standard methods for grouping participants in poor, middle, and rich income groups (Dalvi et al., 2020).

Data Analysis

Data were cleaned, organized, coded, and subjected to statistical analysis using STATA version 16 (StataCorp, 2019). After data cleaning, 15,139 observations were subjected to analysis (**Table 1**). However, the tables of results may include a smaller number of observations depending on the number of missing observations in the variables included in the specific analysis. Descriptive statistics, such as frequency, mean, median, and standard deviation, were used to present the knowledge and practices among participants. Inferential statistical tools, such as tetrachoric correlation, Z-statistics, and ordered probit regression (Daykin and Moffatt, 2010), were performed to examine the influence of various factors on consumption habits

TABLE 1 | Demographic information on the sampled respondents.

City	Sample size	Men (%)	Women (%)	Average age (%)	Low income (%)	Middle income (%)	High income (%)
Ahmedabad	1,496	22.8	77.2	41.9	7.6	79.3	13.1
Bengaluru	1,127	48.5	51.5	42.8	5.5	87.9	6.6
Chennai	1,502	44.2	55.8	39.9	19.2	56.8	24.0
Delhi	3,999	28.4	71.6	42.1	7.0	82.0	11.0
Hyderabad	1,509	45.5	54.5	40.6	15.2	66.7	18.1
Kolkata	1,501	41.1	58.9	39.6	17.2	59.1	23.7
Mumbai	4,005	42.3	57.7	40.5	13.6	69.2	17.2
Total	15,139	37.6	62.4	41.1	11.6	72.9	15.5

TABLE 2 | Recognition of millets and sorghum in each city (% of respondents).

Crop	Ahmedabad		Bengaluru		Chennai		Delhi		Hyderabad		Kolkata		Mumbai	
	W	M	W	M	W	M	W	M	W	M	W	M	W	M
	n = 1,105	n = 287	n = 630	n = 579	n = 767	n = 602	n = 2,661	n = 923	n = 737	n = 597	n = 796	n = 569	n = 2,104	n = 1,461
Pearl millet*	24.8	50.5	57.8	56.5	30.3	39.2	21.1	39.3	42.5	48.6	28.8	36.2	49.8	51.7
Sorghum*	62.8	55.7	58.2	60	48	40.8	56.8	47.5	54.7	50.1	46.8	45	77.8	77.7
Finger millet*	36	42.2	59.2	58.4	46.4	54	38.9	42.4	54.8	58.9	44	49.5	79.5	81.6
None	0	0	0	0	0	0	0	0	0	0	0	0	2.7	2.3

**Total percentage does not add up to 100 as the responses on all the three millets were separated and added to pearl millet, finger millet, and sorghum. W, women; M, men.*

and perceptions toward millets. The dataset is currently stored in Dataverse and managed by ICRISAT (https://doi.org/10.21421/D2/JUMRM8).

RESULTS

Gender

Among the respondents, 9,453 were women and 5,686 were men, and their average age was 41.2 years. Overall, a higher proportion of women were interviewed as they constituted the majority of grocery shoppers in the town (**Table 1**). In particular, women constituted an even higher proportion of grocery shoppers in Ahmedabad and Delhi.

Recognition of Millets

Although not presented in the table, 89% of the participants reported that they knew millets. Among the participants who responded “yes” to consuming millets, most of them did not recognize it when shown the picture of sorghum, pearl millet, and finger millet (**Supplementary Figure 1**). An example response from a 28-year-old female was “I have been drinking ragi malt (a milk drink made from finger millet) almost every day for the past 3 years since my first pregnancy, but I had no idea what ragi (finger millet) looked like, and today, I see it in the picture that you showed to me, and this is fabulous, and thanks to smart food for all the good work.”

Although not shown in the table, among the three crops, sorghum had the highest recognition rate (59.3%) when shown images of the crop and the grain, followed by finger millet (54.8%). There was no difference in recognition of millets between age groups, though these crops were a traditional staple. To our surprise, the youngest group, below 20 years, had similar or higher recognition of the crops, especially pearl and finger millets, than other age groups.

Recognition of all the three crops among both women and men was the highest in Mumbai, followed by Bengaluru, the lowest being in Kolkata (**Table 2**).

Awareness of Nutritional Value

It was identified that the awareness of millets was prevalent in the last 4 years, and that more than 23% of the respondents mentioned they had heard about or started eating millets <5 years before.

TABLE 3 | Health consciousness of the participants.

Question	Answer	% of respondents
Do you think millets are healthy?	Yes	40.0
	Maybe	47.7
	No	9.4
	Don’t know	3.3
What do you think is healthy about millets?	Good for women	22.4
	High in iron	20.0
	High in calcium	15.5
	Good for diabetes	12.5
	Good for pregnancy	10.0
	Good for babies	7.0
	Good for cancer	2.6
	Other reasons (e.g., good for bone, hair, skin health)	10.0
How health conscious are you?	Very	34.9
	Reasonably	55.6
	Not so or not at all	9.9

When asked whether they thought millets were healthy, less than a half (40%) of the respondents were sure that millets were healthy (**Table 3**). On being asked what they thought was healthy about millets, the answers included: it is good for the general health of women (22%), is high in iron (20%), is high in calcium (15.5%), and is good for diabetes (12.5%). The rest of the participants (30%) thought that it was good for bone health, cancer, during pregnancy, and for babies (**Table 3**). Most (91%) of the respondents said that they were reasonably or highly health conscious (56.6 or 34.4%, respectively; **Table 3**).

Sources of Information

When asked what their source of information on health and foods was, by far the most influential was social sources, the largest being social media with 50.7% of the participants opting for it, while TV shows were the sources for 38.7%, and family and friends were the sources for 36.9%. The other influential information sources were courses (33.9%), newspapers (15.3%), and books (14.8%; **Figure 1**).

Consumption

Although there was a considerable proportion of consumers eating millets frequently (49.6% consumed 1 or more times per week), there was also a reasonable proportion of people who had never or almost never consumed millets (34.9% consumed millets never or up to two times a year; **Figure 2**). The city-wise frequency of consumption of millets was linked to the recognition of these crops in each city, as shown in **Table 3**. Bengaluru led in terms of consumption, while Ahmedabad and

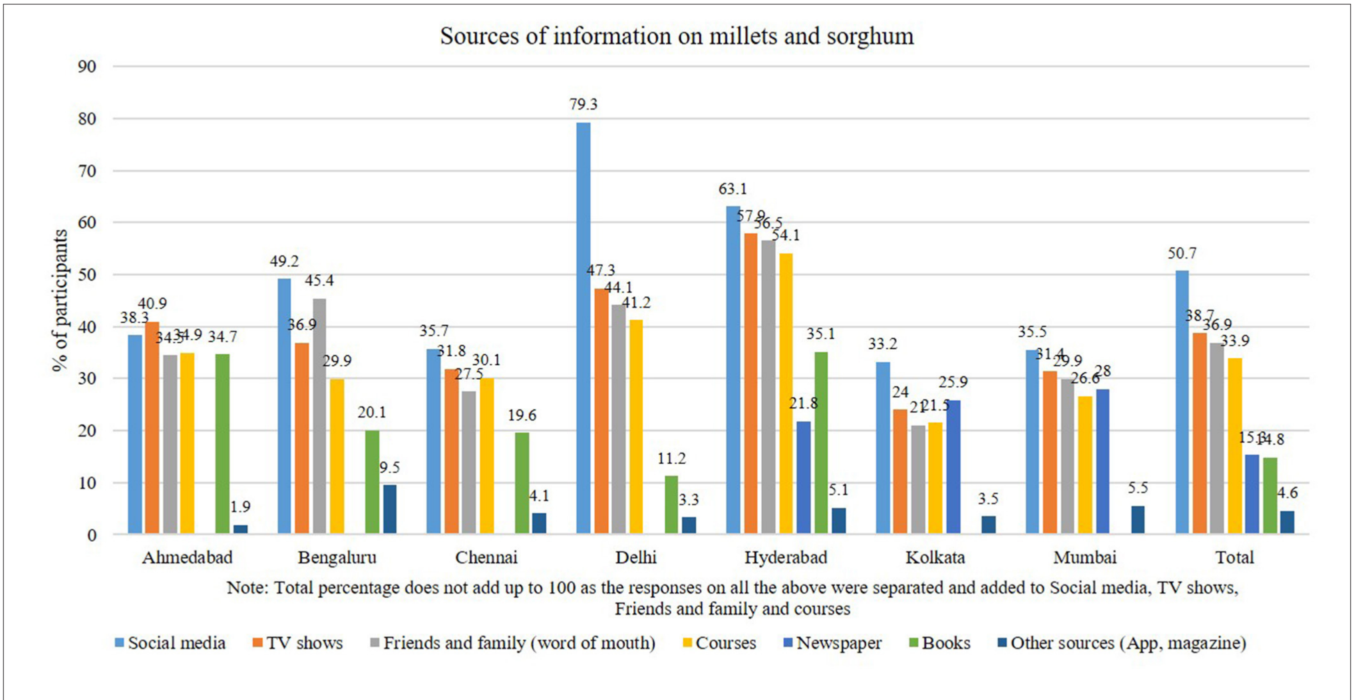

FIGURE 1 | Participants sources of information on millets and sorghum.

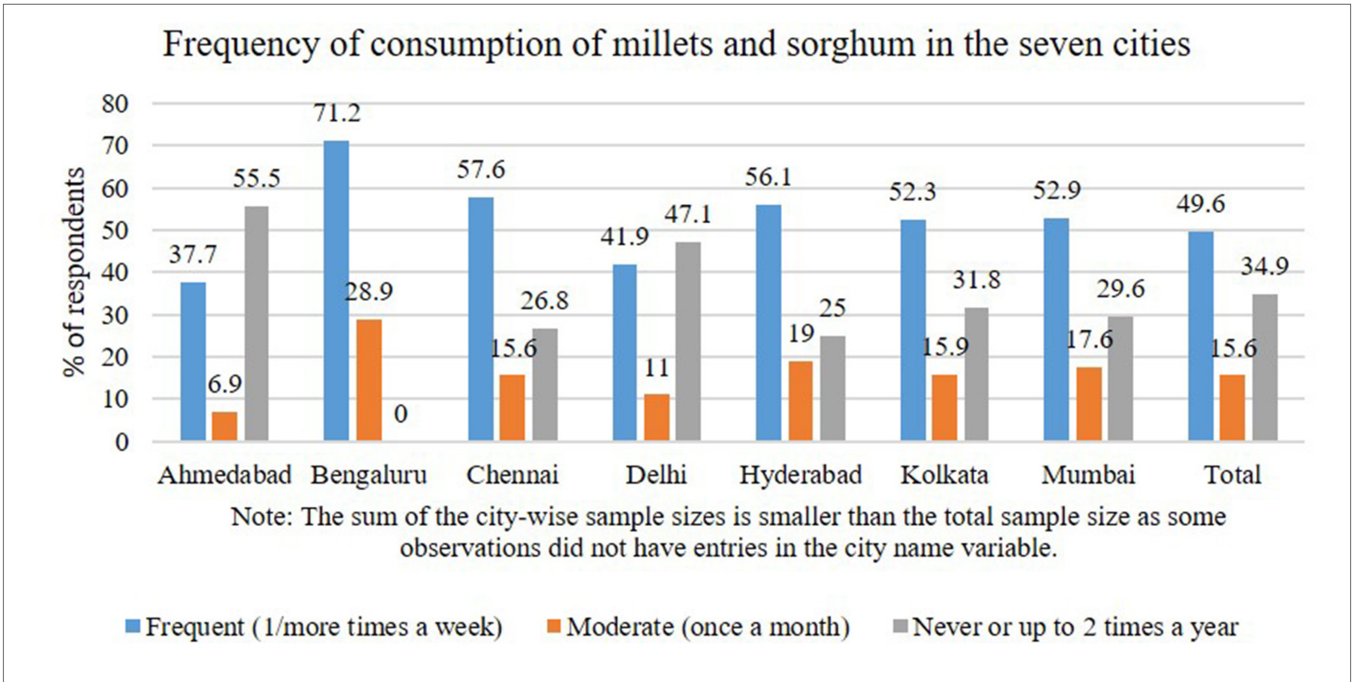

FIGURE 2 | Frequency of consumption of millets and sorghum by participants from seven cities.

Delhi had the lowest consumption frequencies. In Bengaluru, no respondent mentioned that they never/rarely consumed millets. In fact, it was the only city where all the respondents consumed these crops at least once a month. Approximately 71.2% of the respondents in Bengaluru consumed millets at least once a week. This was followed by 57.6 and 56.1% of the respondents being frequent consumers and 26.8 and 25% never/rarely consuming millets in Chennai and Hyderabad, respectively. Mumbai and Kolkata ranked next with 52.9 and 52.3% being frequent consumers and 29.6 and 29.6% never/rarely consuming. Delhi and Ahmedabad had the lowest frequency of consumption with 41.9 and 37.7% consuming frequently and 47.1 and 55.5% never/rarely consuming millets, respectively (**Figure 2**). This indicates that South India is a much greater consumer of millets.

How Millets Are Eaten

The most common form in which millets were eaten at the pan India level was as ready-to-eat food, as reported by 45.6% of the respondents. Breakfast porridge, which is one of the traditional forms of consumption, stood at the top at 38.3%. Ahmedabad and Bengaluru had the largest consumption of ready-to-eat food, with up to 65.7 and 63.4% of the respondents saying that this was the most common form in which they consumed millets (**Figure 3**).

Reasons for Consumption

Figure 4 presents the reasons stated for the consumption of millets. The major reason at the pan India level was the prevalence of health problems including but not limited to diabetes, heart conditions, bone health, and general health. The proportion was especially large in Ahmedabad (48.1%) and Delhi

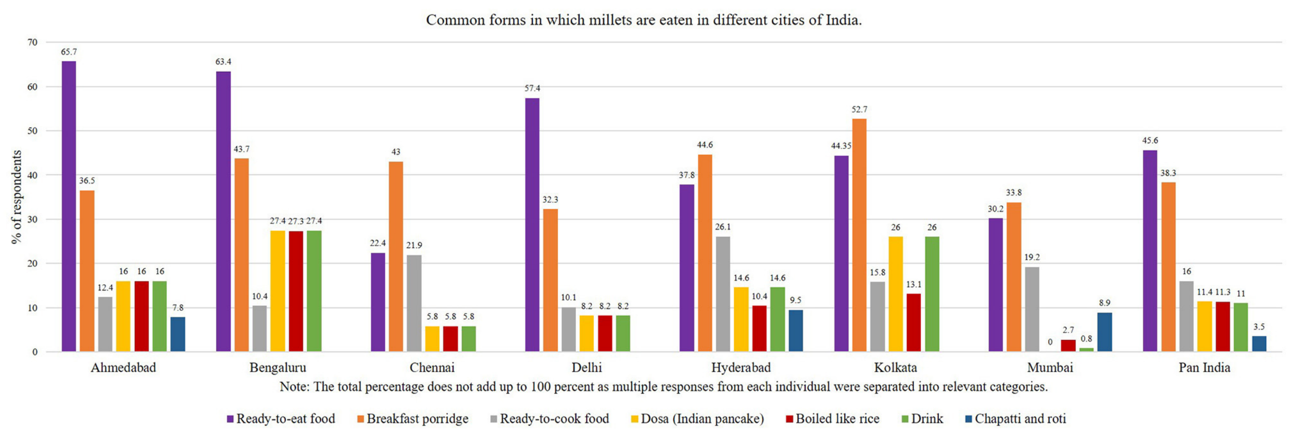

FIGURE 3 | Common forms in which millets are eaten by participants of the survey in different cities of India.

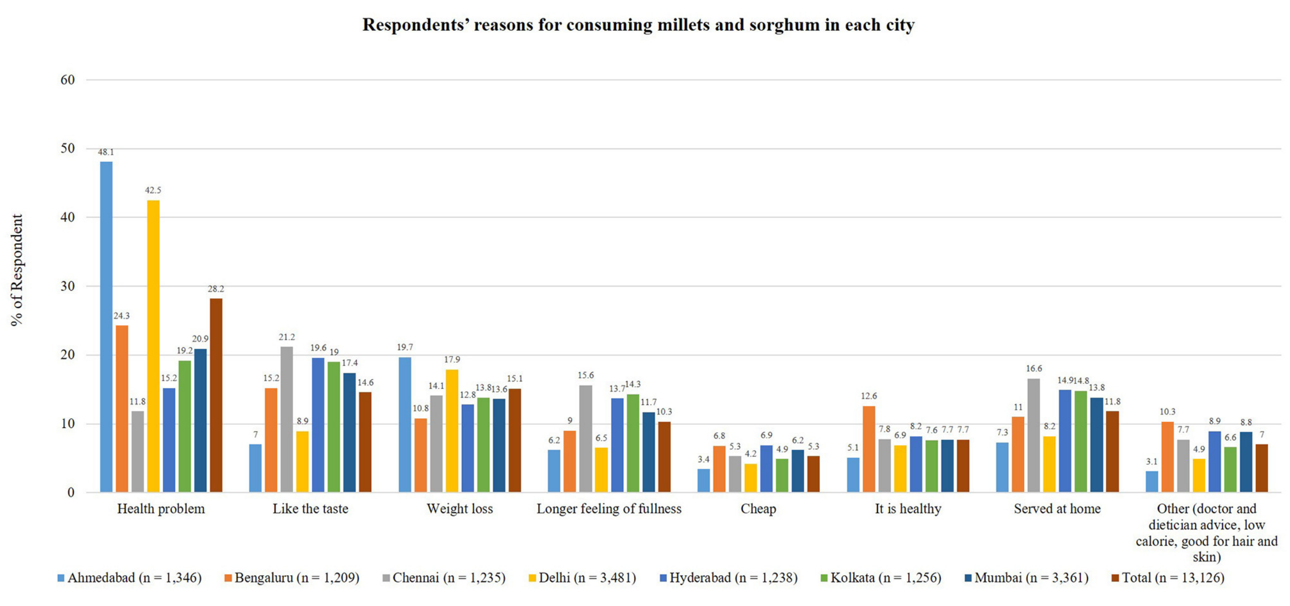

FIGURE 4 | Respondents reasons for consuming millets and sorghum in each city.

(42.5%). The only two cities where this was not the number one reason were Chennai and Hyderabad, where taste preference was the major reason for eating millets. The second major reasons for eating millets pan India were for weight loss (15.1%) and its taste (14.6%).

However, the reason for eating it based on taste preference was not consistent across cities and ranged widely (7–21%); it was predominant in Ahmedabad and Delhi that had lower frequency of consumption. Health and fitness were together chosen as the major reasons for eating millets by at least 58.0% of the respondents. However, few said that they ate it just because it was healthy (7.7%); this trend was consistent across all the cities, ranging from 5.1 to 12.6%. This indicates that a specific health problem is an influential driver for people to eat millets, rather than just because millets are healthy. More women tended to attribute their consumption of millets to health issues and weight loss (**Table 4**), while men tended to consume it simply because it was served at home. Furthermore, low income respondents tended to consume millets for the taste and subsistence and not to lose weight; neither did they find millets cheap.

The most prevalent health reasons for eating millets included weight loss (the top ranked reason especially a primary reason by women 30–50 years old); diabetes (many diabetic patients said that their doctor had recommended eating millets); blood pressure; cancer; skin diseases (this was noted particularly from Bangalore and Mumbai participants who believed that sorghum and finger millet were good for skin and hair growth, and some doctors who participated in the survey said that they ate finger millet a few times a week for better skin); eye sight problem; stronger bones; and healthy pregnancy and baby growth.

TABLE 4 | Relationship between reasons for consuming millets and sorghum and the demographic profile of the respondents: tetrachoric correlation coefficients and *p*-values ($n = 15{,}513$).

Stated reason for millets and sorghum consumption	Gender dummy (female = 1; otherwise = 0)	Low income dummy (low income = 1; otherwise = 0)
I have a health problem	0.507*** (0.000)	−0.304*** (0.000)
Taste	−0.035** (0.037)	0.167*** (0.000)
For weight loss	0.187*** (0.000)	−0.093*** (0.000)
Longer feeling of fullness	−0.105*** (0.000)	0.137*** (0.000)
Cheap	−0.155*** (0.000)	−0.012 (0.716)
Feeling healthy	−0.122*** (0.000)	0.049** (0.048)
Served at home	−0.360*** (0.000)	0.060*** (0.006)
Other (doctor and dietician advice, low calorie, good for hair and skin)	−0.127** (0.026)	−0.010 (1.000)

*Numbers in parentheses are p-values. ***p < 0.01, **p < 0.05.*

Reason for Not Consuming More

The survey further enquired why people did not consume (more) millets. Overall, the family eating custom at home was the most frequently stated reason by nearly 40% of the respondents. Only Kolkata and Chennai differed in this trend, both citing limited availability as the key reason and its high price as another. It is worth noting that only 3% of the respondents in Bengaluru cited limited availability of millets as a concern, as the state is the largest producer of millets in India. Kolkata and Chennai had smaller production of millets. Chennai was also the place where respondents had the lowest awareness of all three millets.

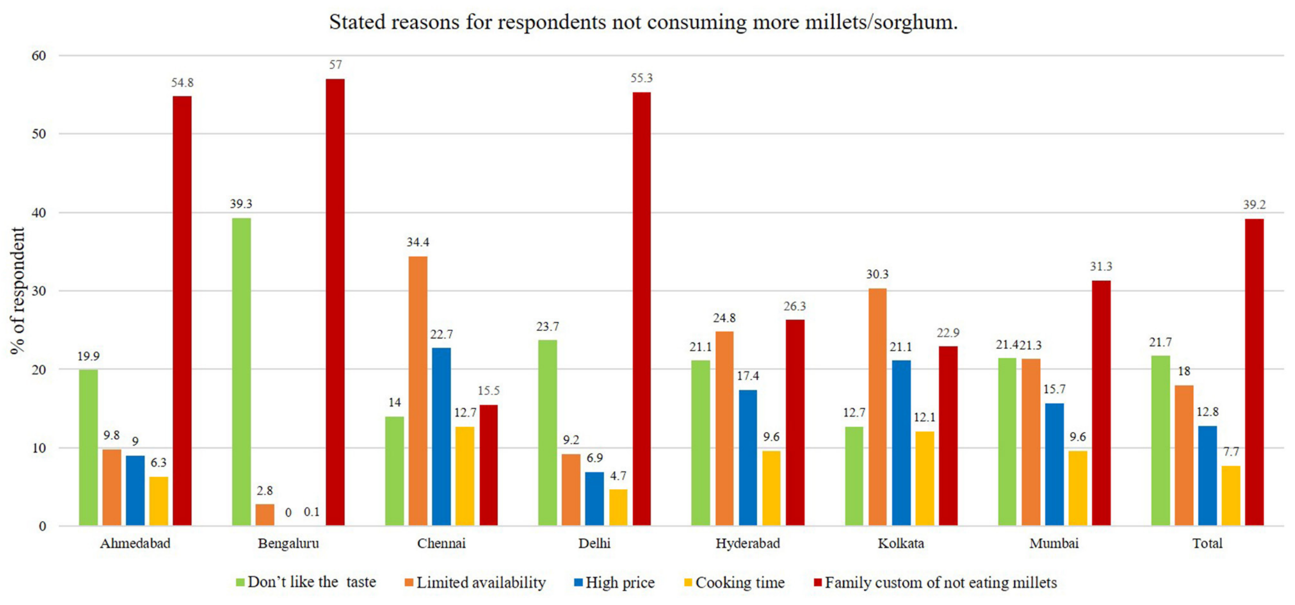

FIGURE 5 | Stated reasons for respondents not consuming more millets/sorghum.

TABLE 5 | Tetrachoric correlation between respondents' reasons for not consuming millets/sorghum and their demographic profile.

Stated reason for not consuming everyday	Gender dummy (female = 1; male = 0)	Low income dummy (low income = 1; otherwise = 0)	Middle class dummy (middle class = 1; otherwise = 0)	High income dummy (high income = 1; otherwise = 0)
Don't like the taste	0.084*** (0.000)	−0.198*** (0.000)	0.134*** (0.000)	−0.035* (0.060)
Limited availability	−0.279*** (0.000)	0.197*** (0.000)	−0.285*** (0.000)	0.153*** (0.000)
High price	−0.046*** (0.008)	0.150*** (0.000)	−0.248*** (0.000)	0.151*** (0.000)
Cooking time	−0.224*** (0.000)	0.181*** (0.000)	−0.152*** (0.000)	0.009 (0.340)
Family custom of not eating millets	0.408*** (0.000)	−0.225*** (0.000)	0.292*** (0.000)	−0.147*** (0.000)

*Numbers in parentheses are p-values. ***p < 0.01, *p < 0.10.*

In Bengaluru, the major reasons cited for not eating (more) millets were their taste and family dietary customs. Pan India, the second major reason for not consuming more millets was the taste, cited by 22% of the respondents. Cooking time of millets was of significance in all the cities (**Figure 5**).

Table 5 shows the tetrachoric correlation between the reasons stated for infrequent consumption of millets and the demographic profile of the respondents. Unexpectedly, women who did not frequently consume millets tended to attribute it to a dislike of the taste and family custom, while men attributed it to the limited availability, high price, and long cooking time. Both low and high income groups showed similar tendencies, whereas the middle income group exhibited a distinct set of dispositions. The latter group, when not consuming millets frequently, attributed it to unfavorable taste and family custom. **Table 6** shows the Spearman's non-parametric correlation between the frequency of consumption and the reasons stated for not increasing the frequency. Those who consumed less frequently were more likely to mention family custom as the reason. In contrast, those who consumed more frequently tended to mention taste, high price, non-availability in markets, and long cooking time as reasons for not increasing the frequency of consumption.

TABLE 6 | Spearman's coefficient of correlation between frequency of consumption and their reason for not consuming more millets and sorghum ($n = 13,840$).

Stated reason for not consuming more	Spearman's rho	*p*-value
Don't like the taste	0.1634	0.000
Limited availability	0.1347	0.000
High price	0.0822	0.000
Long cooking time	0.0836	0.000
Family custom of not eating millets	−0.3442	0.000

Production and Consumption

Table 7 shows the state-level production of millets, as well as their levels of consumption, in the cities studied. Karnataka was the largest producer of these crops, followed by Maharashtra, Gujarat, and Tamil Nadu, confirming the concentration of production in southern states of India. Except in Gujarat, urban consumers in Karnataka and Maharashtra consumed these crops at relatively high frequency, with "once or twice a week" being the median. Despite Gujarat being the largest producer of pearl millet, it exhibited the smallest consumption, clearly showing that its large production had little impact on urban consumption. On the other hand, consumers in Delhi consumed these crops moderately (once a month), but their production was small. In contrast to these cities, Telangana and West Bengal consumed millets frequently despite less production.

Table 8 examines how state-wise per capita production was related to the frequency of consumption of millets through ordered probit regression. The covariates included in the model were the age dummy, gender dummy, and income dummy. The standard errors were clustered at the site level to account for intra-site repetition in the value of production. The results showed no statistically significant relationship between state-wise per capita production and the frequency of consumption in the urban areas, where age, gender, and income factors were controlled for. Lastly, younger people and women consumed millets less frequently, whereas people with low incomes consumed millets more frequently. An investigation of the distributional effects (**Table 9**) to ascertain the marginal effects of state-wise per capita production on different levels of consumption frequency revealed that production and consumption exhibited a U-shaped non-linear relationship, suggesting that older people consumed more millets when the consumption level is in the low range. Likewise, being a woman and having low income was associated with consuming the crops less frequently.

DISCUSSION AND POLICY IMPLICATIONS

Millet campaigns and promotions implemented in the past few years may be the reasons for the higher recognition of millets among the young age group. The individual ability to recognize

TABLE 7 | Production (tons) of millets and sorghum per state population in 2015–2016 and their frequency of consumption.

City	State/union territory	Production of finger millet per state population ('000 tons)	Production of pearl millet per state population ('000 tons)	Production of sorghum per state population ('000 tons)	Production of small millets per state population ('000 tons)	Production of the four crops per state population ('000 tons)	Median frequency of millets/sorghum consumption in the city
Ahmedabad	Gujarat	0.23	11.92	2.09	0.30	14.54	Rare
Bengaluru	Karnataka	18.07	2.21	14.52	0.15	34.95	Frequent
Chennai	Tamil Nadu	3.49	1.82	6.02	0.47	11.80	Frequent
Delhi	Delhi	–	0.21	0.17	–	0.37	Moderate
Hyderabad	Telangana	0.01	0.06	0.85	0.01	0.93	Frequent
Kolkata	West Bengal	0.13	0.00	–	0.02	0.15	Frequent
Mumbai	Maharashtra	0.77	2.75	11.17	0.26	14.94	Frequent

Population data are based on authors' extrapolation of census data Chandramouli, 2011.

TABLE 8 | Frequency of consumption of millets and sorghum and production per state population: ordered probit regression.

Variable	Coeff.	Robust SE	Z-statistic	*p*-value
Log of production per capita (Ln kg)	0.0382	0.0324	1.18	0.237
Age dummy (1 if <30 years = 1; 0 otherwise)	−0.2597***	0.0471	−5.52	0.000
Gender dummy (1 if female = 1; 0 otherwise)	−0.5140***	0.1178	−4.37	0.000
Income dummy (1 if poor; 0 otherwise)	0.2402***	0.0706	3.40	0.001
Cutoff point 1	−0.7047	0.0495		
Cutoff point 2	−0.2865	0.0650		

*Dependent variable: Consumption frequency, where 3 = frequent (1 or more times a week), 2 = moderate (once a month), and 1 = rare (never or up to 2 times). n = 13,840; Wald χ^2 =173.04 (p = 0.000); Log Likelihood = −13,456; Pseudo R^2 = 0.0301. ***p < 0.01; **p < 0.05.*

millets varied across states, in line with the diverse agro-ecologies and contrasting cropping patterns across India (Tsusaka and Otsuka, 2013). Frequent consumers were in Bengaluru, which is not surprising given its reputation for being an advanced city for organic and health foods including millets (Anbukkani et al., 2017). It may be noted that Karnataka state, of which Bengaluru is the capital, was the first Indian state to have a Millet Mission and to launch the annual national millet fair in January 2017, which promoted traditional millets to be brought back as staples and popular modern food.

Although millets can be easily incorporated into almost all popular rice- and wheat-based recipes, one of the reasons for not consuming millets regularly is the lack of knowledge on how to incorporate or cook them. Currently, a few recipes are widely used in communities to cook millets. These include finger millet balls, finger millet porridge, millet chapattis/rotis, and finger millet malt. The relationship between demographic characteristics and the attitude of farm women toward value-added products of finger millet were studied previously (Kowsalya et al., 2017), wherein it was concluded that when farm women were well-trained, there was a favorable attitude toward value addition of finger millet-based products. This, along with the consumer survey results, suggests the need for training on value addition for traditional smart foods, such as millets (Anitha et al., 2019a,b), and that preparing millets in a culturally appropriate manner helps in improving their acceptability. The model for introducing millets in communities and schools in an acceptable manner and how they can change perceptions and influence food preferences in a positive manner have been demonstrated previously (Diama et al., 2020; Wangari et al., 2020). Equally important is understanding the initial knowledge, practices, and individual attitude toward these traditional crops while planning and implementing any nutrition-related interventions using them. A study (Singh and Raghuvanshi, 2012) on knowledge, perceptions, and practices by 111 diabetes individuals reported that 55% of the participants consumed millets because it was a habit from childhood and only 26% of them reported that they ate it because they liked it. However, 11% of the respondents reported that they had no reason to consume millets. The reason for not consuming these grains was either because they were not prepared at their homes (20%) or were not part of their food from childhood (26.6%). Some of them reported that it was advised not to consume them, and 20.6% of them reported that there was no particular reason to not consume them. This shows that if millets were not eaten from childhood, there was no incentive or trigger for consuming them, hence the importance of raising awareness and inculcating good practices from childhood in order to develop a healthy population. However, the current study clearly demonstrated that the major reason for consuming millets was the prevalence of a health issue. A small percentage of people (11.8%) said that it was consumed due to the habit of it being eaten at home; while a significant percentage of people (39.2%) said they did not eat millets because of cultural habits of it not being eaten at home. This highlights the importance of influencing attitudes and practices for millets to become a common food in the

TABLE 9 | Marginal effects of state-wise per capita production of millets and sorghum on log odds of frequency of consumption in the urban areas (n = 13,840).

Frequency of consumption	State-wise per capita production of millets and sorghum		Age dummy (1 if <30 years; 0 otherwise)		Gender dummy (1 if female; 0 otherwise)		Income dummy (1 if poor; 0 otherwise)	
	Marginal effects	*p*-value	Marginal effects	*p*-value	Marginal effects	*p*-value	Marginal effects	*p*-value
Frequent (1 or more times a week)	0.0146 (0.0123)	0.236	−0.0992*** (0.0187)	0.000	−0.1965*** (0.0420)	0.000	0.0918*** (0.0257)	0.000
Moderate (once a month)	−0.0010 (0.0011)	0.350	0.0068 (0.0041)	0.100	0.0135*** (0.0044)	0.002	−0.0063*** (0.0018)	0.000
Rare (never or up to twice a year)	−0.0136 (0.0114)	0.233	0.0925*** (0.0166)	0.000	0.1830*** (0.0458)	0.000	−0.0855*** (0.0270)	0.002

*Wald χ^2 =169.65 (p = 0.000); McFadden's Pseudo R^2 = 0.032. ***p < 0.01, **p < 0.05, *p < 0.10. Numbers in parentheses are robust standard errors.*

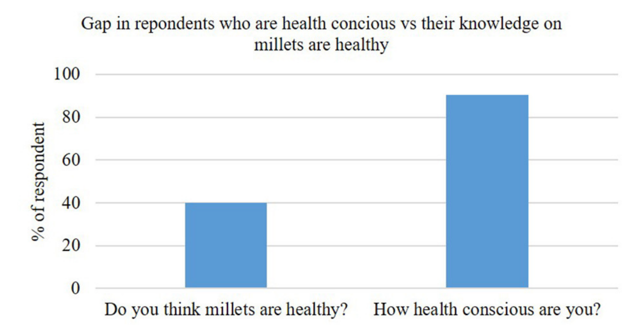

FIGURE 6 | Gap in respondents who are health conscious vs. their knowledge on millets health benefit.

household, resulting in more consumption. The result also shows that targeting the person/people who most influence what is eaten at home can have a multiplier effect on consumption.

In summary, health- and wellness-related issues were by far the major factors influencing consumption of millets in urban areas, with 58% of the interviewees stating so. Consumers with a health issue seemed to be the lowest hanging fruit with regard to market segments that were early adopters of millets, especially women. Consumers wanting to lose weight were also early adopters, again especially among women. Similar results were observed in the previous studies on consumer motives, attitude and purchase preferences for health and organic food products, showing that the health factor was the most important motive behind their selection and purchase (Syah and Yuliati, 2017; Nandi et al., 2016).

Furthermore, a large gap was identified between people who were health conscious (91%) and those who knew that millets were healthy (40%; **Figure 6**). While some health benefits of millets were recognized, such as its role in preventing lifestyle-related non-communicable diseases, there were several other health benefits not well-known previously. A recent systematic review and global meta-analysis of 65 studies on 11 types of millets shows that millets help manage and reduce the risk of developing type 2 diabetes (Anitha et al., 2021a). Another systematic review and meta-analysis of 19 studies shows that long-term consumption of millets can reduce cholesterol, triglyceride, low-density lipoprotein cholesterol (LDL-C), very low-density lipoprotein cholesterol (VLDL-C), high-density lipoprotein cholesterol (HDL-C), body mass index (BMI), and hypertension, which is another piece of evidence of its potential in reducing the risk of developing cardiovascular diseases (Anitha et al., 2021b). Their richness in iron also helps in improving hemoglobin levels and iron status of the body (Anitha et al., 2021c). In particular, finger millet is rich in calcium and zinc, which are essential for growth and immunity. These nutritional and therapeutic effects of millets are vital and provide alternative ways to solve some of the global public health issues. The proportion of people aware of health benefits of millets being 40% implies a large proportion of potential consumers and a significant knowledge gap. This result is also supported by an earlier study in Tamil Nadu that people had little knowledge of millets in comparison with oats (Sreedhar and Shaji, 2017). Learning the actual health and nutritional benefits would increase consumption of millets. It is recognized that reaching the mainstream market may be challenging and would require more than just awareness raising.

However, as family customs strongly influence what people eat at home, influencing the decision makers at households would have a ripple effect on consumption and be a major way to reach male consumers. Taste was observed to be a major reason why the respondents did/did not eat more millets, indicating that health awareness alone would not significantly boost millet consumption. Together, these insights showed the need for tasty products and simple recipes made from millets. As millets are mostly eaten as traditional porridge, there seem to be abundant opportunities to grow the market through ready-to-eat and ready-to-cook convenience foods and cookies, among others.

Overall, it is recognized that the market for millets would expand with appropriate awareness campaigns targeting different segments, especially through social media channels that were found to be the major sources of information on health and food. It would also be important to introduce tasty, modern, and convenient products into markets that are more easily accessible.

The findings of this study may be useful for policymakers as well as different stakeholders, e.g., food companies, government entities, nutritionists, development organizations, and researchers, who intend to promote consumption of millets under various government programs by improving awareness and delivering marketing campaigns. Specific consumer segments should be identified based on the research evidence, while the understanding of consumer awareness and rationale for eating or not eating millets may potentially be valuable inputs to public health debate. In particular, this may be useful for the Government of India Millet Mission, individual state Millet Missions, and the potential upcoming UN International Year of Millets (2023). The information may also help design nutrition behavioral change programs in urban settings.

The shopping centers where the surveys were conducted had good footfalls, with a wide variety of visitors. Nevertheless, the data were geographically localized, and the interviewees resided mainly in major urban cities. Future studies should attempt to obtain similar data on consumption, knowledge, and attitudes at the rural and peri-urban levels to compare various consumer segments and to develop better understanding of millet utilization. Moreover, repeated studies should be conducted to track these changes over time and the influences on changed behavior.

CONCLUSION

Little has been formally studied about urban consumers' knowledge, attitudes, and practices related to millets despite growing health consciousness among people, increasing non-communicable diseases in India, and the nutritional potential of millets. The survey involving over 15,000 face-to-face interviews across seven major cities in India is arguably the largest survey on consumers about millets. A key aim of this study was to understand the motivation of consumers and how best to position millets in any campaigns while planning agriculture-based nutrition interventions to improve the market, consumption, and nutritional status. The findings imply a need to more actively promote the benefits of millets and to create awareness of various ways of cooking millets or creating millet products to satisfy taste preferences and change the perception of millets, which would in turn lead to an increase in their consumption.

DATA AVAILABILITY STATEMENT

The original contributions presented in the study are included in the article/**Supplementary Materials**, further inquiries can be directed to the corresponding author/s.

ETHICS STATEMENT

Ethical review and approval was not required for the study on human participants in accordance with the local legislation and institutional requirements. The participants provided their written informed consent to participate in this study.

AUTHOR CONTRIBUTIONS

JK-P: conceptualization, methodology, investigation, review and editing, and resources. SA, TWT, and RB: validation, formal analysis, data curation, and writing original draft. KM, RH, and MB: methodology, validation, and first analysis. SU, PK, SN, and AJ: writing—review and editing. All authors contributed to the article and approved the submitted version.

FUNDING

This research was funded by Smart Food endowment fund, ICRISAT.

ACKNOWLEDGMENTS

This work was undertaken as part of the Smart Food initiative led by ICRISAT, FARA, CORAF, FANRPAN, and APAARI and coordinated jointly in India with IIMR. The authors are grateful to a range of people for their inputs: the expert panel for reviewing the survey—Ravula Padmaja, Jerome Bossuet, and Carl Hinrichsen (ICRISAT staff/consultants) and Sridhar Iriventi (Founder and Managing Director of Go Bharathi Agro Industries); and Ismail Mohamad (ICRISAT) for generating the map.

SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fsufs.2021.680777/full#supplementary-material

REFERENCES

Anbukkani, P., Balaji, S. J., and Nithyashree, M. L. (2017). Production and consumption of minor millets in India- a structural break analysis. *Ann. Agric. Res*. 38, 1–8.

Anitha, S., Botha, R., Kane-Potaka, J., Givens, D. I., Rajendran, A., Tsusaka, T. W., et al. (2021b). Can millet consumption help to manage hyperlipidaemia and obesity - a systematic review and meta-analysis. *Front. Nutr*. doi: 10.3389/fnut.2021.700778

Anitha, S., Govindaraj, M., and Kane-Potaka, J. (2019a). Balanced amino acid and higher micronutrients in millets complements legumes for improved human dietary nutrition. Cereal Chemistry. 1-11. doi: 10.1002/cche.10227

Anitha, S., Htut, T. T., Tsusaka, W. T., Jalagam, A., and Kane-Potaka, J. (2019b). Potential for smart food products: use of millets and pigeonpea to fill the nutrition gap in rural Myanmar. *J. Sci. Food Agric*. 100, 394–400. doi: 10.1002/jsfa.10067

Anitha, S., Kane-Potaka, J., Botha, R., Givens, D. I., Binti Sulaiman, N. L., Upadhyay, S., et al. (2021c). Millets can have a major impact on improving iron status, haemoglobin level and in reducing iron deficiency anaemia - a systematic review and meta-analysis. *Front. Nutr*. Submitted.

Anitha, S., Kane-Potaka, J., Tsusaka, T. W., Botha, R., Rajendran, A., Givens, D. I., et al. (2021a). A systematic review and meta-analysis of the potential of millets and sorghum for managing and preventing diabetes mellitus. *Front. Nutr*. doi: 10.3389/fnut.2021.687428

Basavaraj, G., Parthasarathy Rao, P., Bhagavatula, S., and Ahmed, W. (2010). Availability and utilization of pearl millet in India. *J. SAT Agric. Res*. 8, 1–7.

Chandramouli, C. (2011). *Census of India 2011*. Office of Registrar General and Census Commissioner. Available online at: http://www.census2011.co.in (accessed February 14, 2020).

Creswell, J. W., and Creswell, J. D. (2017). *Research Design: Qualitative, Quantitative, and Mixed Methods Approaches*. Thousand Oaks, CA: Sage Publications.

Dalvi, M. T., Khairnar, R. M., and Kalghatgi, R. S., (2020). An update of B.G. Prasad and Kuppuswamy Socio economic status classification scale for Indian population. *Indian J. Pediatr*. 87, 567–568. doi: 10.1007/s12098-020-03200-7

Daykin, R. A., and Moffatt, G. P. (2010). Analyzing ordered responses: a review of the Ordered Probit Model. *Understand. Stat*. 1, 157–166. doi: 10.1207/S15328031US0103_02

Diama, A., Anitha, S., Kane-Potaka, J., Htut, T. T., Jalagam, A., Kumar, P., et al. (2020). "How the Smart Food concept can lead to transformation of food systems and combat malnutrition? - different approaches in Africa, globally, and a case study from Myanmar with lessons learnt for creating behavior change in diets," in *Hidden Hunger and the Transformation of Food Systems. How to Combat the Double Burden of Malnutrition?,* ed H. K. Biesalski (Basel: Karger), 149–158. doi: 10.1159/000507494

Edge, M. S., Jones, J. M., and Marquart, L. (2005). A new life for whole grains. *J. Am. Dietet. Assoc*. 105, 1856–1860. doi: 10.1016/j.jada.2005.10.022

Irianto, H. (2015). Consumers' attitude and intention towards organic food purchase: an extension of theory of planned behavior in gender perspective. *Int. J. Manage. Econ. Soc. Sci*. 4, 17–31.

Jager, J., Putnick D. L., and Bornstein M. H. (2017). More than just convenient: the scientific merits of homogeneous convenience samples. *Monogr. Soc. Res. Child Dev*. 82, 13–30. doi: 10.1111/mono.12296

Kane-Potaka, J. (2018). "Breaking the food-system divide with Smart Food: good for you, the planet and the farmer: reshaping agriculture for better nutrition," in *The Agriculture Food Nutrition Health Nexus, Proceedings of the Crawford Fund 2018 Annual Conference* (Canberra, ACT), 13–14. Available online at: https://www.crawfordfund.org/wp-content/uploads/2019/01/2018-Crawford-Fund-Conference-Proceedings.pdf

Kane-Potaka, J., and Kumar, P. (2019). "Smart food - food that is good for you, the planet and the farmer," in *State of India's Livelihoods Report 2019* (New Delhi: Access Development Services), 71–82. Available online at: https://livelihoods-india.org/publications/all-page-soil-report.html#; www.smartfood.org/wp-content/uploads/2020/05/SOIL-Smart-Foods.pdf

Kowsalya, K. S., Lalitha, K. C., Preethi., and Ahmed, T. (2017). Impact of demographic characteristics on attitude of farm women towards value added products of ragi. *Int. J. Curr. Microbiol. Appl. Sci*. 6, 1188–1194. doi: 10.20546/ijcmas.2017.603.138

Longvah, T., Ananthan, R., Bhaskarachary, K., and Venkaiah, K., (2017). *Indian Food Composition Table*. Hyderabad: National Institute of Nutrition.

Nambiar, S. V., and Patwardhan, T. (2014). Millets in diabetes - Emic Views. *Int. J. Pure Appl. Biosci*. 2, 89–97.

Nandi, R., Bokelmann, W., Gowdru, N. V., and Dias, G. (2016). Consumer motives and purchase preferences for organic food products: empirical evidence from a consumer survey in Bangalore, South India. *J. Int. Food Agribus. Market.* 28, 74–99, doi: 10.1080/08974438.2015.1035470

Parthasarathy Rao, P., Basavaraj, G., Ahmad, W., and Bhagavatula, S. (2010). An analysis of availability and utilization of sorghum grain in India. *J. SAT Agric. Res.* 8, 1–9.

Radhika, G., Van, D. M. R., Sudha, V., Ganesan, A., and Mohan, V. (2009). Refined grain consumption and the metabolic syndrome in urban Asian Indians (Chennai Urban Rural Epidemiology Study 57). *Metab. Clin. Exp*. 58, 675–681. doi: 10.1016/j.metabol.2009.01.008

Roche, E., Conner, D., Kolodinsky, M. J., Buckwalter, E., Berlin, L., and Powers, A. (2012). Social cognitive theory as a framework for considering farm to school programming. *Childhood Obes.* 8, 357–363. doi: 10.1089/chi.2012.0035

Rooney, L. W. (2010). "Virtues of sorghum: utilization and supply chain management," in *INTSORMIL Presentations*. Available online at: https://digitalcommons.unl.edu/intsormilpresent/28

Saleh, S. M. A., Zhang, Q., Chen, J., and Shen, Q. (2013). Millet grains: nutritional quality, processing, and potential health benefits. *Comprehens. Rev. Food Sci. Food Saf*. 12, 281–295. doi: 10.1111/1541-4337.12012

Singh, P., and Raghuvanshi, R. S. (2012). Finger millet for food and nutritional security. *Afr. J. Food Sci*. 6, 77–84. doi: 10.5897/AJFSX10.010

Sreedhar, S. K. P., and Shaji, S. (2017). Comparative KAP study on ragi, a forgotten wonder grain with oats among South Indian families. *Curr. Res. Diab. Obes. J*. 3, 1–4. doi: 10.19080/CRDOJ.2017.03.555601

StataCorp. (2019). *Stata Statistical Software: Release 16*. College Station, TX: StataCorp, L. L. C.

Syah, A., and Yuliati, L. N. (2017). The influence of values and attitude toward healthy food selection at student of Bogor Agricultural University. *J. Consum. Sci*. 2, 57–65. doi: 10.29244/jcs.2.2.57-65

Tsusaka, W. T., and Otsuka, K. (2013). "The declining impacts of climate on crop yields during the Green Revolution in India: 1972 to 2002," in *An African Green Revolution: Finding Ways to Boost Productivity on Small Farms,* eds K. Otsuka and D. F. D. Larson (dordrecht: Springer), 71–94. doi: 10.1007/978-94-007-5760-8_4

Umanath, M., Balasubramaniam, R., and Paramasivam, R., (2018). Millets consumption probability and demand in india an application of heckman sample selection model. *Econ. Affairs* 63, 1033–1044. doi: 10.30954/0424-2513.4.2018.29

Ushakumari, R. S., Latha, S., and Malleshi, G. N. (2004). The functional properties of popped, flaked, extruded and roller-dried foxtail millet (*Setaria italica*). *Int. J. Food Sci. Technol.* 39, 907–915. doi: 10.1111/j.1365-2621.2004.00850.x

Vetriventhan, M., Azevedo, V. C. R., Upadhyaya, H. D., Kane-Potaka, J., Anitha, S., Prabhakar Govindaraj, M., et al. (2020). Genetic and genomic resources, and breeding for accelerating improvement of small millets: current status and future interventions. *Nucleus* 63, 217–239. doi: 10.1007/s13237-020-00322-3

Wangari, C., Mwema, C., Siambi, M., Silim, S., Ubwe, R., Malesi, K., et al. (2020). Changing perception through a participatory approach by involving adolescent school children in evaluating Smart Food dishes in school feeding programs real-time experience from Central and Northern Tanzania. *Ecol. Food Nutr*. 59, 472–485. doi: 10.1080/03670244.2020.1745788

Willett, W., Rockström, J., Loken, B., Springmann, M., Lang, T., Vermeulen, S., et al. (2021). Food in the Anthropocene: the EAT-Lancet Commission on healthy diets from sustainable food systems. *Lancet Commiss*. 393, 447–492. doi: 10.1016/S0140-6736(18)31788-4

Conflict of Interest: KM was employed by company SR Global Media and Research Pvt. Ltd. RH was employed by Thought Folks Digital.

The remaining authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's Note: All claims expressed in this article are solely those of the authors and do not necessarily represent those of their affiliated organizations, or those of the publisher, the editors and the reviewers. Any product that may be evaluated in this article, or claim that may be made by its manufacturer, is not guaranteed or endorsed by the publisher.

frontiers in Nutrition

SYSTEMATIC REVIEW
published: 17 August 2021
doi: 10.3389/fnut.2021.700778

Can Millet Consumption Help Manage Hyperlipidemia and Obesity?: A Systematic Review and Meta-Analysis

Seetha Anitha[1], Rosemary Botha[2], Joanna Kane-Potaka[1], D. Ian Givens[3], Ananthan Rajendran[4], Takuji W. Tsusaka[5] and Raj Kumar Bhandari[6]*

[1] Smart Food Initiative, International Crops Research Institute for the Semi-Arid Tropics (ICRISAT), Patancheru, India, [2] Development Strategy and Governance Division, International Food Policy Research Institute (IFPRI), Lilongwe, Malawi, [3] Institute of Food, Nutrition and Health, University of Reading, Reading, United Kingdom, [4] Food Chemistry Division, National Institute of Nutrition (NIN), Hyderabad, India, [5] Organization for Advanced and Integrated Research, Kobe University, Kobe, Japan, [6] National Technical Board of Nutrition, Government of India (GoI), New Delhi, India

OPEN ACCESS

Edited by:
Kathleen L. Hefferon,
Cornell University, United States

Reviewed by:
Sapna Langyan,
National Bureau of Plant Genetic Resources (ICAR), India
Somnath Mandal,
Uttar Banga Krishi Viswavidyalaya, India

***Correspondence:**
Seetha Anitha
s.anitha@cgiar.org;
dr.anithaseetha@gmail.com

Specialty section:
This article was submitted to Nutrition and Sustainable Diets, a section of the journal Frontiers in Nutrition

Received: 26 April 2021
Accepted: 05 July 2021
Published: 17 August 2021

Citation:
Anitha S, Botha R, Kane-Potaka J, Givens DI, Rajendran A, Tsusaka TW and Bhandari RK (2021) Can Millet Consumption Help Manage Hyperlipidemia and Obesity?: A Systematic Review and Meta-Analysis. Front. Nutr. 8:700778. doi: 10.3389/fnut.2021.700778

Many health benefits of millets (defined broadly to also include sorghum) have been advocated, including their roles in managing and preventing diabetes; however, the effects of millets on hyperlipidemia (high lipid levels) have been underrecognized. A systematic review and meta-analysis were conducted to collate available evidence of the impacts of millets consumption on lipid profile, namely total cholesterol (TC), triacylglycerol, high-density lipoprotein cholesterol (HDL-C), low-density lipoprotein cholesterol (LDL-C), and very-low–density lipoprotein cholesterol (VLDL-C). The results from 19 studies showed that the consumption of millets for periods as short as 21 days to 4 months reduced levels of TC, triacylglycerol, LDL-C, and VLDL-C ($p<0.01$) by 8.0, 9.5, 10 and 9.0%, respectively. Four studies demonstrated that millets consumption brought TC and triacylglycerol levels to the normal levels (<200 and <150 mg/dl, respectively). Furthermore, upon consumption of millet-based meals, there was a 6.0% increase in the HDL-C 4.0 and 5.0% reduction in systolic and diastolic blood pressure, and 7.0% reduction in body mass index (BMI). This evidence, leads us to conclude that consumption of millets reduces hyperlipidemia and hence hypertension, and raises the levels of HDL-C (good cholesterol), which can be beneficial for managing the associated risk of developing hypertension and atherosclerotic cardiovascular diseases in future.

Systematic Review Registration: The protocol of this systematic review has been registered in the online registration platform called "research registry" with the unique identification number "reviewregistry1123."

Keywords: millets, hyperlipidemia, cholesterol, triacylglycerol, lipid profile

INTRODUCTION

Cardiovascular disease (CVD) is one of the leading causes of morbidity and mortality globally, accounting for 30% of deaths (1) worldwide, in which blood lipid profile plays a major role. Elevated levels of total cholesterol (TC), low-density lipoprotein cholesterol (LDL-C), very-low–density lipoprotein-cholesterol (VLDL-C), and triacylglycerol lead to progression of CVD and an increase of mortality. Among these, LDL-C is a major modifiable risk factor for atherosclerotic CVD (2), whereas, some types of the high-density lipoprotein cholesterol (HDL-C) are considered "good" cholesterol. Therefore, it is important to look at the ratio of TC to HDL-C in assessing the risk of CVD (3, 4). Along with lifestyle management, it is also vital to focus on a diet that can lower the risk of CVD by regulating metabolism and managing the lipid profile.

In developing countries, cereals typically occupy a major portion of the nutritionally unbalanced plate, which tends to be dominated by milled rice, maize, and refined wheat providing readily available carbohydrates. In addition, the so-called "junk food" and other unhealthy foods made from refined flour and fatty ingredients introduce large amounts of saturated fats into the body which, combined with sedentary lifestyles, can worsen the health of individuals. Lipid metabolism can be severely affected in individuals who are pre-diabetic, have type 2 diabetes mellitus, or other metabolic syndromes. Therefore, diet-based interventions for diabetes and other metabolic syndromes should contain ingredients that have a low glycemic index (GI) and the potential to correct metabolic abnormalities, such as deleterious lipid metabolism.

Millets are recognized as smart foods (5) as they fulfill the criteria of being "good for you," "good for the planet," and "good for the farmer," Millets are traditional nutritious staple foods which can diversify staples and the overall diet when introduced, thereby playing a key role in controlling the levels of lipids in blood, managing metabolic disorders, such as diabetes and hyperlipidemia, and reducing the potential risk of developing CVD.

A recent systematic review and meta-analysis of low GI millets (including sorghum) and their effects on managing and reducing the risk of developing type 2 diabetes shows that millets have a beneficial effect on various outcomes, such as the fasting and post-prandial blood glucose levels, insulin index, and glycated hemoglobin (HbA1c) marker levels (6). This ability to manage and prevent diabetes may also help toward the management of hyperlipidemia. However, further, studies are needed to understand the effects of the consumption of millets on blood lipid management. This systematic review and meta-analysis are aimed at collating science-based evidence from randomized controlled trials and self-controlled clinical trials conducted through dietary intervention and cross-sectional studies on use of millets and their effects on lipid profile.

Review question: Does consumption of millet(s)-based foods help in managing blood lipid profile compared with regular, non-millet diets?

METHODS

Study Period

The systematic review and meta-analysis were conducted from October 2017 to March 2021. The protocol of this systematic review has been registered in the online registration platform called "research registry" with the unique identification number "reviewregistry1123."

Information Sources

The study used the 27-item Preferred Reporting Items for Systematic Reviews and Meta-analyses (PRISMA) checklist (7) for every step of data collection, extraction, and analysis.

TABLE 1 | Search strategy and keywords used to identify relevant papers.

Number	Criteria and keywords used for search
1	Boolean logic such as "AND," "OR," "NOT" were used.
2	Efficacy of millets on lipid profile. Replaced the word "millets" with the names of millets, such as "barnyard millet," "foxtail millet," "finger millet," "pearl millet," "proso millet," "brown top millet," "little millet," "kodo millet," "teff," "job's tears," "fonio"
3	Impact of consuming millets on lipid profile
4.	Efficacy of millets on total triacylglycerol levels in humans. Replaced the word "total triacylglycerol" with "cholesterol," "LDL-C," "HDL-C," "VLDL-C"

Only studies that were published in the English language were considered. The scoping study was conducted using PubMed and MEDLINE to check for any existing studies on this topic and whether there were any overlaps with the research question as per the guidelines of (8). Search engines including Google Scholar, Scopus, Web of Science, PubMed, and CAB abstract were used to identify studies relevant to the research question. Search results obtained through a search strategy and keywords (**Table 1**) were further screened for relevance of the study, completeness of information, and quality of research, based on inclusion and exclusion criteria. If, after collecting the full papers, any required data were missing, the authors of the papers were contacted and complete information was requested for use in the meta-analysis.

Inclusion Criteria

The PRISMA flow diagram (**Figure 1**) shows the steps involved in the inclusion and exclusion of the studies. The articles published between 2011 and the first quarter of 2021, which fulfilled the criteria of being (1) Randomized controlled trials, self-controlled clinical trials, and/or cross-sectional studies conducted to test the efficacy of millets on TC, triacylglycerols, LDL-C, VLDL-C, and/or HDL-C; (2) studies that had information on any or all of the outcomes, including levels of TC, triacylglycerol, LDL-C, VLDL-C, and HDL-C; (3) studies on any age group or gender of any population to test the efficacy of millets on blood lipid profile; (4) studies that assessed the effects of millets on blood lipid profile along with or without weight, BMI, and systolic and/or diastolic blood pressure; and (5) only peer-reviewed journal articles were selected and included in the current systematic review.

Exclusion Criteria

(1) Review articles were excluded from further consideration. (2) Animal studies were excluded. (3) In the case of incomplete data, the authors were contacted. If complete data were still not accessed, the study was excluded.

Data Extraction

Each study was labeled with author details and year. The age group and gender of the participants were recorded along with the country, study method, sample size, type, and form of millets studied. The numerical variables considered for analysis included mean TC level, triacylglycerol, VLDL-C, LDL-C, and HDL-C

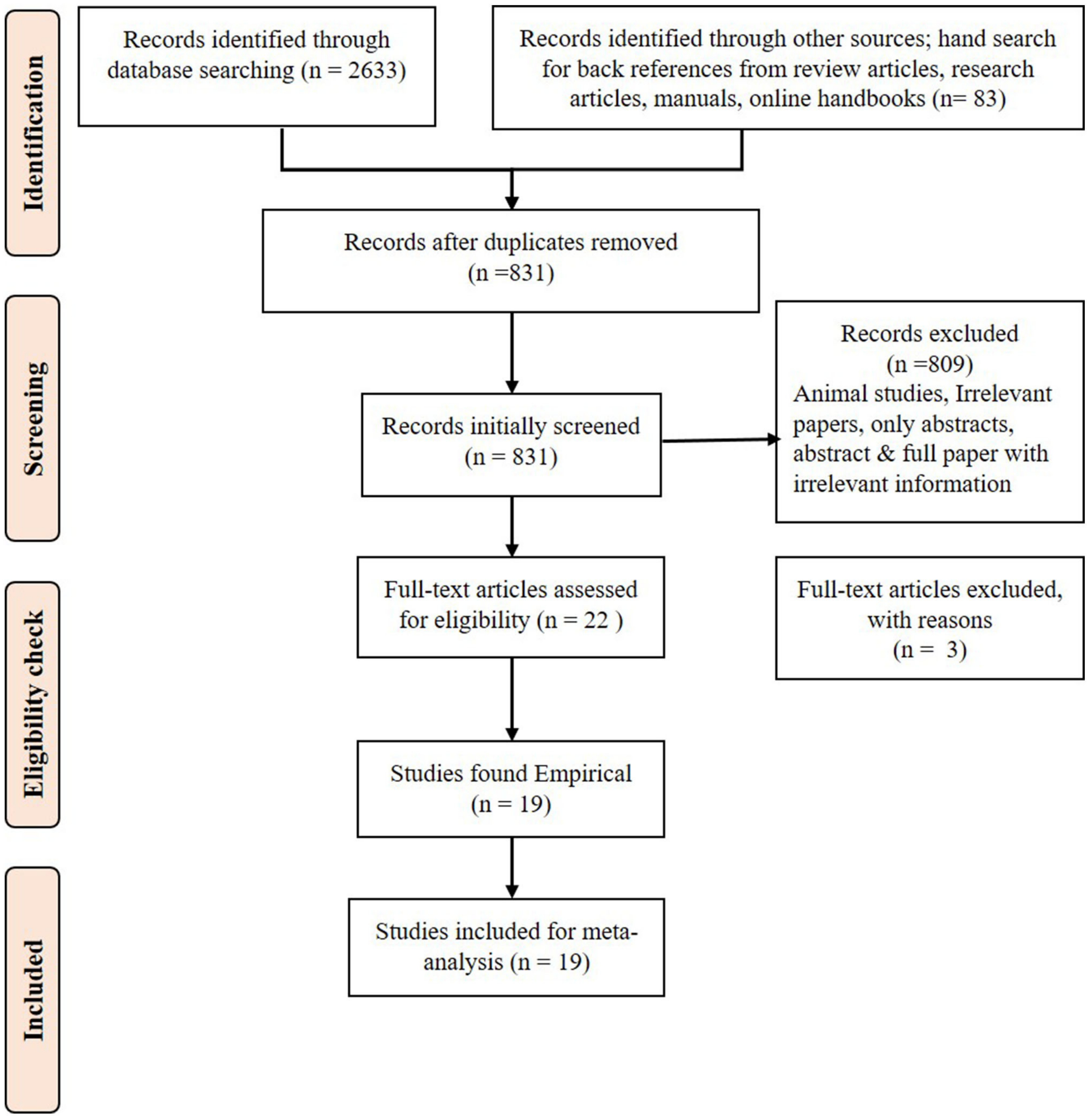

FIGURE 1 | PRISMA flow diagram for systematic review.

in mg/dl, and weight gain or loss in kg. Systolic and diastolic blood pressure was recorded in mmHg. The data were then entered into Excel spread sheets as per guidelines provided by Harrer et al. (9). If standard error was recorded, it was converted to standard deviation (SD). Similarly, if the data were presented in different units, they were converted to the same unit to maintain uniformity. For example, cholesterol, and triacylglycerol concentrations given in mmol/L were converted to mg/dl to ensure the same unit of measurement is used for all the measurements.

Quality Assessment of the Studies

Using the eight-item Newcastle–Ottawa Scale (10, 11), the quality of each study was assessed by two investigators, and any disagreement was resolved by discussing it with a third reviewer.

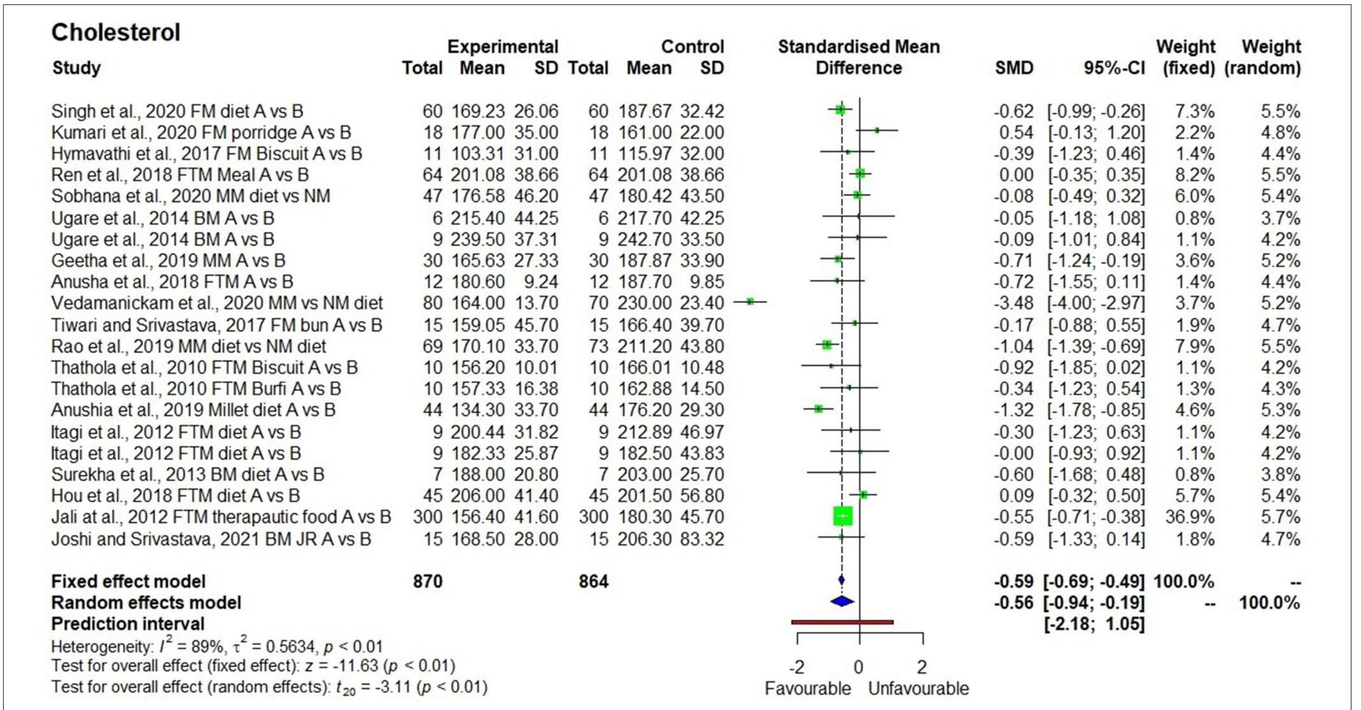

FIGURE 2 | Effect of consuming millets on total cholesterol level (pre- vs. post-treatment or intervention vs. control diet).

The researchers also applied the principle of Bell et al. (12) to further strengthen the quality assessment.

Risk of Bias Assessment

A funnel plot was used to assess publication bias if any. Other biases such as selection bias, detection bias, attrition bias, and reporting bias were assessed using the guidelines provided in Cochrane Handbook online version 6.2, (2021) for systematic reviews of interventions (13, 14).

Summary Measures and Result Synthesis

Groups of individuals who were fed with millet-based meals for certain study periods (28 days to 4 months) were considered as intervention groups. The initial baseline measurement taken on these individuals was considered as the control measurement or pre-intervention measures, which consisted of regular rice- and wheat-based diets. Therefore, the before and after effects on primary outcomes, such as HDL-C, VLDL-C, LDL-C, TC, and triacylglycerol levels, and secondary outcomes such as weight, BMI, and systolic and diastolic blood pressure were included in the meta-analysis to measure the standard mean difference (SMD) and heterogeneity (I^2). The significance of the results was determined using the fixed effect model for small samples, and/or single-source information with low heterogeneity among studies, and the random effect model for other cases (15, 16) and a p-value <0.05 indicates the significance of the effect. Results of both the fixed effect and random effect models were captured in each forest plot. A meta-analysis was conducted using the software R Studio version 4.0.4 (2021) to obtain forest plot along with heterogeneity (I^2) and the overall test effect in the fixed effect and random effect models and funnel plots to determine publication bias (9, 17).

Descriptive statistics results such as mean, SD, and a percentage increase or decrease in TC, LDL-C, VLDL-C, HDL-C, triacylglycerol, weight, BMI, and systolic and diastolic blood pressure were calculated for both the intervention samples and control samples.

Subgroup Analysis

Subgroup analysis was conducted for different types of millets used in the study.

RESULTS

Finger millet, foxtail millet, barnyard millet, and/or a mixture of millets (finger millet and little millet) were used in the 19 studies (18–36) that were included in one or more outcomes of meta-analysis. It was observed that consumption of millet-based food for a duration varying from 21 days to 4 months had a significant reducing effect on TC, triacylglycerol, VLDL-C, and/or LDL-C levels. Two cross-sectional studies included in the systematic review documented millet consumption information for up to 2 years. The consumption of millets was either in the form of biscuits, burfi (sweet), porridge, buns, boiled with water (similar to rice), roti (flatbread), dumpling, or upma. The amount of millets provided to the intervention groups varied from 50 to 200 g (dry weight basis) either in one meal or divided into two meals per day.

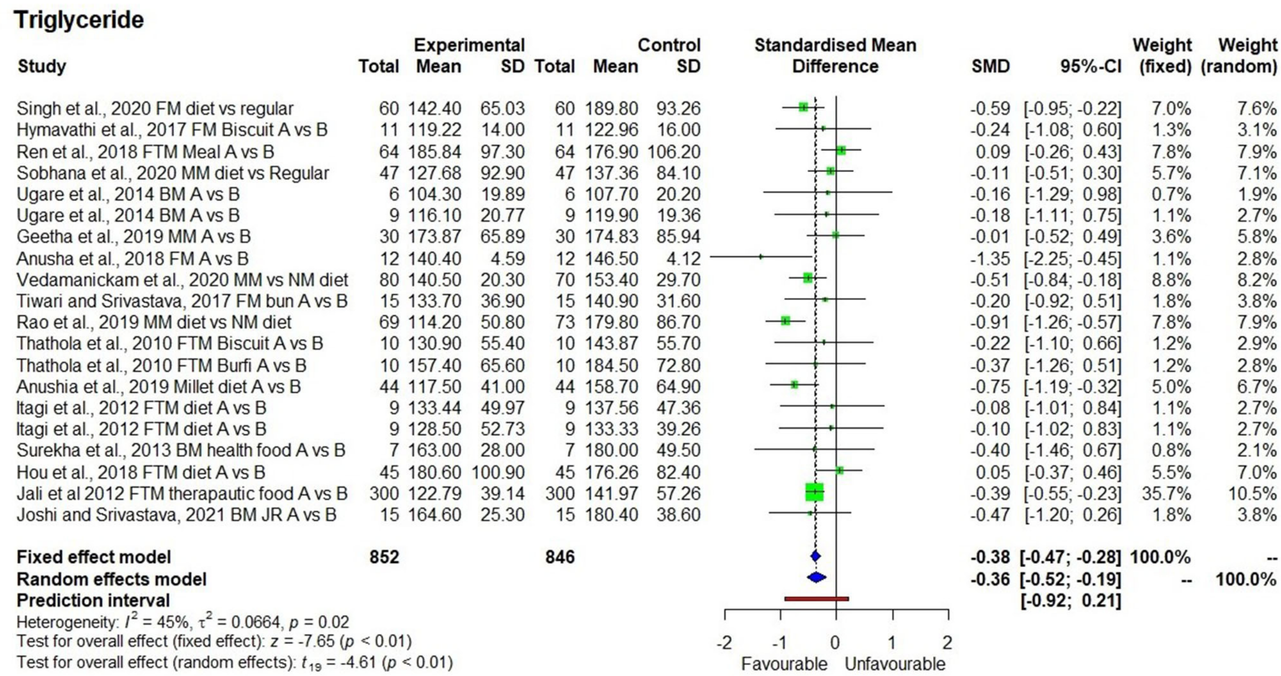

FIGURE 3 | Effect of consuming millets on triacylglycerol level (pre- vs. post-treatment or intervention vs. control diet).

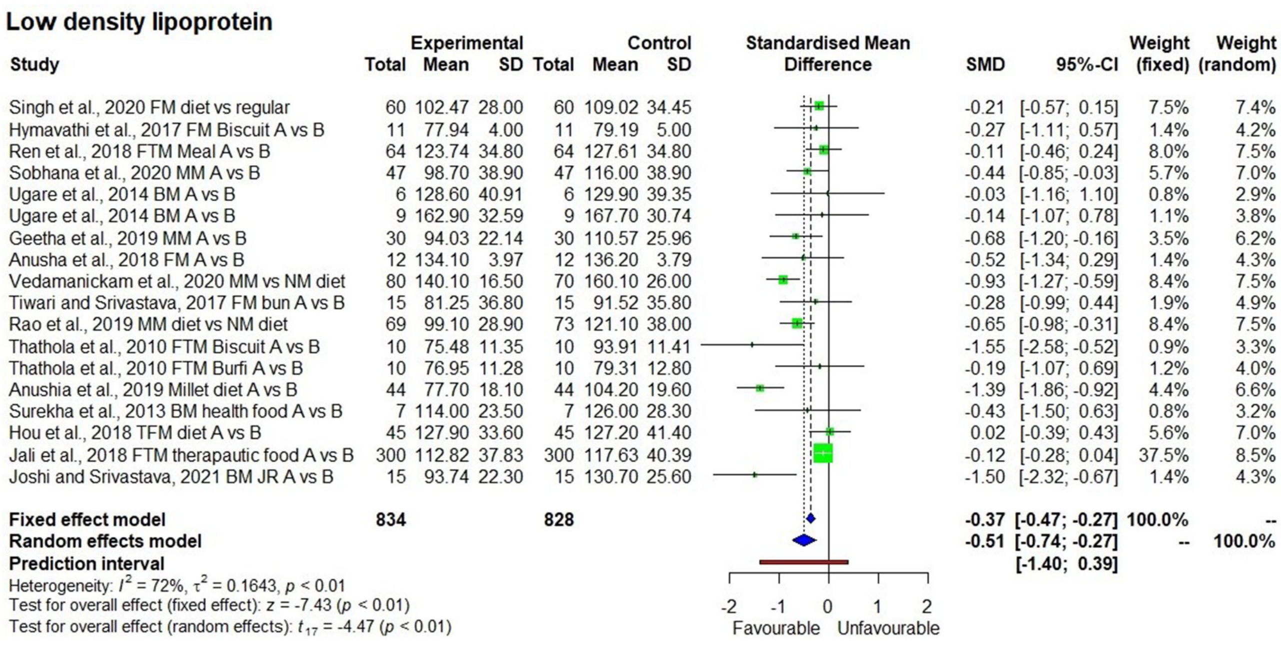

FIGURE 4 | Effect of consuming millets on low-density lipoprotein-cholesterol (LDL-C) level (pre- vs. post-treatment or intervention vs. control diet).

The meta-analysis conducted on an outcomes-generated forest plot shows a significant reducing effect in TC levels after the consumption of a millet-based diet for 21 days to 4 months ($p = 0.01$) with SMD of −0.56, 95% class interval of −0.94 to −0.19, and I^2 value of 89% confirming the heterogeneity (**Figure 2**). Descriptive statistics conducted for 870 samples, and 21 observations from the 18 studies showed that the TC levels decreased by 8% from 189.5 ± 27.7 to 174.8 ± 28.9 mg/dl. Three

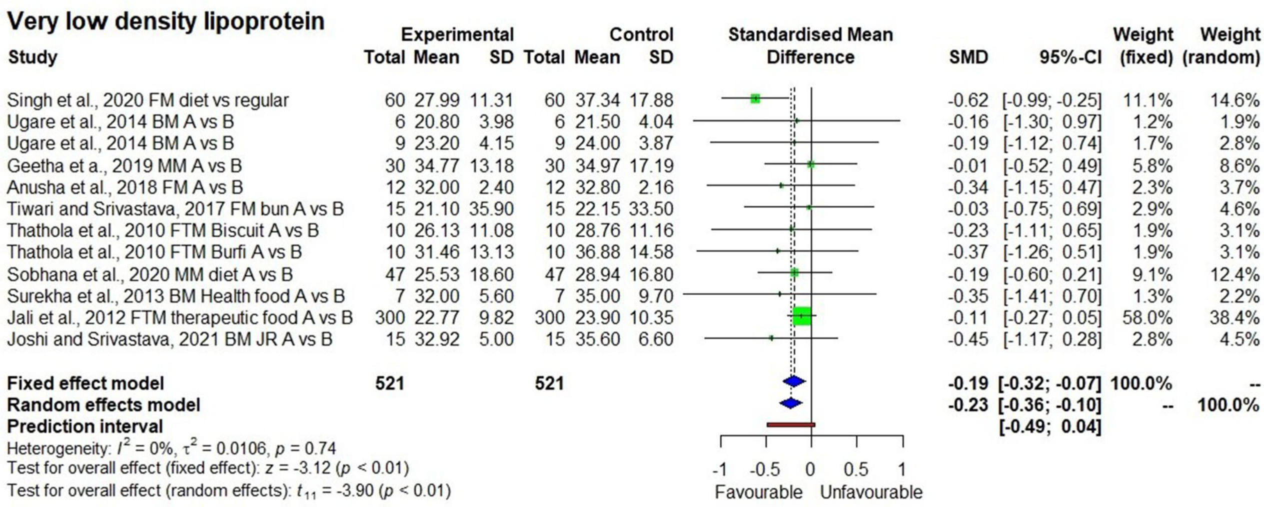

FIGURE 5 | Effect of consuming millets on very-low–density lipoprotein-cholesterol (VLDL-C) level (pre- vs. post-treatment or intervention vs. control diet).

studies demonstrated the reduction in TC levels from 215.8 ± 12.5 mg/dl (>200 mg/dl hence hypercholesterolemia) to 167.5 ± 3.2 mg/dl (<200 mg/dl hence normal).

The consumption of millets for a period of 21 days to 2 years had a significant reducing effect on ($p < 0.01$) triacylglycerol levels with SMD of −0.38 and 95% confidence interval (CI) of −0.47 to −0.28. As the heterogeneity was low ($I^2 = 45\%$), the fixed effect model was used to interpret the results (**Figure 3**). Nonetheless, in both the fixed effect and random effect models, the effects were significant ($p < 0.01$). Descriptive statistics for 852 samples and 20 observations from the 17 studies showed a decrease in triacylglycerol levels by 9.5% from 154.3 ± 24.5 to 139.8 ± 23.5 mg/dl. There were four studies that demonstrated the reduction in hypertriacylglycerolemia (>150 mg/dl) to normal triacylglycerol levels (<150 mg/dl) after consumption of millets for 3 months with a mean reduction from 170.4 ± 17.2 to 128.6 ± 14.9 mg/dl (24.5% reduction) (18, 26, 28, 30).

The consumption of millet-based diets for long periods of time also had a significant reducing effect ($p < 0.01$) on LDL-C levels with I^2 value of 72% (moderate heterogeneity among studies) with SMD of −0.51 and 95% CI of −0.74 to −0.27 (**Figure 4**). The LDL-C levels from 834 samples in 18 observations from the 16 studies were 118.2 ± 23.9 mg/dl, compared with 106.7 ± 25.4 mg/dl at the baseline (10% reduction). Particularly, five studies demonstrated the reduction in LDL-C from a moderately elevated level of 116.5 ± 10.0 to a normal level of 92.6 ± 8.7 mg/dl. One long-term cross-sectional study showed a reduction from the high level of LDL-C (160.1 ± 26 mg/dl) to the moderately high level (140.1 ± 16.5 mg/dl).

For VLDL-C meta-analysis, I^2 value was 0% indicating no heterogeneity between the studies. However, both the fixed effect and random effect models showed a significant reducing effect in VLDL-C levels with $p<0.01$ (**Figure 5**). It is evident from 521 samples, 12 observations of the 10 studies that VLDL-C levels decreased by 9.0% from 30.2 ± 6.0 to 27.5 ± 4.9 mg/dl.

In contrast to other outcomes, the HDL-C levels (**Figure 6**) had a significant increasing effect after consumption of millet-based foods for a long period ($p < 0.01$) with SMD of 0.32, 95% CI of 0.12–0.52. Descriptive statistics showed that HDL-C levels slightly increased (6.0%) from 43.4 ± 7.5 to 46.0 ± 7.6 as found in 763 samples in 18 observations from the 15 studies.

The ratio of TC to HDL-C was kept below 5 (ideal) by consuming barnyard millet–based meals for 28 days to two months (32, 35), where the TC:HDL-C ratio was reduced from 5.1 ± 0.14 to 4.2 ± 0.26.

Although, descriptive statistics showed a mean reduction in weight by 1.5 ± 0.4 kg among 208 study participants who consumed the millet-based diet, the effect on reduction was not statistically significant in a meta-analysis ($p = 0.15$). There were only four studies that measured systolic blood pressure, which showed a significantly reducing effect after consuming a millet-based diet ($p < 0.01$). Diastolic blood pressure showed a significantly reducing effect after consumption of millet-based diet ($p < 0.01$). BMI had significantly reducing effect by 7.0% after consumption of millet-based diets for 3–4 months ($p < 0.05$) with no heterogeneity observed among the studies ($I^2 = 0\%$) and 95% CI of −0.34 to −0.0.02 (**Figures 7–10**).

Publication bias was assessed using the funnel plot, and the observed asymmetry was adjusted using the trim and fit model to account for the small sample size effect until symmetry was achieved ($p < 0.0001$).

DISCUSSION

Hypertension, hyperlipidemia, diabetes, and smoking are the main risk factors for atherosclerotic CVD (37, 38). A recent systematic review on the effects of millets in managing and reducing the risk of developing type 2 diabetes showed that millets have beneficial effects on diabetes by providing low GI meals, reducing fasting and post prandial blood glucose levels

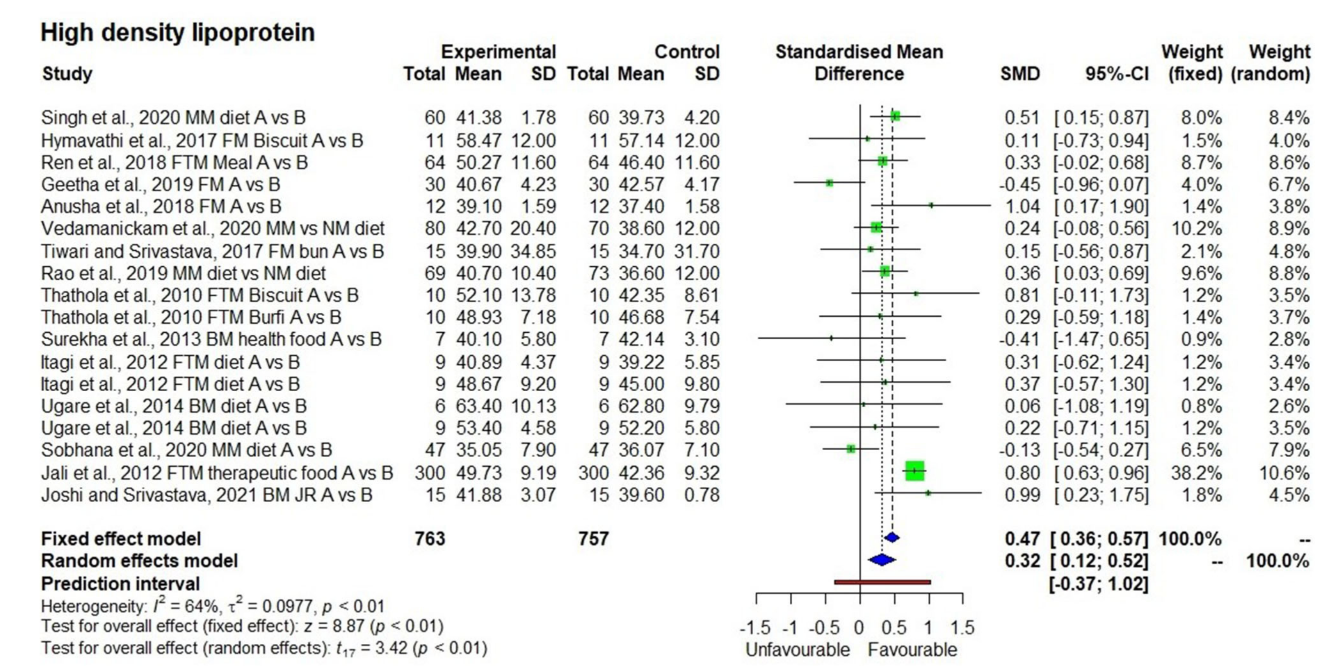

FIGURE 6 | Effect of consuming millets on high-density lipoprotein-cholesterol (HDL-C) level (pre- vs. post-treatment or intervention vs. control diet).

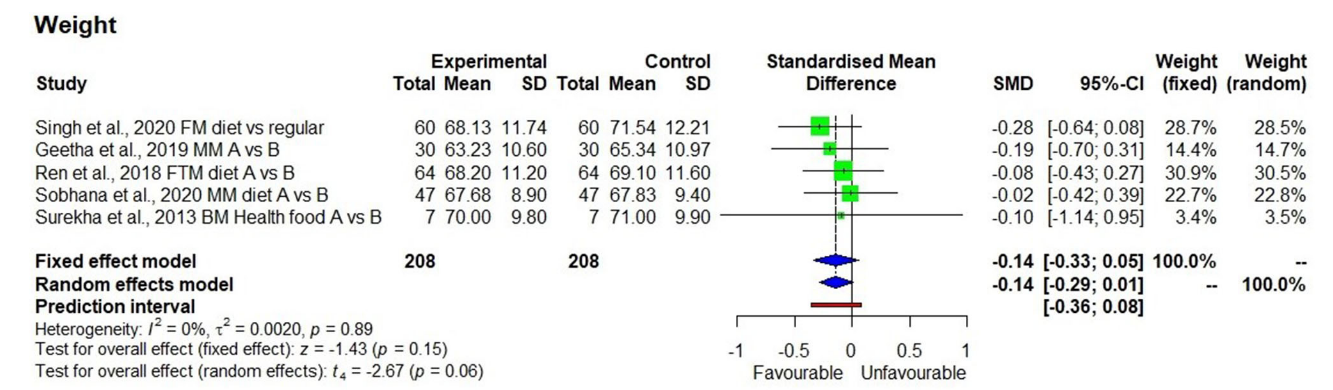

FIGURE 7 | Effect of consuming millets on weight of the participants (pre- vs. post-treatment or intervention vs. control diet).

as well as the glycated hemoglobin level (HbA1c) (6). The current systematic review focused on the effect on blood lipids as another important beneficial outcome of millet consumption. The meta-analysis showed that the ingestion of low (46.7 ± 12.0%) GI millet-based food had significant reductions in the levels of TC, triacylglycerol, LDL-C, and VLDL-C. Among other factors, the levels of glucose (and other simple carbohydrates) and saturated fats, and inappropriately controlled diabetes and metabolic syndromes are the causes of hyperlipidemia (37). Millets, being a low-GI food (6), reduce the blood glucose available for the synthesis of triacylglycerols (27). Moreover, millets also reduced VLDL-cholesterol which is a carrier of triacylglycerol in plasma, thereby it further reduced the triacylglycerol levels. This implies that millets play a key role in reducing triacylglycerol levels.

Overall, the 17 studies demonstrated an average of 9.5% reduction in triacylglycerol levels, and particularly four of the studies (18, 26, 28, 30) demonstrated that the plasma triacylglycerol levels reduced from a hypertriglyceridemic (>150 mg/dl) condition to normal (<150 mg/dl) when a millet-based meal was consumed once a day for 3 months instead of the regular rice- and/or wheat-based diets. Although, two cross-sectional studies that recorded more than 2 years of millet consumption showed that there was a reduction (26, 28), further, long-term randomized controlled trials would be necessary to confirm this.

LDL-C is considered as a major risk factor for CVD (39). Even with medication, residual LDL-C is associated with the risk of CVD. In the current study, consuming millets was shown to significantly reduce the LDL-C

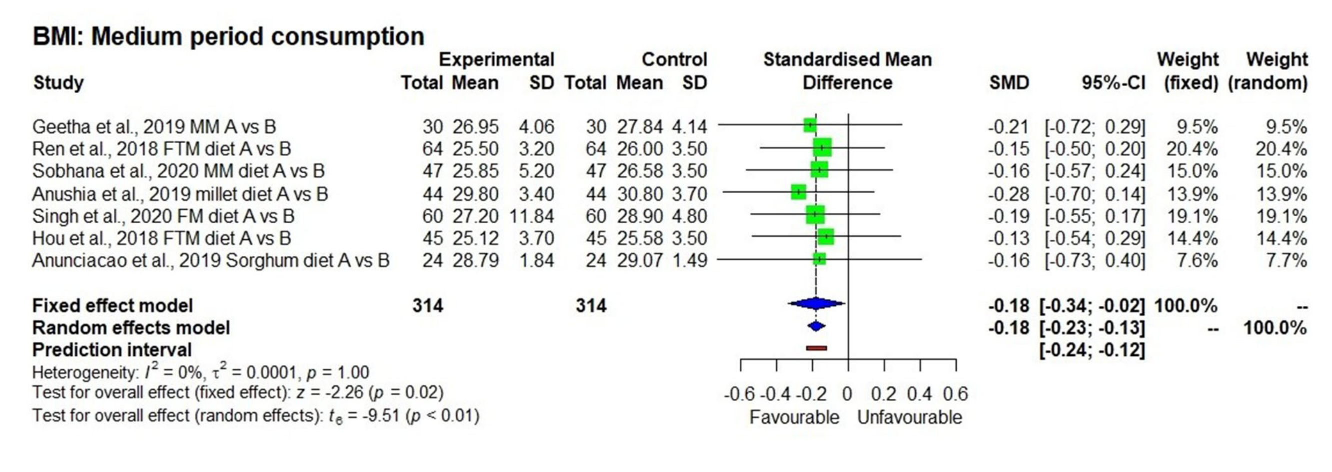

FIGURE 8 | Effect of consuming millets on BMI of the participants (pre- vs. post-treatment).

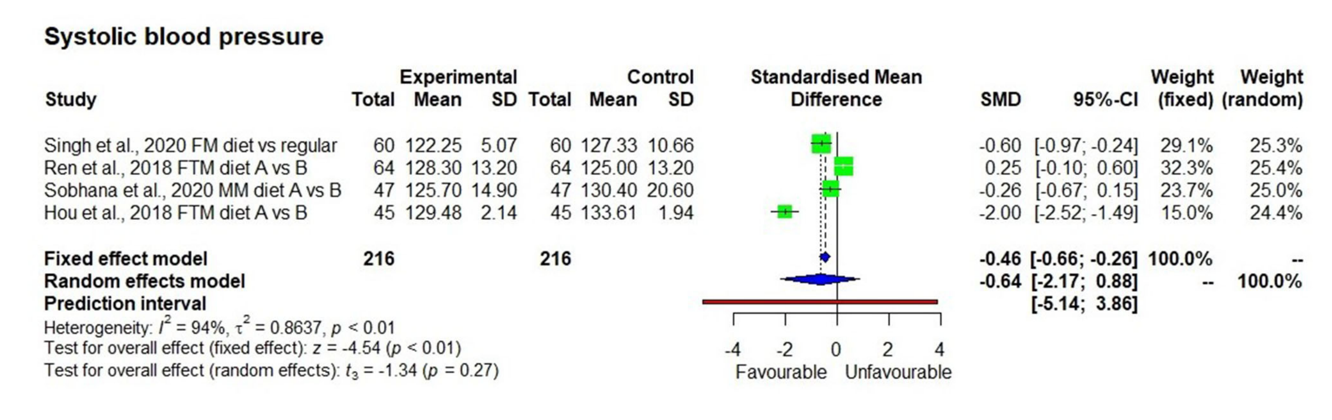

FIGURE 9 | Effect of consuming millets on systolic blood pressure of the participants (pre- vs. post-treatment or intervention vs. control diet).

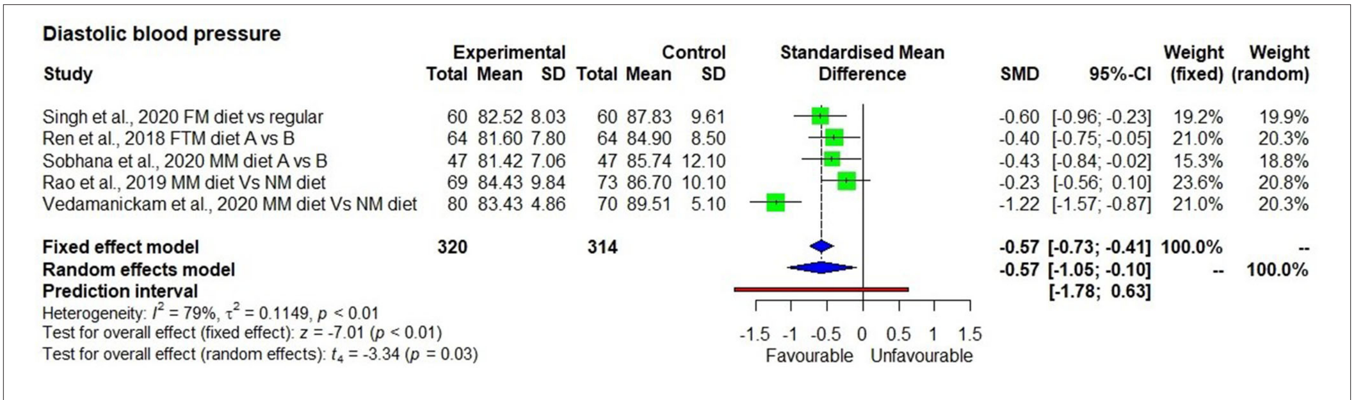

FIGURE 10 | Effect of consuming millets on diastolic blood pressure of the participants (pre- vs. post-treatment or intervention vs. control diet).

levels, and long-term consumption resulted in efficient reduction (26), indicating that long-term consumption of millets can help manage LDL-C levels and reduce the risk of CVD.

Reduction in the levels of LDL-C, VLDL-C, triacylglycerol, and TC is associated not only with the consumption of low-GI millets that produce a low glucose response and reduce the availability of glucose for triacylglycerol formation (27),

TABLE 2 | Fatty acid profile of the millets in comparison to other staple foods.

Type of grain	Linoleic acid[a] (mg/100 g)	Oleic acid[b] (mg/100 g)	Mono-unsaturated fatty acids (mg/100 g)	Poly-unsaturated fatty acids (mg/100 g)	Saturated fatty acids (mg/100 g)
Finger millet	362 ± 15	585 ± 36	585 ± 36	431 ± 21	317 ± 17
Pearl millet	1844 ± 57	585 ± 36	1047 ± 40	1984 ± 55	875 ± 35
Sorghum	508 ± 18	314 ± 14	314 ± 40	524 ± 18	163 ± 6
Kodo millet	576 ± 18	291 ± 7	297 ± 7	597 ± 18	246 ± 2
Little millet	1230 ± 43	868 ± 24	868 ± 24	1277 ± 48	589 ± 40
Maize, dry	1565 ± 18	700 ± 18	706 ± 17.4	1606 ± 18.5	413 ± 5.6
Rice, raw milled	234 ± 46	109 ± 21	117 ± 6.6	253 ± 13.2	184 ± 8.9
Wheat flour, refined	325 ± 7	51 ± 3	51 ± 3	343 ± 8	99 ± 2
Wheat flour, whole	697 ± 20	149 ± 8	149 ± 8	742 ± 19	206 ± 8

Source: Longvah et al. (40).
[a]Essential omega-6 fatty acid, beneficial for cardiovascular health, metabolism, and immune functions.
[b]A non-essential mono-unsaturated omega-9 fatty acid.

but also with the consumption of unsaturated fatty acids from millets. The content of unsaturated fatty acids (both mono- and poly-unsaturated) is 2–10 times higher in millets compared with refined wheat and milled rice (**Table 2**). Some millets have 2.5, 5.7, and 7.8 times more unsaturated fatty acids, especially poly-unsaturated fatty acids compared with milled rice, whole wheat, and refined wheat. Intake of unsaturated fatty acids helps maintain high HDL-C levels. In addition, intake of poly-unsaturated fatty acids, which are essential in our diet, helps in lowering LDL-C levels and thereby lowering the risk associated with CVD (41, 42). Therefore, it suggests that replacing milled rice and refined wheat flour with millets bearing low GI and high poly-unsaturated fatty acids will help in reducing the risk of CVD. In this systematic review, there was 6.0% increase in HDL-C levels after consumption of millet-based meals as compared with regular rice or wheat meals. Thus, there is a need for further long-term randomized controlled trials to assess the impacts on HDL-C levels over an extended period of time. It is also noted that the refining process of teff, a type of millet, decreases the unsaturated fatty acid content and increases the risk of CVD (43).

Diastolic hypertension is common in individuals with components of the metabolic syndrome such as diabetes and hyperlipidemia, and diastolic blood pressure is the best predictor for future risk of CVD (44, 45). Five studies (18, 21, 22, 26, 28) showed that the average diastolic blood pressure in 320 subjects was slightly higher (86.9 ± 1.8 mmHg) than normal (<80 mmHg) at the beginning of the intervention, which was later decreased by 5% (from 86.9 ± 1.8 to 82.7 ± 1.3 mmHg), along with a decrease in lipids, after consuming a millet-based diet for 3 months compared with a regular rice/wheat-based diet. This suggests that consumption of a millet-based diet and diversification of staples with millet will help in reducing the risk of hypertension and associated CVD.

Obesity is a major concern as it raises the risk of CVD and type 2 diabetes. There was no statistically significant reduction in weight observed in the meta-analysis ($p = 0.15$), although, there was a reduction in the mean weight by 1.5 kg in 3 months. This suggests that more long-term studies with a larger sample size are needed for further investigation. Moreover, not all the studies that reported BMI also reported the actual weight.

On the other hand, it was evident from eight studies that on average there was a 7.0% reduction in BMI (28.5 ± 2.4 to 26.7 ± 1.8 kg/m^2) in initially overweight and obese people, showing the possibility of returning to a normal BMI range (<25). Among the eight studies, six were randomized controlled trials conducted for 3–4 months, whereas, two others were cross-sectional studies conducted for 1–2 years. In the randomized controlled trials, there was no heterogeneity ($I^2 = 0.0\%$) observed between the studies, which could be due to the smaller number of studies, sample size, and the influence of geographical distribution (Asian population, mostly Indian). Moreover, there was no variation in effect size, with SMD of −0.18 for both fixed effect and random effect models. The result was significant both in fixed effect and random effect models ($p < 0.05$). Therefore, it was evident that millet-based food consumption can help reduce the degree of overweight and obesity (>30 BMI). In the open-label, self-controlled clinical trial by Ren et al. (21), they observed that a reduction in BMI to be associated with a significant reduction in body fat mass (from 22.1 ± 7.1 to 21.1 ± 7.2 kg) within 12 weeks of foxtail millet consumption compared with only regular diet with no significant reduction in body muscle mass, indicating that consumption of millet was particularly targeting the fat in the body. Another study also showed a significant reduction in body fat mass from 21.6 ± 0.96 to 20.92 ± 0.98 kg by consuming whole foxtail millet-based meals for 12 weeks compared with a regular diet (33).

Furthermore, studies also showed that consumption of millet-based foods caused satiety (32). Ren et al. (21) showed that consumption of foxtail millet for 12 weeks increased the blood leptin levels significantly (from 8.3 ± 6.4 to 9.6 ± 7.0 ng/ml), which is the indication of hunger suppression and reduced energy intake by altering the nervous system signals and blood glucose metabolism. The satiety is also due to slower gastric emptying time (32, 46, 47) and the high fiber content of millet (40). This characteristic may reduce sudden spikes in blood glucose levels, leading to decreased availability of glucose for triacylglycerol synthesis, thereby reducing triacylglycerol levels. This would need detailed research to further investigate and quantify the variables and impacts.

Hyperlipidemia is associated with inflammation, leading to lipotoxicity and progression of CVD. Some markers that might contribute to endothelial dysfunction include leptin, interleukin-6 (IL-6), and adiponectin. Potential tools for risk assessment include serum high-sensitivity C-reactive protein (hs-CRP), fasting insulin, tumor necrotic factor-α (TNF-α), IL-6, leptin, and adiponectin (48). A study conducted on foxtail millet consumption for 12 weeks (18) showed a decrease in inflammatory marker IL-6 from 6.2 ± 9.4 to 4.8 ± 5.5 pg/ml and TNF-α from 2.6 ± 5.5 to 1.4 ± 0.5 pg/ml. Another study on finger millet consumption for 12 weeks (18) showed a significant reduction in IL-6 (from 4.9 ± 0.7 to 1.60 ± 0.5 mmol/l) ($p = 0.000$) and TNF-α (from 7.8 ± 1.2 to 3.9 ± 0.6 mmol/l) ($p = 0.016$). However, Sobhana et al. (22) demonstrated that consumption of a millet-based diet for 3 months did not significantly reduce hs-CRP (from 0.45 ± 0.078 to 0.43 ± 0.064 µg/ml), which is not a significant reduction. More long-term studies are recommended to examine this effect. However, the reduction in IL-6 and TNF-α suggests that millet consumption can reduce inflammation caused by hyperlipidemia and diabetes.

Two studies examined the impacts of millets consumption on plasma antioxidant capacity. Hymavathi et al. (20) demonstrated significant increases in the levels of antioxidants such as superoxide dismutase from 79 ± 11 to 82 ± 12 U/ml, and reduced glutathione (GSH) level from 10.9 ± 2 to 11.9 ± 2 U/L, suggesting that consumption of finger millet could suppress stress levels in both diabetic and normal individuals. Consumption of millets for 8 weeks increased the ferric ion–reducing antioxidant power (19) from 679.5 ± 120.3 to 763.9 ± 105.3 ($p < 0.05$) and Trolox equivalent antioxidant capacity from 296.9 ± 122.8 to 431.0 ± 55.4 ($p < 0.05$). In other words, antioxidant capacity improved in healthy individuals upon consumption of millets. Similar studies should be conducted on pre-diabetic, diabetic, and hyperlipidemic individuals to understand the effects of tissue damage from metabolic syndromes.

In this systematic review, although, studies examined the impacts of millet consumption on blood lipid profile, none of the included studies focused on millet consumption and its impact on hyperlipidemia in other related disease conditions, such as non-alcoholic fatty liver disease (NAFLD), which is predicted to become a major cause of liver-related morbidity and mortality by 2030 (49). Consumption of whole grains increases the intake of unsaturated fatty acids and dietary fiber, which enhances the fullness or satiety value, which, in turn, could have a beneficial effect on reducing energy intake and hyperlipidemia, and reducing the risk of CVD and NAFLD (49). Further, research on long-term millet consumption and its association with inflammatory markers and reduction in hyperlipidemia, especially in NAFLD individuals, would help to construct dietary plans to reduce risks of development and progression of NAFLD and CVD.

Recommendations

Overall, it is recommended that millet-based diets be designed and then promoted for management and prevention of atherosclerotic CVD as well as weight management. It would be beneficial to diversify major staples with millets across Africa and Asia, because millets have nutritional and health benefits, such as low GI and high levels of several necessary micro- and macronutrients (e.g., iron, zinc, calcium, and protein). Additionally, millets are a "smart food": not only "good for you" but also "good for the planet" and "good for the farmer," i.e., environmentally sustainable and climate-smart, with a lower carbon footprint. Therefore, they should also be part of solutions for reforming the food systems. This will help contribute to a range of UN Sustainable Development Goals, such as Zero Hunger, Good Health and Well-being, Responsible Consumption and Production, and Climate Action.

Priority research to address the limitations of this study or build more evidence includes the following. (1) Only five millets were assessed, namely finger millet, foxtail millet, barnyard millet, sorghum, and little millet combined with other millets in a meal. There are other millets and a range of varieties that are grown globally and expected to bear similar effects on managing hyperlipidemia. It is thus important to generate evidence with the unstudied crops/varieties. (2) Out of the 19 studies, two were conducted in China, one in Sri Lanka, and one in Brazil. The remaining 15 studies were from India, alluding to a geographical limitation. Millets are grown and consumed in all inhabited continents, especially across sub-Saharan Africa and South Asia. It will be useful to generate further evidence in different sub-populations. (3) The duration for randomized controlled trials varied from 21 days to 4 months. Research over a year or two will provide useful evidence. (4) No study was conducted on the effects of processing and cooking of millets on the lipid profile. It is critical to establish such a linkage. (5) Only three studies determined TC to HDL-C ratio, which is a key marker for CVD. Therefore, it is recommended to include all relevant parameters while implementing dietary interventions and assessing the impacts on hyperlipidemia and CVD. (6) The number of products in each category (baking, boiling, etc) was very small in the current systematic review hence the effect of processing on lipid profile was not evaluated which could be considered in future research. (7) As millets were identified to reduce hyperlipidemia, millets are expected to have a role in reducing or preventing NAFLD, which has not been studied to date.

CONCLUSIONS

The systematic review executed in this study provides a strong evidence that millet consumption can improve blood lipid profile and thus exerts beneficial effects on management and prevention of hyperlipidemia, reduction in high blood pressure, weight, and BMI as well as an overall reduction in associated risk of CVD. Millets should therefore be integrated into nutrition and health strategies, utilized to diversify staples across Africa and Asia, and promoted as broader solutions in food system reforms. Further, analysis, customized to the situation, should be undertaken to ensure that millets are appropriately integrated into initiatives for maximized effectiveness.

DATA AVAILABILITY STATEMENT

The original contributions presented in the study are included in the article/**Supplementary Material**, further inquiries can be directed to the corresponding author/s.

AUTHOR CONTRIBUTIONS

SA and JK-P: conceptualization, methodology, data extraction, and writing. SA, RB, and TWT: methodology, data extraction, analysis, and interpretation. DIG, AR, and RKB: methodology, risk assessment, writing, and reviewing the manuscript until final stage. All authors contributed to the article and approved the submitted version.

FUNDING

The systematic review is supported by Smart Food Endowment Fund, ICRISAT.

ACKNOWLEDGMENTS

The authors would like to acknowledge Dr. Jali MV who sent in missing data to support this meta-analysis, and Dr. Ram B Singh who clarified having used finger millet in his study. The authors acknowledge Ms. Rajani Kumar, ICRISAT, for editing the manuscript. The authors also thank Mr. Ramesh Kotnana for sourcing scientific articles.

SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fnut.2021.700778/full#supplementary-material

REFERENCES

1. Zhao X, Wang D, Qin L. Lipid profile and prognosis in patients with coronary heart disease: a meta-analysis of prospective cohort studies. *BMC Cardiovasc Disord.* (2021) 21:1–15. doi: 10.1186/s12872-020-01835-0
2. Ralph B, Hyperlipidemia and cardiovascular disease: new insights on lipoprotein(a). *Curr Opin Lipidol.* (2019) 30:260–1. doi: 10.1097/MOL.0000000000000594
3. Gordon DJ, Probstfield JL, Garrison RJ, Neaton JD, Castelli WP, Knoke JD, et al. High-density lipoprotein cholesterol and cardiovascular disease. Four prospective American studies. *Circulation.* (1989) 79:8–15. doi: 10.1161/01.CIR.79.1.8
4. Castelli WP, Anderson K, Wilson PW, Levy Lipids D. and risk of coronary heart disease. The Framingham Study. *Ann Epidemiol.* (1992) 2:23–8. doi: 10.1016/1047-2797(92)90033-M
5. Poole N, Kane-Potaka J. *The Smart Food Triple Bottom Line – Starting with Diversifying Staples Including summary of latest Smart Food studies at ICRISAT. Agriculture for Development journal, No. 41.* Tropical Agriculture Association, UK (2020). p. 21–3. Available online at: https://taa.org.uk/wp-content/uploads/2021/01/Ag4Dev41_Winter_2020_WEB.pdf (accessed March 3, 2021).
6. Anitha S, Kane-Potaka J, Tsusaka TW, Botha R, Rajendran A, Givens DI, et al. A systematic review and meta-analysis of the potential of millets and sorghum for managing and preventing diabetes mellitus. *Front Nutr.* (2021) 8:1–18. doi: 10.3389/fnut.2021.687428
7. Mohar D, Liberati A, Tetzlaff J, Altman DG. Preferred reporting items for systematic reviews and meta-analysis: the PRISMA statement. *Open Med.* (2009) 3:123–30. doi: 10.1136/bmj.b2535
8. Atkinson LZ, Cipriani A. How to carry out a literature search for a systematic review: a practical guide. *BJPsych Advan.* (2018) 24:74–82. doi: 10.1192/bja.2017.3
9. Harrer M, Cuijpers P., Furukawa TA, Ebert DD. *Doing Meta-Analysis in R: A Hands-on Guide.* (2019). Available online at: https://bookdown.org/MathiasHarrer/Doing_Meta_Analysis_in_R/ (accessed February 28, 2021).
10. Peterson J, Welch V, Losos M, Tugwell P. *The Newcastle-Ottawa Scale (NOS) for Assessing the Quality of Non-Randomised Studies in Meta-Analyses.* Ottawa: Ottawa Hospital Research Institute (2011).
11. Luchini C, Stubbs B, Solmi M, Veronese N. Assessing the quality of studies in meta-analyses: advantages and limitations of the Newcastle Ottawa Scale. *World J Meta-Anal.* (2017) 5:80–4. doi: 10.13105/wjma.v5.i4.80
12. Bell A, Fairbrother M, Jones K. Fixed and random effects models: making an informed choice. *Qual Quant.* (2019) 53:1051–74. doi: 10.1007/s11135-018-0802-x
13. Higgins JPT, Thomas J, Chandler J, Cumpston M, Li T, Page MJ, et al. (editors). *Cochrane Handbook for Systematic Reviews of Interventions* version 6.2 (updated February 2021). Cochrane, (2021). Available online at: www.training.cochrane.org/handbook (accessed April 13, 2021).
14. Ahn H, Kang E. Introduction to systematic review and meta-analysis. *Korean J Anaesthesiol.* (2019) 71:103–12. doi: 10.4097/kjae.2018.71.2.103
15. Hak T, Van Rhee HJ, Suurmond R. *How to Interpret Results of Meta-Analysis. (Version 1.3).* Rotterdam: The Netherlands: Erasmus Rotterdam Institute of Management. p. 1–2 (2016). Available online at: www.erim.eur.nl/research support/meta-essentials/downloads (accessed April 26, 2021).
16. Isreal H, Richter RP. A guide to understanding meta-analysis. *J Orthop SportsPhys Ther.* (2011) 41:496–504. doi: 10.2519/jospt.2011.3333
17. Balduzzi S, Rücker G, Schwarzer G. How to perform a meta-analysis with R: a practical tutorial? *Evidence Based Mental Health.* (2019) 22:153–60. doi: 10.1136/ebmental-2019-300117
18. Singh RM, Fedacko J, Mojto V, Isaza A, Dewim M., Watanabe S, et al. Effects of millet based functional foods rich diet on coronary risk factors among subjects with diabetes mellitus: a single arm real world observation from hospital registry. *MOJ Public Health.* (2020) 9:18–25. doi: 10.15406/mojph.2020.09.00318
19. Kumari D, Chandrasekara A, Athukorale P, Shahidi F. Finger millet porridges subjected to different processing conditions showed low glycemic index and variable efficacy on plasma antioxidant capacity of healthy adults. *Food Product Process Nutr.* (2020) 2:13. doi: 10.1186/s43014-020-00027-9
20. Hymavathi TV, Rao SK, Boini P, Rajesh P, Rodrigues L, Sampathi S, et al. Profiling of *Eleusine coracana* biscuits for glycemic index, stress and lipid. *MOJ Drug Design Dev Ther.* (2017) 1:52–8. doi: 10.15406/mojddt.2017.01.00010
21. Ren X, Yin R, Hou D, Xue Y, Zhang M, Diao X, et al. The glucose-lowering effect of foxtail millet in subjects with impaired glucose tolerance: a self-controlled clinical trial. *Nutrients.* (2018) 10:1509. doi: 10.3390/nu10101509
22. Sobhana PP, Kandlakunta B, Nagaraju R, Thappatla D, Epparapalli S, Vemula SR, et al. Human clinical trial to assess the effect of consumption of multigrain Indian bread on glycemic regulation in type 2 diabetic participants. *J Food Biochem.* (2020) 44:e13465. doi: 10.1111/jfbc.13465

23. Ugare R, Chimmad B, Naik R, Bharati P, Itagi S. Glycemic index and significance of barnyard millet (*Echinochloa frumentacae*) in type II diabetics. *J Food Sci Technol.* (2014) 51:392–5. doi: 10.1007/s13197-011-0516-8
24. Geetha K, Geetha Yankanchi M, Hiremath N. Effect of high fibre food mix on lipid profile and body weight in obese subjects. *Biomed Res*. (2019) 30:655–8. doi: 10.35841/biomedicalresearch.30-19-257
25. Anusha B, Hymavathi TV, Vijayalaskhmi V, Reddy P, Pradeepa Robert T. Lipid lowering effects of foxtail millet (Setaria italic) Quinoa (Chenopodium quinoawild) in pre-diabetics. *JPRI.* (2018) 24:1–7. doi: 10.9734/JPRI/2018/45285
26. Vedamanickam R, Anandan P, Bupesh G, Vasanth S. Study of millet and non-millet diet on diabetics and associated metabolic syndrome. *Biomedicine.* (2020) 40:55–8.
27. Tiwari N, Srivastava S. Effect of finger millet (Eleusine coracana) buns supplementation on serum glucose and serum lipids level in type 2 diabetics. *Asian J Dairy Food Res.* (2017) 36:337–40. doi: 10.18805/ajdfr.DR-933
28. Rao A, Prabhu D, Krishnamurthy V. Millets in metabolic syndrome-time to tap the potential. *J Evid Based Med Healthc.* (2019) 6:1284–7. doi: 10.18410/jebmh/2019/266
29. Thathola A, Srivastava S, Singh G. Effect of foxtail millet (*Setaria Italica*) supplementation on serum glucose, serum lipids and glycosylated haemoglobin in type 2 diabetics. *Diabetol Croat.* (2011) 40:23–8.
30. Anushia K, Uma Mageshwari J, Trueman P, Viswanathan V. The effect of millet supplementation on weight and lipid profile. *TAPI J.* (2019) 13:1–7.
31. Itagi S, Naik R, Bharati P, Sharma P. Readymade foxtail millet mix for diabetics. *Int J Sci Nat.* (2012) 3:47–50.
32. Surekha N, Chimmad BV, Naik SR. Hypoglycaemic and hypolipidemic efficacy of barnyard millet (Echinochloa frumentacea Link) based health food. *Asian J Home S.* (2013) 8:383–7.
33. Hou D, Chen J, Ren X, Wang C, Diao X, Hu X, et al. A whole foxtail millet diet reduces blood pressure in subjects with mild hypertension. *J Cereal Sci.* (2018) 84:13–9. doi: 10.1016/j.jcs.2018.09.003
34. Jali MV, Kamatar MY, Jali SM, Hiremath MB, Naik RK. Efficacy of value added foxtail millet therapeutic food in the management of diabetes and dyslipidamea in type 2 diabetic patients. *Recent Res Sci Technol.* (2012) 4:03–4.
35. Joshi S, Srivastava S. Hypoglycemic and hypolipidemic effect of barnyard millet consumption in type 2 diabetic subjects. *Int J Curr Microbiol App Sci.* (2021) 10:467–77.
36. Anunciacao PC, Cardoso LDM, Alfenas RDCG, Queiroz VAV, Carvalho CWP, Martino HSD, et al. Extruded sorghum consumption associated with a caloric restricted diet reduces body fat in overweight men: A randomized controlled trial. *Food Res Int*. (2019) 119:693–700. doi: 10.1016/j.foodres.2018.10.048
37. Sandesara PB, Virani SS, Fazio S, Shapiro MD. The forgotten lipids: triacylglycerols, remnant cholesterol, and atherosclerotic cardiovascular disease risk. *Endocr Rev*. (2019) 40:537–57. doi: 10.1210/er.2018-00184
38. Marston NA, Giugliano RP, Im K, Silverman MG, O'Donoghue ML, Wiviott SD, et al. Association between triacylglycerol lowering and reduction of cardiovascular risk across multiple lipid-lowering therapeutic classes - a systematic review and meta-regression analysis of randomized controlled trials. *Circulation.* (2019) 140:1308–17. doi: 10.1161/CIRCULATIONAHA.119.041998
39. Michael DS, Deepak LB. "Cholesterol-Years" for ASCVD Risk Prediction and Treatment. *J Am Coll Cardiol.* (2020) 76:1517–20. doi: 10.1016/j.jacc.2020.08.004
40. Longvah T, Ananthan R, Bhaskarachary K, Venkaiah K. *Indian Food Composition Table*. Hyderabad: National Institute of Nutrition (2017). p. 1–578.
41. Micha R. & Mozaffarian D. Saturated fat and cardiometabolic risk factors, coronary heart disease, stroke, and diabetes: a fresh look at the evidence. *Lipids.* (2010) 45:893–905. doi: 10.1007/s11745-010-3393-4
42. Nettleton JA, Brouwer IA, Geleijnse JA et al. Saturated fat consumption and risk of coronary heart disease and ischemic stroke: a science update. *Ann Nutr Metabol.* (2017) 70:26–33. doi: 10.1159/000455681
43. Yilmaz HO, Arslan M. Teff: nutritional compounds and effects on human health. *Acta Sci Med Sci.* (2018) 2:15–8.
44. Tverdal A. Systolic diastolic blood pressures as predictors of coronary heart disease in middle aged Norwegian men. *Br Med J.* (1987) 294:671–3. doi: 10.1136/bmj.294.6573.671
45. Franklin SS. The importance of diastolic blood pressure in predicting cardiovascular risk. *J Am Soc Hypertens.* (2007) 1:82–93. doi: 10.1016/j.jash.2006.11.004
46. Alyami J, Ladd N, Pritchard SE, Hoad CL, Sultan AA, Spiller RC, et al. Glycaemic gastrointestinal and appetite responses to breakfast porridge from ancient cereal grains: a MRI pilot study in healthy human. *Food Res Int.* (2017) 118:49–57. doi: 10.1016/j.foodres.2017.11.071
47. Alyami J, Whitehouse E, Yakubov GE, Pritchard SE, Hoad CL, Blackshaw E, et al. Glycaemic gastrointestinal hormonal and appetitive responses to pearl millet or oats porridge breakfasts: a randomized crossover trial in healthy humans. *Br J Nutr.* (2019) 122:1142–54. doi: 10.1017/S0007114519001880
48. Chang CJ, Jian DY, Lin MW, Zhao JZ, Ho LT, Juan CC. Evidence in obese children: contribution of hyperlipidemia, obesity, inflammation, insulin sensitivity. *PLoS ONE.* (2015) 10:e0125935. doi: 10.1371/journal.pone.0125935
49. Dorosti M, Heidarloo AJ, Bakhshimoghaddam F, Alizadeh M. Whole-grain consumption and its effects on hepatic steatosis and liver enzymes in patients with non-alcoholic fatty liver disease: a randomised controlled clinical trial. *Br J Nutr.* (2020) 123:328–36. doi: 10.1017/S0007114519002769

Conflict of Interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's Note: All claims expressed in this article are solely those of the authors and do not necessarily represent those of their affiliated organizations, or those of the publisher, the editors and the reviewers. Any product that may be evaluated in this article, or claim that may be made by its manufacturer, is not guaranteed or endorsed by the publisher.

frontiers in Nutrition

SYSTEMATIC REVIEW
published: 14 October 2021
doi: 10.3389/fnut.2021.725529

Millets Can Have a Major Impact on Improving Iron Status, Hemoglobin Level, and in Reducing Iron Deficiency Anemia–A Systematic Review and Meta-Analysis

Seetha Anitha[1]*, Joanna Kane-Potaka[1], Rosemary Botha[2], D. Ian Givens[3], Nur Liana Binti Sulaiman[3], Shweta Upadhyay[4], Mani Vetriventhan[1], Takuji W. Tsusaka[5], Devraj J. Parasannanavar[6], Thingnganing Longvah[6], Ananthan Rajendran[6], Kowsalya Subramaniam[7] and Raj Kumar Bhandari[8]

[1] Smart Food Initiative, International Crops Research Institute for the Semi-Arid Tropics (ICRISAT), Patancheru, India, [2] Development Strategy and Governance Division, International Food Policy Research Institute (IFPRI), Lilongwe, Malawi, [3] Institute of Food, Nutrition and Health, University of Reading, Reading, United Kingdom, [4] UNICEF, Lilongwe, Malawi, [5] Ostrom Center for Advanced Studies on Natural Resources Governance, Asian Institute of Technology, Pathumthani, Thailand, [6] National Institute of Nutrition (NIN), Hyderabad, India, [7] Food Science and Nutrition, Avinashilingam Institute for Home Science and Higher Education for Women, Coimbatore, India, [8] National Technical Board of Nutrition, Government of India (GoI), New Delhi, India

The prevalence of iron deficiency anemia is highest among low and middle-income countries. Millets, including sorghum, are a traditional staple in many of these countries and are known to be rich in iron. However, a wide variation in the iron composition of millets has been reported, which needs to be understood in consonance with its bioavailability and roles in reducing anemia. This systematic review and meta-analysis were carried out to analyze the scientific evidence on the bioavailability of iron in different types of millets, processing, and the impact of millet-based food on iron status and anemia. The results indicated that iron levels in the millets used to study iron bioavailability (both *in vivo* and *in vitro*) and efficacy varied with the type and variety from 2 mg/100 g to 8 mg/100 g. However, not all the efficacy studies indicated the iron levels in the millets. There were 30 research studies, including 22 human interventions and 8 *in vitro* studies, included in the meta-analysis which all discussed various outcomes such as hemoglobin level, serum ferritin level, and absorbed iron. The studies included finger millet, pearl millet, teff and sorghum, or a mixture of millets. The results of 19 studies conducted on anaemic individuals showed that there was a significant ($p < 0.01$) increase in hemoglobin levels by 13.2% following regular consumption (21 days to 4.5 years) of millets either as a meal or drink compared with regular diets where there was only 2.7% increase. Seven studies on adolescents showed increases in hemoglobin levels from 10.8 ± 1.4 (moderate anemia) to 12.2 ± 1.5 g/dl (normal). Two studies conducted on humans demonstrated that consumption of a pearl millet-based meal significantly increased the bioavailable iron ($p < 0.01$), with the percentage of bioavailability being 7.5 ± 1.6, and provided bioavailable iron of 1 ± 0.4 mg. Four studies conducted on humans showed significant increases in ferritin level ($p < 0.05$) up to 54.7%. Eight *in-vitro* studies

Edited by:
RAKESH BHARDWAJ,
National Bureau of Plant Genetic Resources (ICAR), India

Reviewed by:
Christèle Icard-Vernière,
Institut de Recherche Pour le Développement (IRD), France
Arivalagan Manivannan,
Indian Institute of Horticultural Research (ICAR), India

***Correspondence:**
Seetha Anitha
s.anitha@cgiar.org;
dr.anithaseetha@gmail.com

Specialty section:
This article was submitted to Nutrition and Sustainable Diets, a section of the journal Frontiers in Nutrition

Received: 15 June 2021
Accepted: 03 September 2021
Published: 14 October 2021

Citation:
Anitha S, Kane-Potaka J, Botha R, Givens DI, Sulaiman NLB, Upadhyay S, Vetriventhan M, Tsusaka TW, Parasannanavar DJ, Longvah T, Rajendran A, Subramaniam K and Bhandari RK (2021) Millets Can Have a Major Impact on Improving Iron Status, Hemoglobin Level, and in Reducing Iron Deficiency Anemia–A Systematic Review and Meta-Analysis. Front. Nutr. 8:725529. doi: 10.3389/fnut.2021.725529

showed that traditional processing methods such as fermentation and germination can improve bioavailable iron significantly ($p < 0.01$) by 3.4 and 2.2 times and contributed to 143 and 95% of the physiological requirement of women, respectively. Overall, this study showed that millets can reduce iron deficiency anemia.

Keywords: iron status, hemoglobin level, millets, sorghum, meta-analysis

INTRODUCTION

Iron deficiency anemia is a serious global public health problem. As per the World Health Organization (WHO) report, worldwide, 42% of pregnant women, 30% of non-pregnant women (aged 15–50 years), 47% of preschool children (< 5 years), and 12.7% of young men (> 15 years) are anaemic. Iron deficiency anemia (IDA) adversely affects the growth and cognitive development in children; cognitive, physical, and psychological health in non-pregnant women, and maternal and neonatal outcomes in pregnant women. Its prevalence among women between the ages of 15 and 49 is more than 40% in most Asian and African countries (1). Many factors cause IDA including, gut health, dietary iron deficiency, bioavailability, folic acid deficiency, Vitamin C, Vitamin A, and Vitamin B12 deficiency. In addition, hookworm infestation and malaria also contribute to the increase in the prevalence of IDA among Asian and African countries (2).

Three major approaches are followed to control IDA globally, which are supplementation with iron and folic acid tablets, fortification and natural food-based approaches. Despite the wide implementation of the first two approaches, IDA remains a serious malnutrition problem with an increasing trend globally. The third approach mainly focuses on dietary diversification and enrichment of diets with naturally iron-rich foods without the potential side effects of artificial additives.

In developing countries, milled rice, wheat, and maize replaced the traditional nutritious crops. Refined foods are abundant in starch but lack nutrients, especially micronutrients such as iron (Fe) and zinc (Zn). Given that a major part (>80%) of the diet in developing countries (3) comprises low iron staple food, achieving sufficient intake of iron through the remaining 20% of the diet is impractical. Therefore, it is important to diversify the staple food by including naturally iron-rich food crops such as millets (4). In addition, millets have a 2.3 to 4.0 times more dietary fiber (6.4 ± 0.6 to 11. 5 ± 0.6 g/100g) compared with refined rice and refined wheat (5), which acts as food for beneficial gut microbiome that improves abundance and alters the gut composition in a beneficial way (6, 7). Millets have added advantages as they are recognized as a smart food, i.e., not only good for you since it is nutritious and healthy, but also good for the planet because it is environmentally sustainable and good for farmers since it is resilient and climate-smart (8).

Animal sources of haem iron are well known for their high bioavailability. However, it is not always affordable to the poorest segments of the population. Moreover, a vegetarian population require alternate plant sources of highly absorbable iron to tackle iron deficiency. Although millets are recognized as being naturally rich in iron, their nutrient composition varies with the type, variety, and growing conditions. Commonly used food composition tables while providing an overview of the nutrient composition do not include this detail (5, 9, 10). Presenting a single value for iron level in a type of millet can be misleading as iron levels can vary significantly among varieties. Iron levels can be as much as triple in a commonly available variety over another.

Non-haem iron (plant-based) is not absorbed as readily as haem iron (animal-based) in the presence of phytate and tannin in millets. Most of the cereals such as wheat flour, brown rice, and barley contain phytic acid to levels (5) that are far higher than that of millets. However, the phytate content of millets is often overly emphasized. Nonetheless, it is important to understand the bioavailability of iron from millets and its impact on anemia status. Although few studies have investigated the bioavailability of iron by *in vitro* and *in vivo* methods, not all of them are well known or promoted. Moreover, very few studies focus on the overall beneficial effects of millets on anemia, as most studies focus on only one or just a few of the outcomes, such as hemoglobin, absorbed iron, serum ferritin, and serum transferrin levels. Collating this information can provide information on which millets to use and to what extent they can improve iron status and the type of processing that can enhance the bioavailability of dietary iron. It is against this background that this systematic review of published scientific studies on millets was undertaken to investigate the range of iron levels and its bioavailability in order to enable a comparison with major staples such as rice, wheat (both whole grain and refined), and maize. This was followed by a meta-analysis to collate all the science-based evidence available on millets, their effects on iron status, hemoglobin levels in the body, and their related ability to reduce iron deficiency anemia.

Research Questions

Does consumption of millets-based food help improve iron status and hemoglobin levels and reduce iron deficiency anemia? How does this compare with regular non-millet diets?

METHODS

Study Period and Protocol

The systematic review and meta-analysis were conducted from October 2017 to April 2021. The PRISMA checklist was used to write the protocol (11). The protocol was registered through an online "research registry" with the Unique Identification number "reviewregistry1114".

Search, Inclusion, and Exclusion Criteria

Studies written in English and published between 2010 and 2020 were considered. Google Scholar, Scopus, Web of Science,

TABLE 1 | Search keywords used to identify relevant papers.

Number	Search keywords
1	Millets efficacy in reducing anemia
2	Millets "AND" bioavailability of iron
3	Impact of consuming millets on iron status/hemoglobin level
4	Efficacy of millets on hemoglobin level. Repeat the search by replacing the word "millets" with "finger millet," "pearl millet," "sorghum," teff.
5	Efficacy of millets on iron deficiency anemia
6	Effect of consuming millets on ferritin level
7	Effect of germination of millets on iron bioavailability. Repeat the search by replacing the word "millets" with "finger millet," "pearl millet," "sorghum," teff. Repeat the search by replacing the word "germination" with "fermentation," "malting," "processing."
8	Hand search on the references of published article.

PubMed, and CABI abstract were used to find studies relevant to the research questions. The search was conducted using the search strategy and keywords (**Table 1**), which were further screened for relevance to the study, completeness of information and quality of research based on the inclusion and exclusion criteria.

Inclusion Criteria

The following criteria were included: (1) research studies conducted to test the efficacy of millets in reducing anemia and improving hemoglobin, serum ferritin levels, iron status, and/or bioavailability of iron; (2) studies that had information on any or all of the outcomes including levels of hemoglobin, serum ferritin, absorbed iron, and bioavailability of iron; (3) efficacy studies conducted using high iron and/or biofortified varieties of millets; (4) studies conducted on any age group or gender of any population to test the efficacy of millets in reducing iron deficiency; (5) both *in vivo* and *in vitro* studies that assessed the bioavailability of iron, with the two types of studies treated as separate; (6) peer-reviewed journal articles, full MSc or PhD theses submitted to universities, and full research papers from theses if available online.

Exclusion Criteria

Review articles, animal studies, and publications with incomplete data were excluded.

Data Collection

The study used the PRISMA checklist at every step of data collection, extraction, and analysis (**Figure 1**). Only the relevant papers downloaded that addressed the research questions were used. The references in the relevant publications were also checked by hand search to find more related research articles. If only an abstract was found relevant to this study, then efforts were made to download open access articles or collect the full paper. After collecting the full paper, if any required data were missing, the authors were contacted, and the full information was requested for use in the meta-analysis. The data were extracted from the articles and documented in Excel sheets. Using the data, descriptive statistics, regression analysis, forest plots, and publication bias analysis were performed.

Data Items

Each study was labeled with details on the author and the year of publication. The age group and gender of the study participants were recorded, along with the country, study method, sample size, and type and form of millets used. The numerical variables considered for the meta-analysis included mean and standard deviations of levels of hemoglobin, absorbed iron, and serum ferritin. The data were then entered into an Excel sheet as per the guidelines (12, 13).

Summary Measures and Result Synthesis

(1). Pre-and post- intervention or (2). test and control diets impact on each outcome was recorded with mean and standard deviation values and used for meta-analysis. Since it is continuous data, a meta-analysis was performed to measure Standardized Mean Difference (SMD) and heterogeneity (i^2). The significance of the results was determined using the fixed-effect model for a single source of information and the random effect model for other studies. Results from both models were captured in each forest plot. In addition, descriptive statistics such as mean, standard deviation, and percentage change in hemoglobin levels were calculated for both intervention and control samples. Regression analysis was conducted to test the effects of processing such as germination, fermentation, and malting of millets on the bioavailability of iron. The term 'bioavailability' was used to refer to the percentage of iron in the food that is apparently absorbable based on the *in vivo* and *in vitro* protocols used in the studies included in the meta-analysis. The term 'bioavailable iron' was used to represent the amount of apparently absorbed iron per 100 g of food, and has been calculated as:

$$\text{Bioavailable iron (mg/100 g food)} = \text{iron concentration in the food (mg/100 g)} \times \text{\% bioavailability/100.}$$

Bioavailable iron from millets was then compared with the physiological requirement, which is a requirement for absorbed iron (14). The physiological requirement for various age groups to assess whether the bioavailable iron from millets can contribute to the physiological requirement. The physiological requirement of iron was obtained from the recommended dietary allowances book released recently (15) and was calculated based on the assumption that 8% of the iron is absorbed from the Estimated Actual Requirement (EAR) (15).

Study Quality Assessment

Using the eight-item Newcastle-Ottawa Scale (NOS), the quality (16, 17) of each study was assessed by two investigators. Any disagreement was resolved by discussing it with a third reviewer. The researchers also applied the principle stated in the study of (18) to further strengthen quality assessment.

Detailed Data Analysis

A total of 22 human studies were found eligible for the meta-analysis with three outcomes namely hemoglobin level (g/dl), serum ferritin (ng/ml) and total iron absorbed (mg/day).

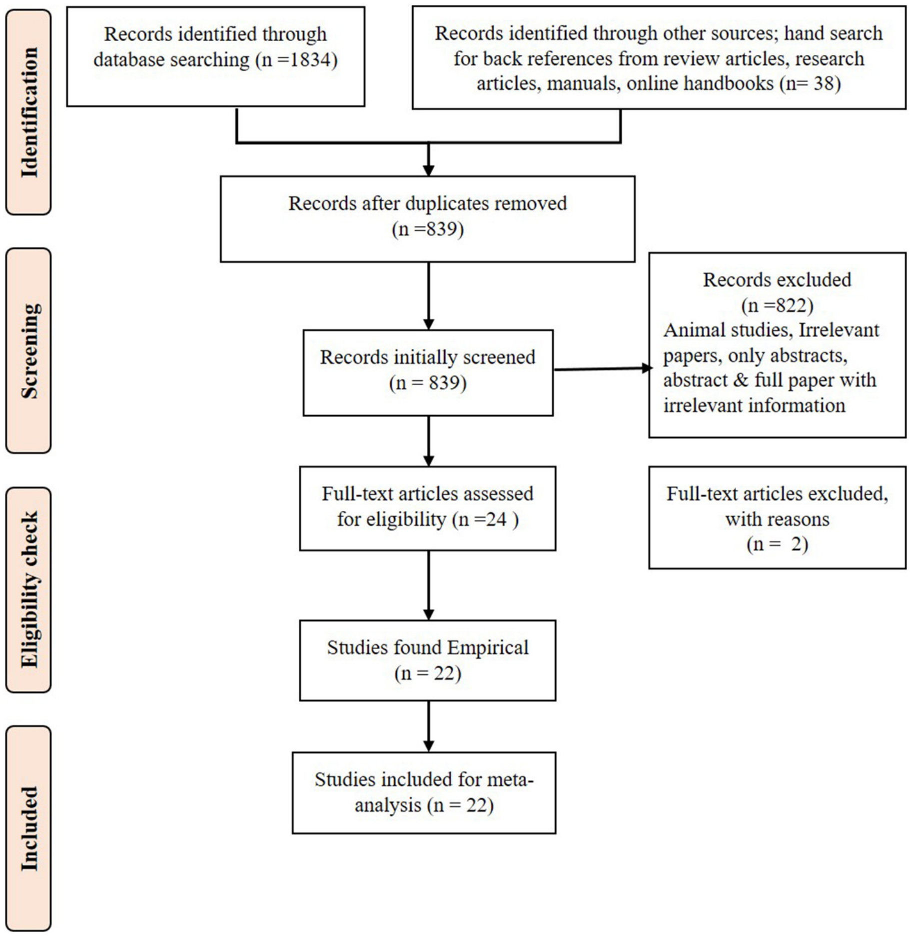

FIGURE 1 | PRISMA flow diagram for systematic review.

Nineteen of these studies (based on various types and forms of millets) were used to determine the effects of consumption of millets on hemoglobin levels, while two studies were used to determine the effects on iron absorption, and four studies were used to measure the effects on serum ferritin levels (a blood protein that contains iron that is commonly tested to indicate the level of iron stored in the body). The iron content of millets was categorized as high if iron content was above 6 mg/100 g (regardless of biofortification), moderate it was from 3 mg/100 g to 6 mg/100 g, and low if below 3 mg/100 g. They were compared with the corresponding control samples which were mostly rice or wheat-based regular diets as well as low iron millets. The heterogeneity of samples and overall test results were included in the forest plots. Both the random effect and fixed-effect models were tested and used to interpret the results and SMD (19–22).

A meta-analysis was conducted using R Studio 4.0.4 (2021) (www.rstudio.com) to obtain a forest plot, heterogeneity, overall test effects in both fixed and random effect models, and funnel plots to determine the publication bias (12, 23).

Subgroup Analysis

Based on the type of millet (finger millet, pearl millet, sorghum, and mixed millets), the duration of the study ('short' if $<$ 4 months while 'long' if $>$ 4 months) and the age of the participants (children, adolescents, and adults) were used for subgroup analysis to assess the effects of consumption of millets on hemoglobin levels.

Risk of Bias Assessment

A funnel plot was used to assess publication bias. Selection, detection, attrition and reporting biases were assessed according to the guidelines provided in the Cochrane handbook for systematic reviews of interventions (24).

RESULTS

Meta-Analysis of Data From Human Studies

There were 22 research papers involving human subjects identified as eligible for the meta-analysis for three outcomes,

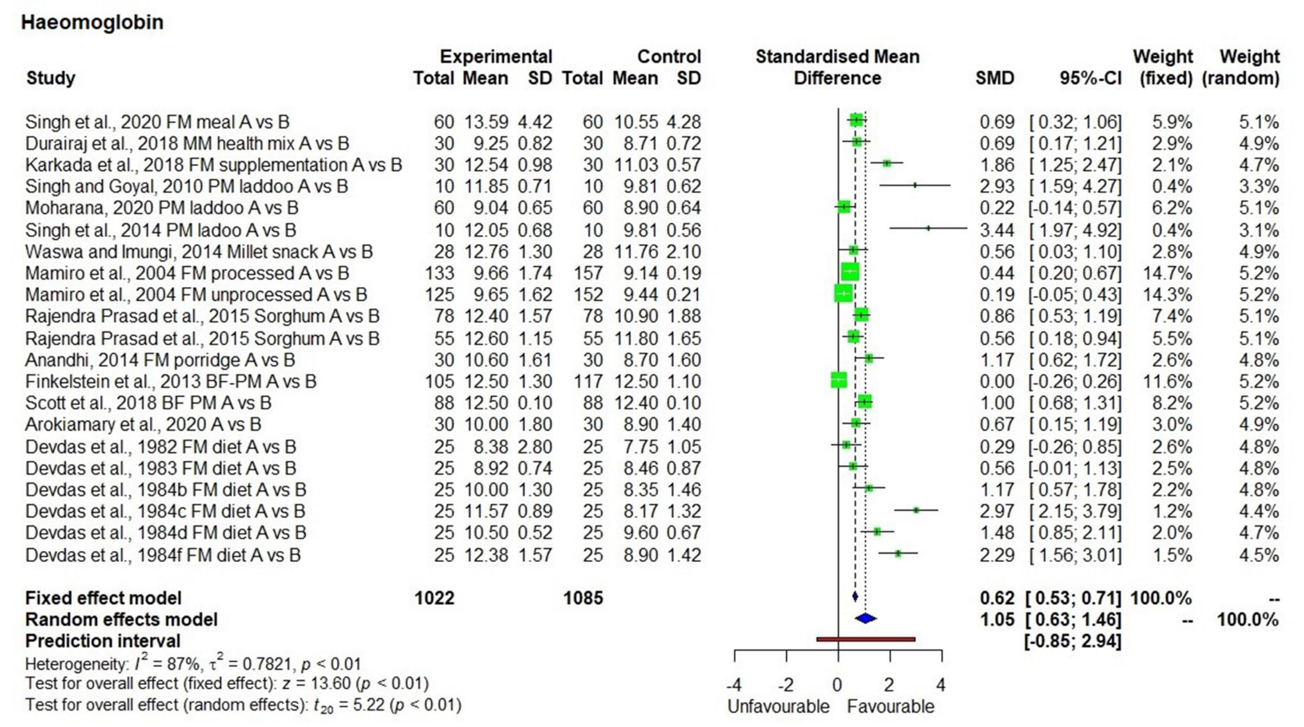

FIGURE 2 | Effect of consuming millets on Hemoglobin level.

namely, hemoglobin levels, serum ferritin levels, and total iron absorbed.

Hemoglobin Level

There were 19 studies (25–43) used to conduct the meta-analysis on hemoglobin levels, which showed high heterogeneity ($I^2 = 87\%$) and statistical significance (**Figure 2**). The hemoglobin levels in 1,022 individuals (from 19 studies) produced SMD of 1.05 at a 95% confidence interval (CI) ranging from 0.63 to 1.46 indicating a significant ($p < 0.01$) overall improvement in hemoglobin levels within the group that had consumed millets for a period ranging from 28 days to 4.5 years. On average, there was a 13.2% increase in hemoglobin levels relative to the baseline in the intervention group who received millet supplementation which is five times higher compared with only a 2.7% increase in hemoglobin levels in the control group who did not receive millet supplementation and were consuming regular rice or wheat-based diet. Seven studies conducted on adolescents showed an increase in hemoglobin levels from 10.8 ± 1.4 (moderate anemia) to 12.2 ± 1.5 g/dl (normal). The studies that qualified for the meta-analysis used finger millet, pearl millet, sorghum, or mixed millets (kodo, little, and foxtail millets). Among the 19 studies, 2 studies used pearl millet which had an iron content averaging 8.6 mg/100 g (44, 45), while the rest of the studies did not indicate the iron content of millets used in meal preparation.

Iron Absorption

The meta-analysis using two studies (44, 45) that measured iron absorption showed that bioavailable iron in the study that had used high iron pearl millet (8.2 mg/100 g grain) was significantly ($p < 0.01$) higher (**Figure 3**) than in the one that had used low iron millets (< 3 mg/100 g grain) with SMD of 1.25 and 95% CI of 0.77 and 1.74, respectively. The bioavailable iron was 1 ± 0.45 mg/day from a dietary iron intake of 14.1 mg/day compared with 0.42 ± 0.27 mg/day from a dietary iron intake of 6.3 mg/day, which is 7.5 ± 1.6 % bioavailability.

Serum Ferritin Level

Four studies (33, 35, 36, 46) measured serum ferritin, which was significantly increased in groups consuming high iron millet-based meals (**Figure 4**), compared with low iron millet-based meals or non-millet-based meals ($p < 0.01$) with moderate heterogeneity among the studies ($I^2 = 76\%$) and SMD of 0.59 and 95% CI of 0.13; 1.06.

There was a 54.7% average increase in serum ferritin levels after the consumption of pearl millet (two studies), sorghum meals (one study), and teff bread (one study). The average iron level in pearl millet-based meal was 8.2 mg/100 g while it was 5.6 mg/100 g in teff bread. The intervention was conducted for 6 weeks using teff bread and 6 months using pearl millet-based meals. The iron levels in sorghum used in the study for 8 months were not indicated.

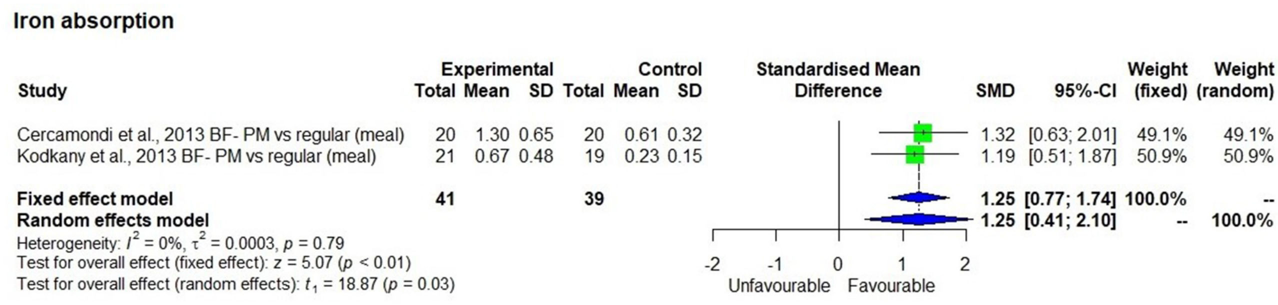

FIGURE 3 | Effect of consuming millet based meal on bioavailable iron content compared to regular meal.

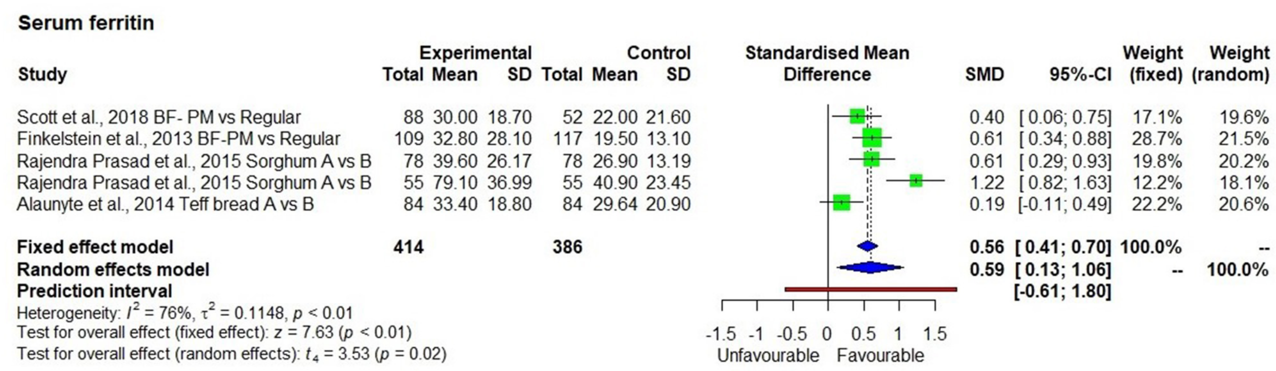

FIGURE 4 | Effect of consuming millet based meal on serum ferritin level.

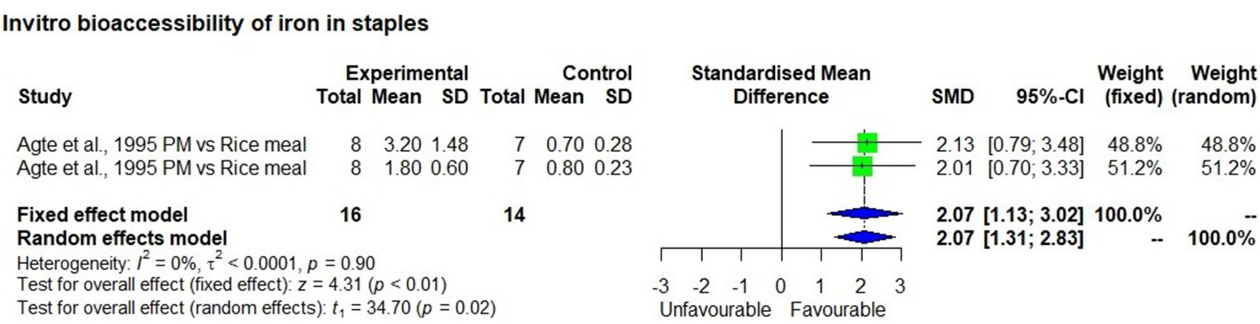

FIGURE 5 | In-vitro bioavailability of iron in millet compared to rice based meal.

Meta-Analysis of *in-vitro* Iron Bioavailability Studies

The meta-analysis included eight studies that measured *in vitro* iron bioavailability in pearl millet and the effects of processing. One study with two observations (47) showed 2.5 mg/100 g bioavailable iron in a high iron pearl millet-based meal, which was significantly higher ($p < 0.01$) than in the rice-based control meal (0.75 mg/100 g) with a bioavailability percentage of 7.5% and 7.9%, respectively (**Figure 5**). Similarly, seven *in-vitro* bioavailability studies showed (48–54), that processing such as germination, fermentation, decortication, expansion (a thermal process that increases the size and volume of the grain) and popping of millet grains had significantly ($p < 0.01$) increased bioavailable iron than those in unprocessed control millet grain (**Figure 6**). The increase in bioavailable iron by fermentation and germination was 3.4 times and 2.2 times higher, respectively than in unprocessed millets.

Additional Statistics

Statistical comparison was conducted to determine the percentage bioavailability of iron and bioavailable iron in high iron pearl millet in two *in vivo* studies (44, 45) and 11 *in*

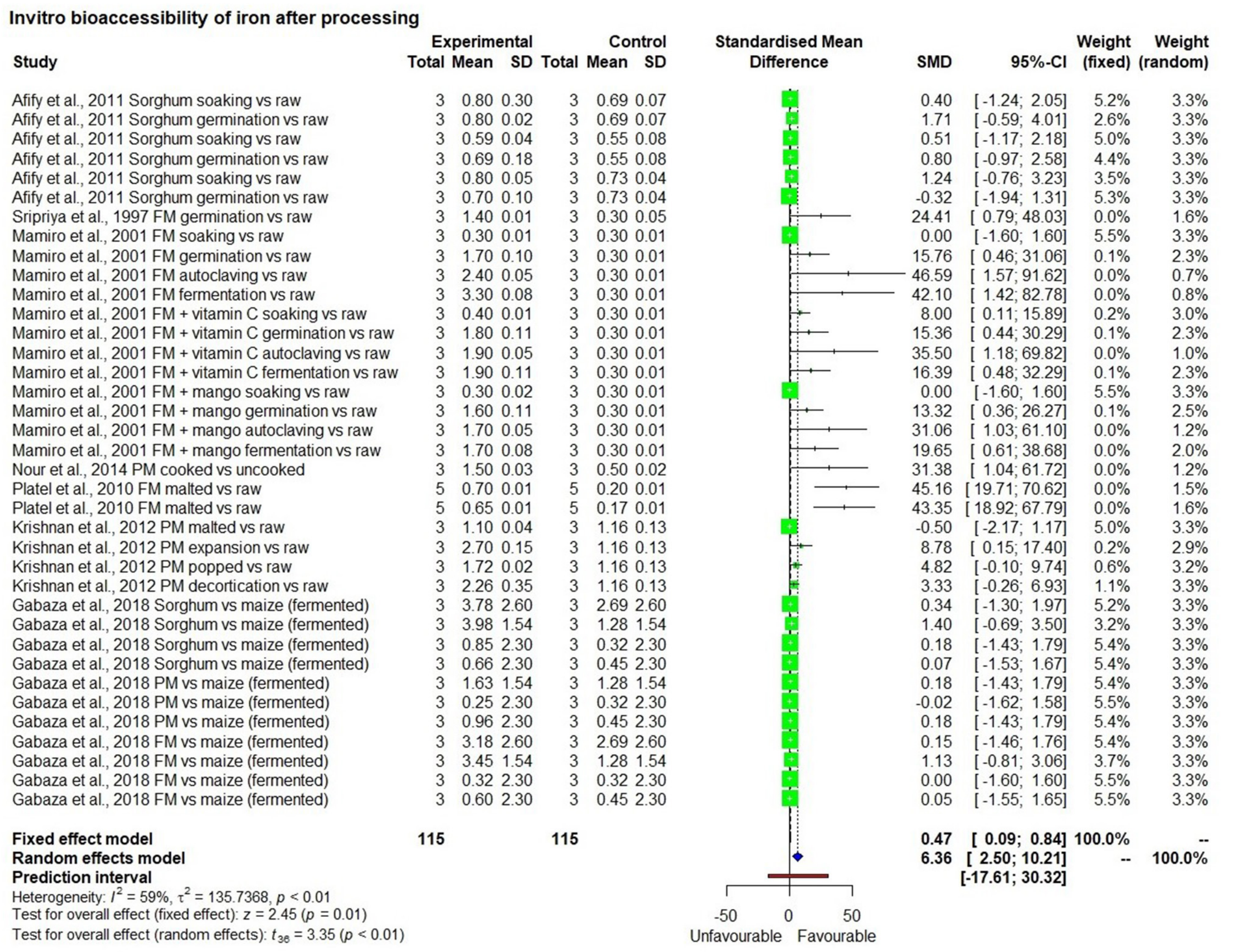

FIGURE 6 | In-vitro bioavailability of iron in millets after processing.

vitro studies on pearl millet, finger millet, and sorghum (47–57). The iron content in the millets used in the *in vitro* studies was 17.27 ± 13.38 mg/100 g. The *in-vitro* studies also showed a significant increase in bioavailable iron (mg/100 g) with the increasing iron content of millets (**Figure 7**). It is also noted some studies shows high iron content in the fermented millets even up to 49.7 mg/100 g (54). On the other hand, based on the two human studies conducted using iron-rich pearl millet (8.3 mg/100 g) showed the bioavailability of 7.5 ± 1.6% with bioavailable iron of 1.0 ± 0.4 mg/100 g, while the concentration of iron in the entire pearl millet-based meals was 14.1 ± 9 mg/100 g.

Regardless of the iron concentration in millets, the overall percentage bioavailability in millets from human studies was 7.22 ± 1.78%, with an overall bioavailable iron content of 0.7 ± 0.45 mg/100 g (**Table 2**).

In this systematic review, the iron content in millets, regardless of biofortification, were organized into low (<3 mg/100 g), moderate (3 to <6 mg/100 g), and high (6 mg/100 g) categories based on their provisions of >30% (high), 15–30% (moderate), and <15% (low) daily iron requirements for adults. The bioavailability from these categories was assessed. The results showed that meals prepared with high iron millets had high bioavailable iron that can provide 100% of the physiological requirement of iron as proposed by ICMR (2020) (**Table 3**). Results from *in vivo* studies showed that bioavailable iron was high in high iron millets (1 ± 0.4 mg/100 g), compared with low iron millets (0.4 ± 0.2 mg/100 g).

Effects of Processing on the Bioavailability of Iron

Processes such as fermentation, germination, and soaking did not affect the total iron content of the grain significantly. Moreover, there was significantly higher bioavailable iron in these processes compared with that in unprocessed millets (**Table 3**). On the other hand, decortication, popping, and malting reduced the iron content. However,

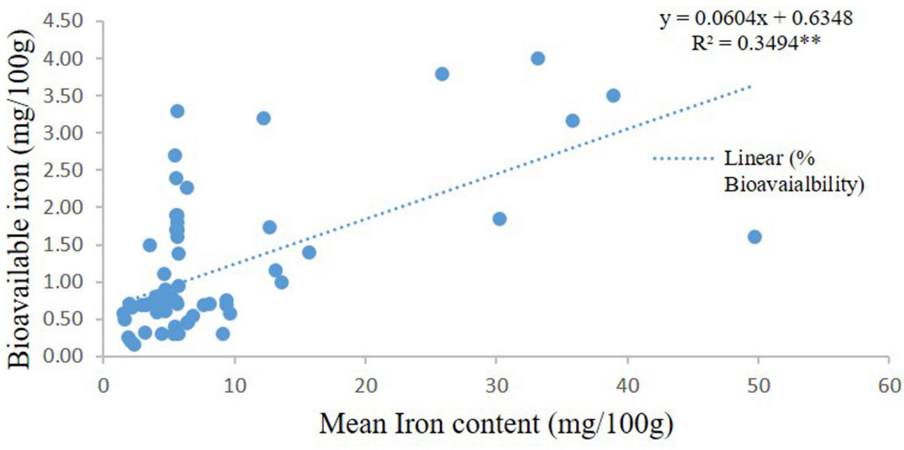

FIGURE 7 | In-vitro bioavailable iron from millets.

TABLE 2 | Iron bioavailability and bioavailable iron in millets based on the type of study (*in vitro* and *in vivo*).

Type of study	Iron content (mg/100 g; mean ± SD)	Iron bioavailability (%) (mean ± SD)	Bioavailable iron (mg/100 g; mean ± SD)
In vitro	8.26 ± 9.31	17.64 ± 12.49	1.13 ± 0.95
In vivo	9.8 ± 6.4	7.22 ± 1.78	0.70 ± 0.40

bioavailable iron was still higher than or equal to that in unprocessed grains.

Millets can provide 100% of the physiological requirement of iron for different age groups (45), though this depends on the type, variety, and the kind of processing undergone. Based on the results from *in vitro* studies, soaking was not significantly associated with an increase in iron bioavailability (**Table 3**). On the other hand, even if the iron content in millets was low, malting increased bioavailable iron by 3.5 times, and thereby bioavailable iron was 1.6 times higher than in unprocessed grains which increased its contribution to the physiological requirement to 1.7 times in adult women (from 39 to 68%). There were 17 observations on fermented millets which showed that the fermentation process did not affect the iron content of grains. However, it increased bioavailable iron by up to 3.4 times (from 0.5 to 1.7 mg/100 g) and can help increase the contribution to the physiological iron requirement by 3.4 times in adult women (from 39 to 143%) (**Table 3**). Fermentation was found to be superior to germination and malting. Germination increased bioavailable iron content by up to 2.2 times compared with unprocessed grains (from 0.5 to 1.1 mg/100 g) and helped meet 95% of the physiological requirement in adult women, which is 2.4 times higher than in unprocessed grains. Adding an absorption enhancing agent such as Vitamin C rich food improved the percentage of iron bioavailability by up to 6.8 times (50). Other processes such as decortication and dephytination using phytase enzyme are industrial processes. While both processes decreased iron content in grains by more than 50%, they increased bioavailable iron by 2.6 and 1.4 times, respectively, thereby increasing their contribution to the physiological requirement in adult women by 2.7 and 1.5 times, respectively.

Popping slightly reduced (3%) the iron content of grains. However, it increased bioavailable iron by 3.4 times and thereby increasing its contribution to the physiological requirement by 3.7 times. Compared with popping, expansion led to a loss of more than 60% of grain iron content while increasing bioavailable iron by 5.4 times compared with unprocessed grains and thereby increased the % contribution to the physiological requirement by 5.8 times in adult women.

It may be noted that processing did not have the same impact in all the studies, possibly due to the difference in the methods used, which needs further evaluation. Fermentation increased mean bioavailable iron content in millets more than all other processing methods.

Three studies conducted on the effects of processing on phytate content showed a reduction in phytate content by 29.7% after germination, 28.1% after soaking, 30.7% after decortication, and 51% after expansion (**Table 4**). This reduction in phytate content increased the bioavailability of iron in these studies. Decortication increased bioavailable iron content by 160% (0.5 mg/100 g to 1.3 mg/100 g).

Germination of finger millet increased bioavailable iron content from 0.4 to 1.3 mg/100 g and decreased tannin content by 50.4%. In pearl millet, cooking reduced tannin content by 5.2% (**Table 4**).

Subgroup Analysis by Type of Millet

A subgroup analysis was performed to determine the effects of consumption of millets on hemoglobin levels based on the type of millets used in the study (finger millet, pearl millet, mixed

TABLE 3 | The effect of processing on the bioavailability of iron in millets and its potential to meet the physiological requirement of iron.

Process	Mean iron content (mg/100 g)	Bioavailability (%)	Bioavailable iron (mg/100 g)	Contribution to the physiological requirement of iron (%) (ICMR, 2020)							
				1–3 yrs	4–6 yrs	7–9 yrs	10–12 yrs girls	13–15 yrs girls	16–17 yrs girls	Adult men	Adult women
***In vivo* human study**											
Boiled/baked meal	6.3 ± 3.1	6.0 ± 1.9	0.4 ± 0.3	105	82	66	31	29	27	44	33
Boiled/baked meal	12.6 ± 6.9	8.3 ± 1.1	1.0 ± 0.4	263	204	164	79	72	68	110	83
***In vitro* method**											
Raw/unprocessed grain	5.4 ± 2.9	9.8 ± 6.8	0.5 ± 0.3	124	96	77	37	34	32	52	39
Boiled meal	23.2 ± 17.2	19.1 ± 20.3	2.2 ± 0.9	570	442	355	171	156	148	238	181
Decorticated grain	4.2 ± 3.2	24.8 ± 15.7	1.3 ± 1.4	332	257	207	99	91	86	139	105
Dephytinized grain	3.3 ± 1.7	24.6 ± 8.7	0.7 ± 0.2	190	148	119	57	52	50	79	60
Fermented grain	14.6 ± 13.8	15.4 ± 13	1.7 ± 1.3	452	351	282	135	124	118	189	143
Germinated grain	5.2 ± 1.0	22.0 ± 8.2	1.1 ± 0.5	300	233	187	90	82	78	125	95
Malted flour	2.9 ± 1.4	28.0 ± 3.5	0.8 ± 0.2	215	167	134	64	59	56	90	68
Popped grain	12.7 ± 0.21	13.4*	1.73 ± 0.0	455	353	284	136	124	118	190	144
Expanded grain	5.5 ± 0.4	49.1*	2.7 ± 0.2	711	551	443	213	194	185	297	225
Soaked grain	4.9 ± 0.7	11.6 ± 6.1	0.5 ± 0.2	140	109	87	42	38	36	58	44

**SD values not available. All in vitro studies are based on 100 g of grain, whereas in vivo studies, they varied from 84 g to 300 g (44, 45, 47–50, 53–55, 57, 58). The quantity required to be consumed can be adjusted to meet 100% of the physiological requirement.*

TABLE 4 | The effects of processing on phytate and tannin content in millets (mg/100 g).

Type of grain	Raw	Germination	Soaking	Decortication	Cooking	Expansion	Popping
Phytate							
Sorghum	584.5 ± 25.4	410.5 ± 15.8	420.3 ± 9.2				
Finger millet	529.0 ± 0.3			363.0 ± 0.2	-	259.0 ± 0.5	549.0 ± 0.5
Pearl millet	203.0 ± 7.25				175.28 ± 2.54		
Reduction in phytate (%)		29.7	28.1	30.7	13.6	51.0	−3.8
Tannin							
Pearl millet	19.0 ± 0.0				18.0 ± 0.0		
Finger millet	973.9 ± 23.0	482.6 ± 12.6					
Reduction in tannin (%)		50.4			5.2		

millets, and sorghum), the duration of the study ('short' or 'long'), and the age group of the participants (children, adolescents, and adult). It was not possible to conduct a subgroup analysis based on the iron content of the grains since only three of the 19 studies on the effectiveness of millets on hemoglobin outcome indicated the iron content in millets used. A subgroup analysis conducted to study the effects of consuming different types of millets on hemoglobin levels showed no significant (**Figure 8**) difference due to using any particular type of millets ($p = 0.48$). Finger millet and pearl millet had similar effects on hemoglobin levels ($p < 0.05$). The effects of using mixed millets could not be estimated due to the small number of studies.

Risk of Bias Assessment

The risk of bias assessment shows major risks coming from blinding of the assessment. As millets have a unique colour, texture, and size it is not possible to conduct the study by blinding the sample. However, it is possible to blind the proportion added and the type of millets included, among other things. The sample size in studies was reasonable, ranging from 10 to 133 samples for assessing the impact of millet consumption on hemoglobin levels. Except for two studies, all the studies had more than 10 samples each. The Trim and Fit method were applied to account for the effects of small sample size on studies until the funnel plot became symmetrical ($p < 0.0001$).

DISCUSSIONS

This study used the term 'high iron millet' to indicate any millet that provides more than 6 mg iron/100 g of grain. Note that the four human interventions investigated were based on biofortified pearl millet. Biofortification is a process that increases the concentration of targeted nutrients in crops through breeding technologies and can

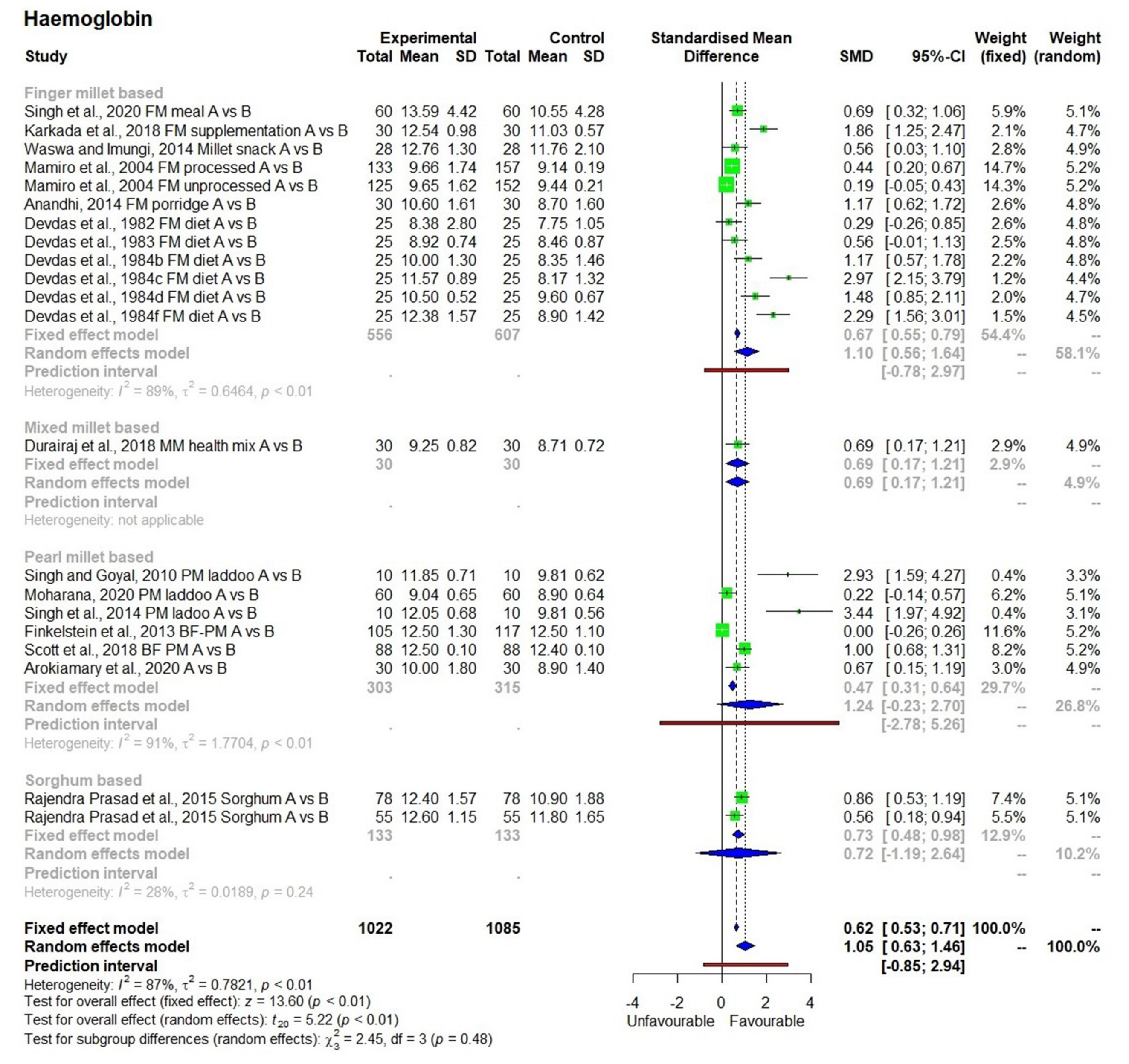

FIGURE 8 | Effect of consuming different types of millets on Hemoglobin level.

be a promising, sustainable, and cost-effective approach to combating micronutrient deficiencies (59). The other human studies and *in vitro* studies used varieties of millets that were commonly available.

All the *in vivo* human studies (lasting 28 days to 6 months), except two studies, used 84 to 300 g of pearl millet or finger millet in the form of a meal: *bhakri*/flatbread, porridge, or *upma* (thick porridge prepared by seasoning and adding spices, with or without vegetables). The meal was provided once or twice a day. It diversified the cereal-based main meal by incorporating millets and increased the intake of iron from the main meal.

Among the 19 studies in the meta-analysis to assess the impact on hemoglobin levels, only 3 studies indicated the levels of iron in millets used. The iron content in the millet grain has a huge impact on bioavailable iron, which in turn impacts the anemia status. It is noteworthy that the consumption of iron-rich pearl millet with 8.3 mg/100 g of iron levels contributed more than 50% of iron to the entire meal with 14.1 ± 9 mg of iron and improved the iron bioavailability with bioavailable iron of 1 ± 0.4 mg/day, compared with the consumption of a low iron pearl millet (< 3 mg/100 g) meal. Regardless of the percentage of bioavailability of iron, if the millet contains high iron, then the amount of bioavailable iron also increases. In general, the percentage bioavailability of iron from plant-based food varies from 1 to 10% (60) and ICMR. (15) also shows that it considered 8% bioavailability of iron from cereal-based diets. This shows that compared with many plant-based foods, millets have an equal or better bioavailability percentage.

However, the same was not the case in *vitro* studies, probably because of the variation in the methodology used.

In vitro Methods Used for the Bioavailability Study

Unlike *in-vivo*, *in-vitro* studies showed significant variation in the bioavailability percentage, with high iron millets having a lower average bioavailability percentage than moderate and low iron millets. However, the bioavailability percentage was generally high, averaging 17.64 ± 12.49 in the *in vitro* studies.

Taking bioavailability percentage into account, bioavailable iron varied significantly by variety and can approximately triple the quantity of iron bioavailable, ranging from 0.54 ± 0.21 in low iron varieties to 1.58 ± 1.24 in high iron varieties.

The huge variation in bioavailability percentage may be explained by the heterogeneity of the four methods adopted in the *in vitro* studies, which were iron solubility, iron dialysability, HCl-extractability, and gastrointestinal models. The *in vitro* methods aimed at mimicking the gastric and small intestinal phase involving the use of pepsin and pancreatin in iron solubility, dialysability and gastrointestinal methods. However, the studies of (49, 57) used HCl-extractability, which did not involve the use of pepsin and pancreatin and may have led to underestimation or overestimation since it did not consider the digestion of minerals in two key areas of the gastrointestinal tract, which distinguishes this method from the three others. The three other *in-vitro* methods also differed in the setup and conditions, which may have contributed to the differential outcome. The *in vitro* study by (50) used two approaches in the iron solubility method in which the usage of either HCl-pepsin or pepsin-pancreatin on mineral extractability, showed differences in the percentage of bioavailability. The differences can be explained by the endpoint measurements. The HCl-pepsin method measured the iron bioavailability at the end of the gastric phase with pH between 1 and 3 and the pepsin-pancreatin method measured the iron bioavailability at the end of the small intestinal phase with pH between 7 and 8. The differences in pH in these two stages may affect the iron solubility and hence the percentage of bioavailability. However, the authors did not outline the methods clearly. Four studies by (47, 53, 55, 56) used the iron dialysability method which was based on equilibrium dialysis. In the absence of *in vivo* epithelial uptake, this *in vitro* method adopted dialysis as a physical separation technique whereby a dialysis membrane was used. It is important to note that the selected molecular cut-off of the dialysis membrane, final pH adjustment, time of incubation, and the method used for iron quantification (colourimetric assay or spectrophotometry) are instrumental for consistency in the results (61). In addition, enzymes' sources, pH and digestion time, are also important parameters for standardization and may alter enzyme activities and possibly the results. The studies by (48, 54, 58) used gastrointestinal models and adopted different approaches in terms of simulated digestion fluids which influence the ionic strength and ratio of samples to buffer. The study of (54), adopted a more recent standardized static *in vitro* digestion model proposed by (62) which is useful to compare results of the inter and intra laboratory. Nevertheless, data validation between *in vitro* and *in vivo* studies may provide information when similar meals and experimental conditions are compared (61). Therefore, *in vitro* methods are useful screening tools to assess iron bioavailability involving a large number of food samples.

Post-harvest iron fortification of pearl millet (artificial fortification) increases the amount of iron available and can be added in large quantities to increase by 32% the total quantity of iron absorbed compared with naturally high iron pearl millet (44). However, a feasible and sustainable approach for long-run implementation would be to release more high iron millet varieties compared with any other method such as fortification and tablet supplementation. The studies showed that, based on the age group, 75 to 100% of the physiological requirement can be achieved through a standard meal prepared using high iron millets. It is noted that fortified foods have processing difficulties such as higher costs of processing and the use of artificial additives for post-harvest fortification. Given that there are many naturally occurring high iron millets, it is important to use them in efficacy studies to generate more science-based evidence and to enable the formulation of policies that would make them available to farmers and also increase the choices for consumers.

Processing had a significant positive impact on the bioavailability of iron (**Table 3**). Of the household and traditional processing methods, fermentation was found to be superior to all other processes for increasing bioavailable iron. Of the commercial processing methods, expansion was found to be superior compared with all other processing methods for increasing bioavailable iron. Generally, dietary inhibitory factors affect the efficiency of iron absorption (63). The major dietary inhibitory factors for iron absorption in millets are phytates and tannin. It is worth noting that millets have similar or lower levels of these anti-nutrients compared with common staples and legumes (5), which are further reduced by processing to positively improve the bioavailability of iron. Studies also showed significantly increased iron bioavailability in millets following different methods of processing. In addition, ascorbate (Vitamin C) in the presence of tannins decreases the chelating properties of tannins and thus increases the bioavailability of iron by up to 6.8 times (50). While one study showed that germination reduced tannin content by 50%, another study revealed a sixfold increase in the bioavailability of iron after the same process. The study of (64) demonstrated in *in vitro* studies that processing eliminated inhibitory factors such as phytates and increased the bioaccessibility of iron. However, *in vivo* studies are required to ascertain if similar effects are achieved using processed millets.

Considering the nutritional quality of all types of millets and their versatile nature of fitting into popular recipes using rice (3), replicating these studies with millets will be useful to identify variation in iron bioavailability and its benefits in reducing IDA.

LIMITATION

Iron contamination of food from post-harvest treatment, storage, and cooking vessels, which could increase the iron content of the grain, was not reported by any studies included in this systematic review. However, it was noted that there have been many studies that do specifically look at or incorporate the impacts of iron contamination from external sources on a variety of different foods (65–67). Especially while reporting high iron levels (>10 mg/100 g), it is important to look at the contamination from an external source (44) to avoid inflated data.

CONCLUSION

The systematic review and meta-analysis showed millets are an excellent source of iron with low-cost potential for reducing iron deficiency anemia. This underlined the need for policymakers to recognize the right varieties and types of millets rich in iron for use as supplement food to counter the high prevalence of anemia in many countries. Selecting the iron rich millet varieties and developing iron-biofortified millet that can provide additional bioavailable iron could be a promising approach to combatting IDA. Incorporating millets as a staple across Asia and Africa could have the potential to make a significant impact on IDA. This can also be applicable in communities where millets are traditional foods but not consumed regularly and access to alternative foods is limited. It can also be concluded that the bioavailability of iron in millets can be further improved through processes such as soaking, germination, decortication, and fermentation which can serve as an effective strategy to reduce iron deficiency anemia.

DATA AVAILABILITY STATEMENT

The original contributions presented in the study are included in the article/**Supplementary Material**, further inquiries can be directed to the corresponding author.

AUTHOR CONTRIBUTIONS

SA and JK-P: conceptualization. JK-P: resource. SA, JK-P, RKB, SU, and MV: data collection, screening, extraction, and analysis. SA, JK-P, IDG, NLBS, and SU: drafting original manuscript. TL, AR, TWT, KS, DJP, and RKB: critical reviewing of protocol and manuscript and editing. All authors contributed to the article and approved the submitted version.

FUNDING

This research was supported financially by the Smart Food endowment fund.

ACKNOWLEDGMENTS

The authors acknowledge Joycelyn M. Boiteau, Division of Nutritional Sciences, Cornell University, Tata-Cornell Institute for Agriculture and Nutrition, the United States for reviewing the case and her valuable inputs that contributed significantly to the quality of the study. Thanks are also due to Dr Colin I Cercamondi, Dr Julia Finkelstein, Dr Jere Haas (Project Investigator of the pearl millet trial) and Mr Amy Fothergill (doctoral student at Cornell University), who provided the data from their studies to support the meta-analysis of iron absorption and hemoglobin levels. Dr Waswa Judith confirmed the usage of finger millet in her study, which is highly appreciated. The authors acknowledge Ms Sitaraman Smitha, ICRISAT, for editing the manuscript.

SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fnut.2021.725529/full#supplementary-material

REFERENCES

1. WHO. *Prevalence Of Anemia Among Children Under 5 Years* (2016).
2. Balarajan Y, Ramakrishnan U, Ozaltin E, Sharkar AH, Subramanian SV. Anemia in low-income and middle-income countries. *Lancet.* (2011) 378:2123–35. doi: 10.1016/S0140-6736(10)62304-5
3. Anitha S, Kane-Potaka J, Tsusaka TW, Tripathi D, Upadhyay S, Kavishwar A, et al. Acceptance and impact of millet-based mid-day meal on the nutritional status of adolescent school going children in a peri urban region of Karnataka state in India. *Nutrients.* (2019) 11:1–16. doi: 10.3390/nu11092077
4. Vetriventhan M, Azevedo VCR, Upadhyaya HD. Genetic and genomic resources, and breeding for accelerating improvement of small millets: current status and future interventions. *Nucl.* (2020) 63:217–239. doi: 10.1007/s13237-020-00322-3
5. Longvah T, Ananthan R, Bhaskarachary K, Venkaiah K. *Indian Food Composition Table*. Hyderabad: National Institute of Nutrition (2017). p. 578.
6. Paganini D, Zimmermann MB. The effects of iron fortification and supplementation on the gut microbiome and diarrhea in infants and children: a review. *Am J Clin Nutr*. (2017) 106:1688S—93. doi: 10.3945/ajcn.117.156067
7. Paganini D, Uyoga MA, Zimmermann MB. Iron fortification of foods for infants and children in low-income countries: effects on the gut microbiome, gut inflammation, and diarrhea. *Nutrients.* (2016) 8:494. doi: 10.3390/nu8080494
8. Poole N, Kane-Potaka J. *The Smart Food Triple Bottom Line – Starting With Diversifying Staples Including Summary Of Latest Smart Food Studies At ICRISAT Agriculture for Development Journal, No. 41, Tropical Agriculture Association, UK. Ps 21–23.* (2020). Available online at: https://taa.org.uk/wp~content/uploads/2021/01/Ag4Dev41_Winter_2020_WEB.pdf (accessed March 3, 2021).
9. Rai KN, Patil HT, Yadav OP, Govindaraj M, Khariwal IS, Cherian B. Dhanshakti-A high-iron pearl millet variety. *Indian Farming*. (2014) 64:32–4.
10. Vetriventhan M, Upadhyaya HD, Azevedo VCR, Jayapal VA, Anitha, S. Variability and trait-specific accessions for grain yield and nutritional traits in

germplasm of little millet (Panicum sumatrense Roth. Ex. Roem. & Schult.). *Crop Sci*. (2021) 61:2658–79. doi: 10.1002/csc2.20527
11. Mohar D, Liberati A, Tetzlaff J, Altman DG. Preferred reporting items for systematic reviews and meta-analysis: the PRISMA statement. *Open Med.* (2009) 3:e1000097. doi: 10.1371/journal.pmed.1000097
12. Harrer M, Cuijpers P, Furukawa TA, Ebert DD. *Doing Meta-Analysis in R: A Hands-on Guide*. (2019). Available online at: https://bookdown.org/MathiasHarrer/Doing_Meta_Analysis_in_R/ (accessed February 28, 2021). doi: 10.1201/9781003107347
13. Atkinson LZ, Cipriani A. How to carry out a literature search for a systematic review: a practical guide. *Bjpsych Advances.* (2018) 24:74–2. doi: 10.1192/bja.2017.3
14. Beaton GH. *Minimum Physiological Requirements And Recommended Dietary Allowances. Joint FAO/WHO/UNU expert consultation on energy and protein requirements, Rome 1-8.* (1981).
15. ICMR. *Recommended Dietary Allowances and Estimated Average Requirements*. (2020). Hyderabad, India: National Institute of Nutrition. p. 1–319.
16. Luchini C, Stubbs B, Solmi M, Veronese N. Assessing the quality of studies in meta-analyses: advantages and limitations of the newcastle ottawa scale. *World J Meta-Anal.* (2017) 5:80–4. doi: 10.13105/wjma.v5.i4.80
17. Peterson J, Welch V, Losos M, Tugwell P. The newcastle-ottawa scale (nos) for assessing the quality of non-randomised studies in meta-analyses. Ottawa: Ottawa Hospital Research Institute (2011).
18. Bell A, Fairbrother M, Jones K. Fixed and random effects models: making an informed choice. *Qual Quant.* (2019) 53:1051–74. doi: 10.1007/s11135-018-0802-x
19. Zlowodzki M, Poolman RW, Kerkhoffs GM, Tornetta P, Bhandari M. How to interpret a meta-analysis and judge its value as a guide for clinical practice? *Acta Orthop.* (2007) 78:598–609. doi: 10.1080/17453670710014284
20. Ahn H, Kang E. Introduction to systematic review and meta-analysis. *Korean J Anaesthesiol.* (2019) 71:103–12. doi: 10.4097/kjae.2018.71.2.103
21. Hak T, Van Rhee HJ, Suurmond R. *How To Interpret Results Of Meta-Analysis. (Version 13) Rotterdam: The Netherlands: Erasmus Rotterdam Institute of Management p 1–2*. (2016). Available online at: http://www.erim.eur.nl/research~support/meta-essentials/downloads (accessed April 26, 2021).
22. Isreal H, Richter RP. A guide to understanding meta-analysis. *J Orthop SportsPhys Ther.* (2011) 41:496–504. doi: 10.2519/jospt.2011.3333
23. Balduzzi S, Rücker G, Schwarzer G. How to perform a meta-analysis with R: a practical tutorial? *Evid Based Ment Health.* (2019) 22:153–60. doi: 10.1136/ebmental-2019-300117
24. Higgins JPT, Thomas J, Chandler J, Cumpston M, Li T, Page MJ, et al. *Cochrane Handbook for Systematic Reviews of Interventions version 6.2 (updated February 2021). Cochrane*. (2021). Available online at: www.training.cochrane.org/handbook (accessed April 13, 2021)
25. Singh RM, Fedacko J, Mojto V, Isaza A, Dewi M, Watanabe S, et al. Effects of millet based functional foods rich diet on coronary risk factors among subjects with diabetes mellitus: a single arm real world observation from hospital registry. *MOJ Public Health*. (2020) 9:18–25. doi: 10.15406/mojph.2020.09.00318
26. Durairaj M, Gurumurthy G, Nachimuthu V, Muniappan K. Balasubramanian, S. Dehulled small millets: the promising nutricereals for improving the nutrition of children. *Matern Child Nutr.* (2019) 15:e12791. doi: 10.1111/mcn.12791
27. Karkada S, Upadhya S, Upadhya S, Bhat G. Beneficial effect of ragi (finger millet) on haematological parameters, body mass index, and scholastic performance among anemic adolescent high- school girls (AHSG). *Compr Child Adolesc Nurs*. (2018) 1–12. doi: 10.1080/24694193.2018.1440031
28. Singh TS, Goyal, M. Impact of an intervention based on pearl millet ladoo on Hemoglobin status of adolescent girls. *Research gate*. (2014) 1–6.
29. Moharana A, Khosla P, Nayak D, Tripathy P. Effect of finger millet [ragi] ladoo consumption on the level of hemoglobin. *Eur J mol clin med*. (2020) 7:1018–22.
30. Singh TS, Goyal M, Sheth M. Intervention trials with pearl millet based iron rich ladoo and iron folic acid (IFA) tablets on hemoglobin status of adolescent females in Bikaner city. *Ethno Med.* (2014) 8:77–82. doi: 10.1080/09735070.2014.11886475
31. Waswa J, Imungi JK. Efficacy of millet based product on management of geophagy: a randomized control trial. *J Food Nutr Disord.* (2014) 3:3–10. doi: 10.4172/2324-9323.1000152
32. Mamiro PS, Kolsteren PW, Van camp JH, Robertfroid DA, Tatala S, Opsomer AS. Processed complementary food does not improve growth or hemoglobin status of rural Tanzanian infants from 6-12 months of age in kilosa district, Tanzania. *Community Int Nutr*. (2004) 1084–90. doi: 10.1093/jn/134.5.1084
33. Prasad MPR, senhur B, Kommi K, Madhari R, Rao MV, Patil JV. Impact of sorghum supplementation on growth and micronutrient status of school going children in southern India–A randomized trial. *Indian J Pediatr.* (2015) 42:51–6.
34. Anandhi S. *Comparative Study TO Assess the Effectiveness of Drumstick Leaves Extract vs Ragi Porridge in Increasing the Level of Hemoglobin Among Women With Iron Deficiency Anemia In Selected Community Area at Kanchipuram District*. Student thesis submitted to MGR medical University, Chennai, Tamil Nadu. (2014). p. 93.
35. Finkelstein JL, Mehta S, Udipi SA, Ghugre PS, LunaSV, Wenger MJ, et al. A randomized trial of iron-biofortified pearl millet in school children in India. *J Nutr.* (2015) 145:1576–81. doi: 10.3945/jn.114.208009
36. Scott SP, Murray-Kolb LE, Wenger MJ, Udipi SA, Ghugre PS, Boy E, et al. Cognitive performance in Indian school going adolescents is positively affected by consumption of iron-biofortified pearl millet: a 6-month randomized controlled efficacy trial. *J Nutr*. (2018) 1462–71. doi: 10.1093/jn/nxy113
37. Arokiamary S, Senthilkumar R, Kanchana S. Impact of pearl millet based complementary food on biochemical and cognitive profiles of school children (5-6 year old). *Eur J Nutr Food Saf.* (2020) 12:24–34. doi: 10.9734/ejnfs/2020/v12i730245
38. Devdas RP, Chandrasekhar U, Bhooma. Nutritional outcomes of a rural diet supplemented with low cost locally available foods – I impact on expectant women. *The Ind J Nutr Dietet.* (1982) 19:111–6.
39. Devdas RP, Chandrasekhar U, Bhooma. Nutritional outcomes of a rural diet supplemented with low cost locally available foods – II Impact on nursing mothers. *The Ind J Nutr Dietet.* (1983) 20:71–8.
40. Devdas RP, Chandrasekhar U, Bhooma. Nutritional outcomes of a rural diet supplemented with low cost locally available foods – IV impact on children studied from birth to pre – school age. *The Ind J Nutr Dietet.* (1984) 21:115–23.
41. Devdas RP, Chandrasekhar U, Bhooma. Nutritional outcomes of a rural diet supplemented with low cost locally available foods – V impact on pre-schoolers followed over a period of four and a half years. *The Ind J Nutr Dietet.* (1984) 21:153–64.
42. Devdas RP, Chandrasekhar U, Bhooma. Nutritional outcomes of a rural diet supplemented with low cost locally available foods – VI impact on school children. *The Ind J Nutr Dietet.* (1984) 21:187–93.
43. Devdas RP, Chandrasekhar U, Bhooma. Nutritional outcomes of a rural diet supplemented with low cost locally available foods – VIII impact on preschool children from a tribal community. *The Ind J Nutr Dietet.* (1984) 21:315–21.
44. Cercamondi CI, Egli IM, Mitchikpe E, Tossou F, Zeder C, Hounhouigan JD, et al. Total iron absorption by young women from iron-biofortified pearl millet composite meals is double that from regular millet meals but less than that from post-harvest iron-fortified millet meals. *J Nutr*. (2013) 143:1376–82. doi: 10.3945/jn.113.176826
45. Kodkany BS, Bellad RM, Mahantshetti NS, Westcott JE, Krebs NF, Kemp JF. Biofortification of pearl millet with iron and zinc in a randomized controlled trial increases absorption of these minerals above physiologic requirements in young children. *J Nutr.* (2013) 143:1489–93. doi: 10.3945/jn.113.176677
46. Alaunyte L, Stojceska V, Plunkett A, Derbyshire E. Dietary iron intervention using a staple food product for improvement of iron status in female runners. *J Int Soc Sports Nutr.* (2014) 11:1–8. doi: 10.1186/s12970-014-0050-y
47. Agte VV, Gokhale MK, Panikar KM, Chiplonkar SA. Assessment of pearl millet vs rice based diets for bioavailability of four trace metals. *Plant Foods Hum Nutr.* (1995) 48:149–58. doi: 10.1007/BF01088311
48. Afify AE-MMR, El-Beltagi HS, El-Salam SMAbd, Omran AA. Bioavailability of iron, zinc, phytate and phytase activity during soaking and germination of white sorghum varieties. *PLoS ONE*. (2011). 6:e25512. doi: 10.1371/journal.pone.0025512

49. Sripriya G, Antony U, Chandra TS. Changes in carbohydrate, free amino acids, organic acids, phytate and HCl extractability of minerals during germination and fermentation of finger millet (*Eleusine coracana*). *Food Chem.* (1997) 58:345–50. doi: 10.1016/S0308-8146(96)00206-3
50. Mamiro PRS, Van Camp J, Mwikya SM, Huyghebaert A. In vitro extractability of calcium, iron, and zinc in finger millet and kidney beans during processing. *Food Chem Toxicol*. (2001) 66:1271–5.
51. Nour AAM, Sokrab AM, Ahmd IAM, Babiker EE. Supplementation and cooking of pearl millet: change in anti-nutrients, and total minerals content and extractability. *Innov Rom Food Biotechnol.* (2014) 15:9–22.
52. Platel K, Epieson SW, Srinivasan K. Bioaccessible mineral content of malted finger millet (Eleusine coracana), wheat (Triticum aestivum), and Barley (Hordeum vulgare). *J Agric Food Chem.* (2010) 58:8100–3. doi: 10.1021/jf100846e
53. Krishnan R, Dharmaraj U, Malleshi NG. Influence of decortication, popping and malting on bioaccessibility of calcium, iron, and zinc in finger millet. *Food scitechnol.* (2012) 48:169–74. doi: 10.1016/j.lwt.2012.03.003
54. Gabaza M, Abraha HS, Muchuweti M, Vandamme P, Raes K. Iron and zinc bioaccessibility of fermented maize, sorghum and millets from five locations in Zimbabwe. *Food Res Int.* (2018) 103:361–70. doi: 10.1016/j.foodres.2017.10.047
55. Priya S, Kowsalya S. In-vitro-bioaccessibility of iron and zinc from millet based convenience foods. *Int J Adv Eng Res Dev.* (2017) 4:158–62. doi: 10.1111/j.1365-2621.2001.tb15200.x
56. Hemalatha S, Platel K, Srinivasan K. Influence of germination and fermentation on bio accessibility of zinc and iron from food grains. *Eur J Clin Nutr.* (2007) 61:342–8. doi: 10.1038/sj.ejcn.1602524
57. Suma PF, Urooj A. Influence of germination on bioaccessible iron and calcium in pearl millet (Pennisetum typhoideum). *J Food Sci Technol.* (2014) 51:976–81. doi: 10.1007/s13197-011-0585-8
58. Lestienne I, Besanc P, Caporiccio B, Lullien-peallerin V, Treache S. Iron and Zinc in Vitro Availability in Pearl Millet Flours (Pennisetum glaucum) with Varying Phytate, Tannin, and Fiber Contents. *J Agri Food Chem.* (2005) 53:3240–7. doi: 10.1021/jf0480593
59. Bouis HE, Hotz C, McClafferty B, Meenakshi JV, Pfeiffer WH. Biofortification: a new tool to reduce micronutrient malnutrition. *Food Nutr Bull.* (2011) 32:S31–41. doi: 10.1177/15648265110321S105
60. Givens DI, Sulaiman NLB, Anitha S. Iron deficiency anemia in India, prevalence, aetiology and solutions: a narrative review. (Unpublished)
61. Aragon IJ, Ortiz D, Pachon H. Comparison between in vitro and in vivo methods to screen iron bioavailability. *CyTA J Food.* (2012) 10:103–11. doi: 10.1080/19476337.2011.596283
62. Minekus M, Alminger M, Alvito P, Balance S, Bohn T, Bourlieu C, et al. Standardized static in vitro digestion method suitable for food – an international consensus. *Food Funct.* (2014) 5:1113–24. doi: 10.1039/C3FO60702J
63. Chiplonkar SA, Agte VV. Statistical model for predicting non-heme iron bioavailability from vegetarian meals. *Int J Food Sci Nutr.* (2006) 57:434–50. doi: 10.1080/09637480600836833
64. Abdalla AA, Ahmed IA, Tinay AHEl. Influence of traditional processing on minerals HCl-extractability of pearl millet (Pennisetum glaucum). *Res J Agric Biol Sci.* (2010) 6: 530–4.
65. Adish AA, Esrey SA, Gyorkos TW, Jean-Baptiste J, Rojhani A. Effect of 610 consumption of food cooked in iron pots on iron status and growth of young children: a randomised trial. *The Lancet*. (1999) 353:712–6. doi: 10.1016/S0140-6736(98)04450-X
66. Geerlings PPD, Brabin BJ, Hart DJ, Fairweather-Tait SJ. Iron contents of malawian foods when prepared in iron cooking pots. *Int J Vitam Nutr Res.* (2004) 74:21–6. doi: 10.1024/0300-9831.74.1.21
67. Harvey PWJ, Dexter PB, Darnton-hill I. The impact of consuming iron from non-food sources on iron status in developing countries. *Public Health Nutr*. (2000) 3:375–383. doi: 10.1017/S1368980000000434

Conflict of Interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's Note: All claims expressed in this article are solely those of the authors and do not necessarily represent those of their affiliated organizations, or those of the publisher, the editors and the reviewers. Any product that may be evaluated in this article, or claim that may be made by its manufacturer, is not guaranteed or endorsed by the publisher.

frontiers in Sustainable Food Systems

REVIEW
published: 27 October 2021
doi: 10.3389/fsufs.2021.727533

A Narrative Review: *In-vitro* Methods for Assessing Bio-Accessibility/ Bioavailability of Iron in Plant-Based Foods

N. Sulaiman[1]*, D. I. Givens[1] and S. Anitha[2]

[1] Institute for Food Nutrition and Health, School of Agriculture Policy and Development, University of Reading, Reading, United Kingdom, [2] International Crops Research Institute for the Semi-Arid Tropics Patancheru, Hyderabad, India

In-vitro measurement has the advantage of rapid and convenient method of screening the iron bioavailability within the range of plant-based foods. It is important to do preliminary screening as it provides information which will be useful to identify promising plant sources of iron before moving to human trials. A review on *in-vitro* methods of bio-accessibility and bioavailability of iron in plant-based foods including fruits, vegetables, cereals and legumes is entailed here. The review will focus on *in-vitro* methods of iron bioavailability in plant-based foods and the effects of inhibitors and processing on the iron bioavailability. The variation of the methods and updates on a recent INFOGEST method used to measure the bioavailability of iron in plant-based foods will also be discussed.

Keywords: ***in-vitro*, iron, bioavailability, bio-accessibility, INFOGEST**

OPEN ACCESS

Edited by:
Kathleen L. Hefferon, Cornell University, United States

Reviewed by:
Ömer Faruk Mizrak, Istanbul Sabahattin Zaim University, Turkey
Jayashree Arcot, University of New South Wales, Australia

***Correspondence:**
N. Sulaiman
nur.sulaiman@reading.ac.uk

Specialty section:
This article was submitted to Nutrition and Sustainable Diets, a section of the journal Frontiers in Sustainable Food Systems

Received: *18 June 2021*
Accepted: *27 September 2021*
Published: *27 October 2021*

Citation:
Sulaiman N, Givens DI and Anitha S (2021) A Narrative Review: In-vitro Methods for Assessing Bio-Accessibility/Bioavailability of Iron in Plant-Based Foods. Front. Sustain. Food Syst. 5:727533. doi: 10.3389/fsufs.2021.727533

INTRODUCTION

In the recent years, a successive increase in encouraging people to consume more plant-based foods has been observed due to their benefit to health as well as being sustainable. In parts of the world especially in low-and middle-income countries (LMIC), plant-based foods contribute to a high proportion of the dietary intake. In these countries, plant-based foods are the main source of macro-and micronutrients including iron. However, inadequate consumption of iron-rich plant-based food, consumption of large quantities of refined food and inadequately fortified food eventually contribute to dietary iron deficiency and poor bio-accessibility/bioavailability of iron to play its functional role in the body. The amount of iron in vegetables, fruits, cereals and legumes range between 0.3–13.9, 0.2–2.8, 1.5–7.6 and 6.2–15.7 mg/100 g respectively (Rousseau et al., 2020). One of the main concerns associated with having plant-based foods as the main source of iron, particularly in LMIC countries where intake of red meat is low, is its limited bio-accessibility/bioavailability from plant-based foods which contributes to iron deficiency problems (Platel and Srinivasan, 2016). Bio-accessibility is the amount of ingested nutrient that is released from the food matrix and potentially available for absorption and physiological function and bioavailability is defined as the amount of ingested nutrient that is absorbed and available for physiological functions (Etcheverry et al., 2012). Both are dependent on digestion and release from the food matrix, but bioavailability is also dependent on absorption by intestinal cells and transport to body cells. In this review, bioavailability will be used to explain both terms despite the difference in the meaning as recognized by most of the studies referenced in here. Moreover, in order for a nutrient to be bioavailable it has to be bio-accessible.

Bioavailability of iron is strongly influenced by the balance between iron inhibitors and enhancers that act as controlling factors for iron absorption. Anti-nutrients such as phytic acid, tannin, calcium and dietary fiber are the main inhibitors of iron absorption. Plant cell walls can act as a physical barrier which reduces the digestibility of the food matrix which can also reduce the mineral bioavailability (Rousseau et al., 2020). Even though many plant-based foods are rich in iron and other minerals, it is important to acknowledge that the release of these nutrients might be limited due to several factors impacting their absorption and therefore bioavailability.

Iron deficiency is one of the most prevalent forms of malnutrition affecting nearly 30% of the world's population including 29% of non-pregnant women, 38% of pregnant women and 43% of children worldwide (WHO, 2020). Low iron intake due to diets that are poor in iron and particularly low bioavailable, non-haem iron can contribute to iron deficiency. Iron deficiency is most likely to occur in LMIC countries. Therefore, it is of great importance to investigate the effects of different plant types and processing methods including cooking, on the bioavailability of minerals to ensure that diets can be formulated to ensure that adequate amounts of nutrients are available for absorption. This will also allow further strategies to be implemented to improve the bioavailability of micronutrients from plant-based foods to address issues on mineral deficiencies.

Generally, there are four main *in-vitro* methods used for measuring iron bioavailability, which are solubility, dialysability, gastrointestinal models and Caco-2 cell models. Each has its own advantages and disadvantages of the endpoint measurement. It is also important to note the form of the plant-based food whether it is consumed as an individual food or within a meal. This review will focus on the four methods used to measure iron bioavailability in plant-based foods either in raw or cooked form.

The aim of the review is to update information on recent *in-vitro* method called INFOGEST (Minekus et al., 2014) along with other commonly used *in-vitro* solubility, dialysability and Caco-2 cell culture methods used as an alternative to human studies. Limitations and recommendation are also discussed.

IRON IN THE DIET

Iron is an essential micronutrient as it plays an important role in oxygen transport, oxidative metabolism, cellular proliferation and other physiological functions (Nair and Iyengar, 2009). Dietary iron exists in two forms, haem and non-haem iron. Haem iron is only found in animal-based foods such as red meat, poultry and seafood and typically about 40% of the iron is in the haem form which has good bioavailability. The other 60% of the iron in animal tissues is in non-haem form. All of the iron in plant-based food is the non-haem form and is less bioavailable (Nair and Iyengar, 2009; Pizarro et al., 2016). According to a review by Rousseau et al. (2020) the absorption of haem iron is facilitated through haem iron specific receptors on the microvilli of the enterocytes where it will split from the porphyrin ring complex once it is absorbed. With non-haem iron, a distinction must be made between iron in ferrous state (Fe^{2+}) and the ferric state (Fe^{3+}). The important step impacting iron bioavailability is the reduction of ferric to ferrous by the action of gastric acid and ferric reductases in the intestinal lumen (Ferruzzi et al., 2020). The transporter carries iron in the ferrous state together with the protons across the mucosal membrane. However, most of the non-haem iron that enters the gastrointestinal tract is in the ferric state which is insoluble and has low bioavailability. In the ferric state, the iron must be reduced before it can be absorbed at the enterocyte (Nair and Iyengar, 2009). However, there are dietary components capable of reducing Fe^{3+} to Fe^{2+} such as ascorbic acid (Blanco-Rojo and Vaquero, 2019).

A review by Hurrell and Egli (2010), found that the percentage of iron bioavailability in mixed diets is estimated to be in the range of 14–18% whereas, it is 5–12% from vegetable diets typical of those in Western countries. This means that, while inadequate intake of iron contributes to the problem of iron deficiency, lower bioavailability of iron from plant-based diets also contributes to this problem. Vegetarians who depend on plant-based diets are more susceptible to iron deficiency due to the lower bioavailability of non-haem iron. According to ICMR-NIN (2020), dietary iron assumed absorbability from plant-based foods varies and is given as 8% for men, women, lactating women and adolescents, 12% for pregnant women, 15% for infants (6–12 months) and 6% for children. Using these values and assuming the concentration of 15 mg Fe/100 g of plant-based foods, 100 g would provide 0.9, 1.2, 1.8 and 2.3 mg/d of absorbable iron for children, men (also for women, lactating women and adolescents), pregnant women and infants respectively. Accordingly, this would only meet the ICMR-NIN (2020) net physiological estimated average requirement [i.e., EAR * (absorbability%/100) of iron for men (0.88 mg/d), non-pregnant and non-lactating women (1.20 mg/d), children (0.36–0.60 mg/d), and young adolescents (0.96 mg/d)]. There are strategies aimed at increasing the iron concentration in major crops such as millets and beans by bio-fortification, however this does not directly translate into an increase in absorbed iron. Moreover, it is important to understand the bioavailability of iron in such new initiatives like bio-fortification, before suggesting for nutrition interventions, dietary plans or policy changes. It is also necessary to understand the effects of enhancers or new enhanced crops by screening their iron bioavailability. To generate evidence-based science on iron bioavailability, *in-vitro* methods are considered simple, cost effective (compared to human efficacy studies) and useful screening tools as a first step to evaluate the bioavailability.

BARRIERS/FACTORS IMPACTING IRON BIOAVAILABILITY FROM PLANT-BASED FOODS

Antinutrients are abundant in plant-based foods and their main role is to act as chemical defense mechanism against pathogens and often interfere with the digestion and absorption of micronutrients. Phytic acid is one of the major inhibitors of iron bioavailability as it forms a complex with iron and is often present in high concentrations (Glahn et al., 2016). It is a principal storage form of phosphate found in plants, particularly in cereals and legumes. The high negative charge present on phytic acid at the small intestinal pH of 6 to 7 facilitates the chelation with iron resulting in insoluble complexes which reduce iron bioavailability (Ferruzzi et al., 2020).

Phenolic compounds such as tannins are also known to impact iron bioavailability. In the case of pearl millet, the iron bioavailability was not only affected by phytate but also by tannins (Lestienne et al., 2005a). Similarly, dietary fiber also contributes to reduced bioavailability as it has the ability to bind minerals.

There is also evidence suggesting that physical barriers such as the cell wall from beans' cotyledons and intracellular matrix contribute a significant barrier to iron absorption (Mamiro et al., 2001; Glahn et al., 2016). Therefore, the *in-vitro* studies could provide an insight to the effects of processing on the bioavailability of iron from various of plant-based sources and the bioavailability of different grain fractions.

A recent review by Glahn and Noh (2021) discussed about the need to redefine the current approach to iron bio-fortification particularly in common beans. According to the author, the current bio-fortification program may not be effective due to lack of data from *in-vitro* methods, animal studies and other analytical techniques to facilitate strategic human studies. The alternative approach to improve the nutritional quality of iron from beans is to understand factors and traits that affect iron bioavailability. The factors include disruption of the cotyledon cell wall in order to release intracellular iron, reduction of phytic acid by using endogenous phytase or soaking prior to consumption, the influence of other components in the diet or within a meal and traits such as seed polyphenolic that enhance iron bioavailability.

Despite the barriers, iron bioavailability can be improved by the addition of enhancers such as ascorbic acid. Many *in-vitro* studies showed a positive enhancement on the bioavailability of iron using ascorbic acid when added to lentils, white beans and carioca beans, yellow beans and black beans (Glahn et al., 2016). In this study, the ascorbic acid was added to the digests at the start of the gastric phase at a concentration of 10 μmol/L. The addition of ascorbic acid enhanced the iron bioavailability in lentil, white bean, carioca bean and pressure-cooked black bean sample. This proves that *in-vitro* measurements are an important tool to study the effects of enhancers or fortifications to improve the bioavailability of iron in plant-based foods.

Furthermore, the bioavailability of iron can be influenced by processing methods such as cooking, which can increase the bioavailability of iron as it may reduce the content of antinutrients such as phytate and when heat is introduced resulting in softening of the food matrix and releasing iron that is bound to protein (Hemalatha et al., 2007a).

In-vitro METHODS OF INVESTIGATING BIOAVAILABILITY OF IRON

As mentioned above, *in-vitro* methods are considered as an appealing alternative to human and animal *in-vivo* studies. They are used as a tool to measure the iron bioavailability in various samples because of the simple, low cost and rapid technique that can be replicated inter-and intra-laboratory. Moreover, human studies present higher variability due to potentially large variability between subjects' iron status. *In-vitro* methods are also useful to assess the effects of processing, bio-fortification, and enhancers on iron bioavailability. Previous studies have used various types of *in-vitro* methods for cereals, legumes and fruits and vegetables.

There are four widely used *in-vitro* methods for accessing bioavailability of iron and the main principal of these methods is to simulate the upper gastrointestinal tract digestion. Iron absorption mainly occurs in the small intestine, hence the endpoint for measuring the iron bioavailability is in the equivalent of upper small intestine (Bohn et al., 2018).

Iron Solubility

The first proposed method for measuring the bioavailability of iron was by Miller et al. (1981) using the principal methods of solubility and dialysability and was later modified by Kapsokefalou and Miller (1991). These models are used to simulate the gastric and intestinal conditions with controlled temperature, agitation, pH, enzyme and chemical composition. The gastric phase is performed with HCl or HCl-pepsin under fixed pH and temperature conditions. The food is incubated at a pH range of 1 to 3 at 37°C for 1 to 3 h. This is followed by an intestinal phase with the addition of pancreatin and bile at neutral pH for between 30 min and 2 h. The solubility method requires centrifugation to separate the supernatant and pellet prior to the measurement of iron in the soluble fraction. The range of pH and time used for incubation directly affect the bioavailability of the iron.

As outlined previously, a high iron content is not always related to high iron bioavailability. A study using *in-vitro* solubility has been reported by Sahuquillo et al. (2003) on iron bioavailability from chickpeas, white beans and lentils using 10 g and 20 g of flour of each legume. This study showed that lentils had the highest total iron content compared to white beans and chickpeas but the lowest iron bioavailability. The authors also mentioned that the comparison of the values with those reported in the literature is difficult due to differences in the methods used. As shown in **Table 1** there were differences in pH, time of incubation, centrifugation force and other additional modifications amongst studies which are important factors for this method.

As mentioned previously, phytates and fibers are inherent factors that reduce iron bioavailability. A study by Lestienne et al. (2005b) explored the effects of these inhibitors on iron bioavailability in pearl millet flour using an *in-vitro* solubility method. The flours were treated with endogenous and exogenous phytase to reduce the phytate content. The iron bioavailability of the different fractions of whole pearl millet grain with low and high fiber and tannin contents were also investigated (Lestienne et al., 2005a). The pH used in gastric phase was 2 for 1 h and the intestinal phase was pH 7 for 2 h. A study by Luo et al. (2010) investigated the effects of phytate degradation in faba bean flour and Luo et al. (2013) on the effects of germination and cooking on faba bean, azuki bean and mung bean sprouts on the iron

TABLE 1 | Iron bioavailability studies using *in-vitro* solubility methods.

Type of study/foods	Conditions (pH, incubation time, centrifugation, buffer)			Iron quantification	Findings	References
	Oral phase	**Gastric phase**	**Intestinal phase**			
Estimating the bio-accessibilities of iron form white beans, lentils and chickpeas with the influence of the sample size	–	Simple assay with 10 g of ground legumes used Double assay 20 g of ground legumes used Pesin, HCl, pH 2, 37°C, 2 h	1M $NaHCO_3$ to raise the pH to 5, pancreatin-bile, 37°C for 2 h Before centrifugation (3,200 rpm 20 min at 20°C, pH was adjusted to 7.2 using 0.5 M NaOH	Flame absorption spectroscopy (FAAS)	Bioavailability (µg/g) *White beans* Simple assay 34.4 Double assay 34.5 *Chickpea* Simple assay 28.9 Double assay 28.1 *Lentils* Simple assay 10.7 Double assay 15.6	Sahuquillo et al. (2003)
Effects of phytase treatment on the iron bioavailability from the whole pearl millet flours	About 2 g of dry sample was precisely weighed in an Erlenmeyer and suspended in 20 ml of distilled water.	After 10 min of conditioning in a shaking water bath at 37°C, pH was adjusted to 2.0 with 1 M HCl solution under magnetic stirring. Next, 1.0 ml of the pepsin solution was added, and the mixture was incubated for 1 h. The pH was then increased to about 4.0 with 0.15 mM PIPES	5.0 ml of the pancreatin-bile, and pH adjusted to 7.0 with the PIPES buffer that allows minimizing pH variation, 2 h. The suspension was then centrifuged at 10,000 g for 30 min at 4°C	AAS	Bioavailability (%) Raw: 9.9% Endogenous phytase (1 h): 22.1% Endogenous phytase (3.5 h): 24.1% Exogenous phytase (1 h): 17.7% Exogenous phytase (3.5 h): 21.4%	Lestienne et al. (2005a)
Assessing the effects of fiber and phytate degrading enzymes on iron solubility from pearl millet flour and grain fractions	–	Pepsin, HCl, pH 2, 1 h	Pancreatin-bile, pH 7, 2 h Centrifuged at 10,000 g for 30 min at 4°C	AAS	Bioavailability (%) Raw 24.9 Endogenous phytase (1 h) 42.0 Endogenous phytase (3.5 h) 37.6 Exogenous phytase (1 h) 37.6 Exogenous phytase (3.5 h) 34.2 Decorticated fraction 41.6 Dephytinized decorticated fraction 66.2 Bran fraction 23.8 Dephytinized bran fraction 34.9	Lestienne et al. (2005b)
Investigating the effects of phytate degradation in faba bean flour on iron bioavailability and in different fractions with or without dephytinisation	α-amylase solution (2 ml) consisting of 12,500 units l^{-1}, 1.5 g l^{-1} NaCl, 1.5 g l^{-1} K_2HPO_4 and 0.5 g l^{-1} Na_2CO_3 (pH 7.0), 30 min, 37°C	Pepsin, HCl, pH 4, 1 h at 37°C	Pancreatin-bile, pH 6, $NaHCO_3$, 30 min at 37°C Centrifuged at 5,000 × *g* for 15 min at 4°C Supernatants were filtered using 0.45 µm membrane	AAS	Bioavailability (%) Raw 32.2 Endogenous phytase (1 h) 70.8 Endogenous phytase (3 h) 78.2	Luo et al. (2010)

(Continued)

TABLE 1 | Continued

Type of study/foods	Conditions (pH, incubation time, centrifugation, buffer)			Iron quantification	Findings	References
	Oral phase	Gastric phase	Intestinal phase			
					Exogenous phytase (1 h) 69.2 Exogenous phytase (3 h) 72.0 Dehulled fraction 28.1 Dephytinized dehulled fraction 58.4 Hull 31.2 Dephytinized hull 33.1	
Effects of soaking and germination on iron bioavailability from three white Sorghum varieties	(5 g) were suspended in 30 ml distilled water using α-amylase solution	Pepsin, lipase, pH 4, 5M HCl, 37°C, 1 h	Adjusted to pH 6.0 using solid $NaHCO_3$, pancreatin-bile, 37°C, 30 min, centrifuged at 3,600 g for 15 min Supernatant filtered through 0.45 mm pore filter	AAS	Bioavailability Raw: 8.02–13.6% Soaked: 14.62–20.75% Germinated: 16.67–20.63%	Afify et al. (2011)
Effects of germination and cooking in faba bean, azuki bean and mung bean	α-amylase solution, 30 min, 37°C	Pepsin, HCl, pH 4, 60 min at 37°C	Pancreatin-bile, pH 6, $NaHCO_3$, 30 min at 37°C Centrifuged at 5,000 × *g* for 15 min at 4°C Supernatants were filtered using 0.45 µm membrane	AAS	Bioavailability (mg/kg) *Faba bean* Raw, soaked, germinated 26, 48 and 60 h: 35.2, 32.1, 32.5, 33.0, 32.6. *Azuki bean* Raw, soaked, germinated 26, 48 and 60 h: 46.8, 42.5, 42.2, 42.4, 42.5 *Mung bean* Raw, soaked, germinated 26, 48 and 60 h: 51.2, 48.6, 47.5, 47.8, 47.5 *Faba bean* Raw, pressure cooking, microwave: 32.6, 30.4, 31.3 *Azuki bean* Raw, pressure cooking, microwave: 42.5, 40.5, 40.8 *Mung bean* Raw, pressure cooking, microwave: 48, 46.5, 46.5	Luo et al. (2013)
Effects of processing and addition of ascorbic acid on the iron bioavailability in finger millet	–	HCL-pepsin: 0.03N HCl, 37°C, 3 h, pH not mentioned, filtered, acid digested	Pepsin-pancreatin: Pancreatin-bile, HCl, pH 5, 2 h at 37°C	AAS	Bioavailability (%) *Finger millet (HCL-pepsin)* Raw, soaked, germinated, autoclaved and	Mamiro et al. (2001)

(Continued)

TABLE 1 | Continued

Type of study/foods	Conditions (pH, incubation time, centrifugation, buffer)			Iron quantification	Findings	References
	Oral phase	Gastric phase	Intestinal phase			
		Pepsin-pancreatin: Pepsin, HCl, pH not mentioned, 2 h at 37°C			fermented: 5.06, 6.01, 29.91, 42.99, 51.98 *Finger millet (Pepsin-pancreatin)* Raw, soaked, germinated, autoclaved and fermented: 4.49, 5.88, 10.57, 4.40, 4.98 Finger millet + Vitamin C (Pepsin-pancreatin) Raw, soaked, germinated, autoclaved and fermented: 5.64, 6.81, 31.20, 33.43, 33.87 *Finger millet + Mango (Pepsin-pancreatin)* Raw, soaked, germinated, autoclaved and fermented: 50.51, 54.66, 77.19, 75.41, 78.55	
Three quinoa seeds (Chenopodium quinoa, wild), sweet unpolished, sweet polished and bitter polished Effect of cooking, soaking, germination and fermentation on iron bioavailability	–	Pepsin, HCl, 37°C, pH 1.8, 90 min	Pancreatin-bile, pH 5, 30 min, Adjusted to pH 6, centrifuged	AAS	Bioavailability (%) *Sweet unpolished quinoa* Raw, cooking, cooking + soaking, cooking + fermentation: 6.6, 6.4, 21.6, 30.7 Germinated seeds Cooking, cooking + fermentation: 19.8, 38.8 *Sweet, polished quinoa* Raw, cooking, cooking + soaking, cooking + fermentation: 7.8, 7.7, 18.5, 26.9 Germinated seeds Cooking, cooking + fermentation: 23.8, 64.0 *Bitter polished* Raw, cooking, cooking + soaking, cooking + fermentation: 9.6, 10.4, 37.3, 48.3 Germinated seeds Cooking, cooking + fermentation: 23.1, 50.7	Valencia et al. (1999)
Dry and green shelled beans (raw and cooked)	–	Pepsin, HCl, 37°C, pH 1.35, 90 min, centrifuged at 3,000 rpm for 45 min, filtered	Pancreatin-bile, pH 7.5, 2 h, centrifuged at 10,000 g for 20 min, filtered	AAS	Bioavailability (%) 38 varieties of raw and cooked beans (3.2 to 3.4%)	Mamiro et al. (2016)

bioavailability but adapted the *in-vitro* solubility method of Kiers et al. (2000) which was slightly different than the method used by Lestienne et al. (2005a,b). Instead of simulating the two key stages of gastric and intestinal phase, the method digested the flours with amylase in the oral phase for 30 min. Additionally, the supernatant was subjected to filtration after centrifugation. The relevance of this modification was to investigate three aspects of digestibility notably solubility, degradability and absorbability. The pH in gastric phase was 4 for 1 h and pH 6 for 30 min in intestinal phase. The iron solubility method used by Lestienne et al. (2005a,b); Luo et al. (2010, 2013) varied in terms of pH, incubation time and centrifugation force used (**Table 1**).

Ascorbic acid has been shown to enhance the iron bioavailability of finger millet and red kidney beans as demonstrated in a study by Mamiro et al. (2001) that used two approaches based on *in-vitro* solubility using HCL-pepsin and pepsin-pancreatin extractability. The former approach measured the iron bioavailability after the gastric phase and the latter measures the iron bioavailability at the end of small intestinal phase producing different values of bioavailability. The HCL-pepsin gives higher value of bioavailability compared to the pepsin-pancreatin method. This means that at lower pH in the gastric phase, the iron is more soluble and as the pH is raised to simulate the intestinal phase, solubility reduces thus lowering the bioavailability. However, it is important to note that most absorption occurs in duodenum so measuring the iron bioavailability at gastric phase may overestimate the value. According to the review by Etcheverry et al. (2012), the iron solubility method can be a good indicator of iron bioavailability as observed by previous studies which assessed the effects of ascorbic acid on bioavailability. This is because ascorbic acid is known to reduce ferric iron which in insoluble, to ferrous iron which is soluble and therefore iron solubility method is a good indicator of measuring iron bioavailability.

The pH and time of incubation in the gastric and intestinal phases in the study by (Valencia et al., 1999) was pH 1.8 and 90 min incubation time followed by pH 5 for 30 min then pH 6 prior to centrifugation (**Table 1**). This study adjusted the pH to 6 due to the high correlation with results from human studies on iron absorption from 20 vegetarian diets. In this study, the effect of different traditional processes including cooking, soaking, germination, and fermentation on the *in-vitro* bioavailability of iron in quinoa products was observed. As seen in all of these studies, *in-vitro* solubility had great flexibility and adaptability depending on the aim of the research.

The *in-vitro* solubility method has also been used to estimate the bioavailability of iron in legume samples. Mamiro et al. (2016) studied 38 different varieties of raw and cooked dry beans and green shelled beans. Diverse variation was observed between different varieties and the green shelled beans showed higher bioavailability compared to dry beans. A study by Sahuquillo et al. (2003) also used this *in-vitro* method to estimate and rank the bioavailability of iron and other minerals including calcium and zinc from white beans, chickpeas and lentils. Afify et al. (2011) also used this method to measure the iron bioavailability from three white sorghum varieties. According to Forbes et al. (1989), the estimation of iron bioavailability using *in-vitro* methods are generally ranked similar to those measured in human studies. This suggests that *in-vitro* methods are useful in comparing and predicting the iron bioavailability in a large number of samples before proceeding to human studies.

However, Pynaert et al. (2006) argued that the iron solubility method was not valid in a study that compared iron bioavailability in infant processed and unprocessed complementary food. This study compared the results obtained by the iron solubility method with gastrointestinal measurements coupled with Caco-2 model with the results obtained from an intervention study with Tanzanian infants. Iron solubility predicted much higher bioavailability in the processed complementary food compared to the unprocessed complementary food. However, these results contrasted with the results of iron status measurements in children who were fed the processed complementary food where no significant differences were observed. This could be due to various reasons including the iron status of the children in the original study, however the author concluded that the iron solubility is a poor predictor of actual iron absorption. In contrast to predictions made from iron solubility data, the results of the Tanzanian intervention study were in agreement with the data generated from the Caco-2 cell model for iron bioavailability. In the intervention study no significant differences were found for growth and iron status parameters between the intervention group and control group fed with unprocessed complementary food.

Iron Dialysability

In the dialysability method, a dialysis tube is introduced in the small intestinal phase and the amount of iron is measured in the dialysate. In theory, any iron that can be liberated to solution under gastric condition is potentially available for absorption across the intestine, hence the amount of solubilized iron can be used as a measure of bioavailability. This method is often chosen due to its simplicity, cost effectiveness and ease of setting up, however it is only limited to assessment of bioavailability.

The dialysability method as developed by Miller et al. (1981) has been used extensively to predict mineral bioavailability. The main difference of this method is the use of a dialysis membrane during the intestinal phase to mimic the differential epithelial uptake of the low molecular and high molecular weight iron. After the gastric phase, the dialysis bag containing $NaHCO_3$ is introduced which allows the slow increase in pH before and during intestinal phase which occurs when food leaves the stomach and enter the duodenum. The use of the dialysis bag eliminates the problem encountered when using centrifugation in *in-vitro* solubility method to separate soluble and insoluble components from complex samples. The dialysable iron is measured at the end of intestinal phase (Cilla et al., 2018).

The study by Argyri et al. (2009) adapted the method introduced by Kapsokefalou and Miller (1991) to develop a new setup for the application of the iron dialysability method. In this setup, six-well plates which can run a parallel digestion over six samples at a time and a ring insert that holds the dialysis membrane are proposed. The other advantage is the use of a smaller volume of 2 ml of samples as compared with the original method which used 20 ml of samples. No significant differences were found in the results obtained between the two approaches which suggests that this setup is convenient to use when large number of samples are analyzed. Another study by Luten et al. (1996) used the iron dialysability method of Miller et al. (1981) to test the repeatability and reproducibility of the method. This study modified the version of Miller et al. (1981) by the important parameters that the Miller study missed, such as pH adjustment.

The dialysability method has also been used in various studies to estimate the iron bioavailability in cereals and legumes, effects of inhibitors and enhancers, different cooking methods and processing of the foods on the bioavailability.

The differences in bioavailability of iron according to different heat treatments (Hemalatha et al., 2007a) and cooking techniques (Sebastiá et al., 2001) on cereals and pulses were observed using this method (**Table 2**). Hemalatha et al. (2007a) looked at the effects of heat processing using either pressure cooking or microwave heating on iron bioavailability from cereals and pulses consumed in India. Generally, iron bioavailability was significantly enhanced in all cereals and pulses following heat treatment. The iron bioavailability of rice and finger millet were higher after microwaving while wheat, sorghum and maize were higher in pressure cooked treatment. Microwave cooking generally produced an even greater increase in iron bioavailability from decorticated chickpea, whole green gram, red gram, black gram, and cowpea. This information is important as cereals and pulses are mostly consumed in a cooked form with pressure cooking being the most common method.

Similarly, Sebastiá et al. (2001) compared the iron bioavailability in other foods using *in-vitro* dialysability method. Beans, lentils and chickpeas were included that were commonly consumed and subject to typical household treatments including soaking in water, traditional cooking methods and microwaving as well as legumes that are commercially marketed as ready to eat (RTE). The differences in the conditions are different dialysis molecular weight cut off and a longer incubation in the intestinal phase. Heat treatments, both traditional and microwave cooking, reduced the iron bioavailability except for RTE legumes which gave the highest iron bioavailability. According to the author, the commercially produced legumes are usually soaked in EDTA to protect iron from reacting with phytic acid and therefore, increased the iron bioavailability.

The effects of different soaking techniques either in water, tartaric acid or calcium hydroxide solution and germination from bran and endosperm rich fractions of pearl millet also used the iron dialysability method to assess the iron bioavailability (Jha et al., 2015). The authors found that there was an improvement in bioavailable iron in acid soaked endosperm and bran fractions and this could be attributed to the decrease in phytate content and solubilisation of insoluble fiber. This type of study provides a useful understanding of the effects of pre-treatments that are traditionally used in households on iron bioavailability.

Apart from the study mentioned earlier, the effects of the inhibitors including phytic acid, tannins, calcium and dietary fiber have also been examined using this iron dialysability method. Hemalatha et al. (2007b) looked at the iron bioavailability of widely consumed cereals and pulses in India and its correlation with phytic acid, tannin, calcium, soluble and insoluble fiber contents. The correlation study found that the phytic acid content of the cereal grains produced a proportionate reduction in iron bioavailability, with the exception of sorghum. However, a similar negative influence of phytate on iron bioavailability from pulses was not evident. Insoluble dietary fiber was also found to interfere with iron bioavailability in pulses. In addition, it appeared that tannins did not have any significant influence on iron bioavailability in either cereals or pulses which contradicted the inhibition by tannins and the reason for this is unclear. Effects of phytase treatment on black gram and green gram was shown to lead to significant increases in iron bioavailability.

Sotelo et al. (2010) assessed the effects of oxalate, phytate and tannins on the iron bioavailability of widely consumed leafy vegetables, cereals, legumes and tubers in raw and cooked form and compared it with animal products (beef and chicken liver, beef, chicken and fish) in Mexico. The phytate/iron molar ratio was used in this study to predict the inhibitory effect on the bioavailability of iron. All samples had ratios more than one which indicated that the iron bioavailability would be impaired by phytates present in these foods. This information is useful for computing the recommended dietary allowances to improve the iron intake.

Fortification is one of the public health strategies to increase iron intake to address iron deficiency. This was demonstrated by Tripathi and Platel (2011) who studied the effect of fortification and enhancers on the iron bioavailability of meals made from fortified finger millet flour. The effects of natural enhancers such as ascorbic acid from amla fruit (gooseberry), (252 ± 34 mg/100 g), (Longvah et al., 2017) on the iron bioavailability from cereals and pulses or a combination of cereals and pulses cooked meal were observed and found various effects depending on the food sample and the amount of enhancers present. The addition of 10 and 30% amla showed an increase of 23 and 75% of iron bioavailability respectively in wheat. Meanwhile, the addition of 10% amla in ragi increased the iron bioavailability by 21%. The addition of 30% amla gave a reduction of 50 and 24% of iron bioavailability in rice and sorghum respectively. A reduction in iron bioavailability of 28–80% was observed in all pulses and 37 to 71% in cereal-pulse combinations with the addition of 10 or 30% amla. The author suggested that the enhancement effect of ascorbic acid on iron bioavailability in amla fruits is inhibited by the presence of tannin (Gowri et al., 2001). A study by Tuyizere et al. (2021) used an iron dialysability method to estimate the iron bioavailability in various wild fruits and vegetables that are abundant in rural areas of Uganda. This information is regarded as necessary to plan the nutritional intervention to improve the bioavailability of iron from these plant species with the addition of enhancers such as ascorbic acid, as well as the effect of processing to improve the intake of iron in the communities. This shows that the application of *in-vitro* methods is wide and flexible and could adapt for many types of study.

The molecular weight cut-off of the dialysis bag in the studies presented in this review varied between 8 and 14 kDa, also the final pH adjustment, time of incubation and the method used for iron quantification (**Table 2**) which makes the comparison between values difficult.

TABLE 2 | Iron bioavailability studies using *in-vitro* dialysability methods.

Type of study/foods	Conditions (pH, incubation time, centrifugation, buffer)			Iron quantification	Findings	References
	Oral phase	Gastric phase	Intestinal phase			
Effects of heat processing on iron bioavailability in rice, finger millet, sorghum, wheat, maize, chickpea, whole and decorticated green gram, whole and decorticated black gram, decorticated red gram, cowpea and French bean	–	Pepsin, pH 2, 37°C, 2 h	Dialysis tube (cut off 10 kDa) containing 25 ml sodium bicarbonate solution, pancreatine-bile mixture, pH 7 37°C for 2 h	AAS	Bioavailability (%) Raw Rice: 8.05 Wheat 5.06 Finger millet: 6.61 Sorghum: 4.13 Maize: 7.83 Whole/decorticated chickpea: 6.89/4.82 Whole/decorticated green gram: 2.24/7.49 Decorticated red gram: 3.06 Decorticated black gram: 2.76 Cowpea: 1.77 French bean: 10.2 Pressure cooked Rice: 12 Wheat 7.03 Finger millet: 7.37 Sorghum: 7.24 Maize: 9.53 Whole/decorticated chickpea: 8.01/4.25 Whole/decorticated green gram: 2.43/8.48 Decorticated red gram: 4.51 Decorticated black gram: 2.40 Cowpea: 3.98 French bean: 11.8 Microwave cooked Rice: 24.1 Wheat: 5.64 Finger millet: 12.2 Sorghum: 2.31 Maize: 6.43 Whole/decorticated chickpea: 5.31/9.06 Whole/decorticated green gram: 4.84/6.51 Decorticated red gram: 7.64 Decorticated black gram: 7.12 Cowpea: 5.63 French bean: 4.05	Hemalatha et al. (2007a)
Effects of calcium, phytate, tannin and fibers on iron bioavailability in rice, finger millet, sorghum, wheat, maize, chickpea whole and decorticated, whole and decorticated green gram, black gram, red gram, cowpea and French bean	–	Pepsin, pH 2, 37°C, 2 h	Dialysis tube (cut off 10 kDa) containing 25 ml sodium bicarbonate solution, pancreatine-bile mixture, pH 7 37°C for 2 h	AAS	Bioavailability (%) Rice: 8.05 Finger millet: 6.61 Sorghum: 4.13 Wheat 5.06 Maize: 7.83 Whole/decorticated chickpea: 6.89/4.82	Hemalatha et al. (2007b)

(Continued)

TABLE 2 | Continued

Type of study/foods	Conditions (pH, incubation time, centrifugation, buffer)			Iron quantification	Findings	References
	Oral phase	Gastric phase	Intestinal phase			
					Whole/decorticated green gram: 2.25/7.49 Black gram: 2.76 Red gram: 3.06 Cowpea: 1.77 French bean: 10.2	
Effects of different processing on iron bioavailability from white beans, chickpeas, lentils and ready to eat legumes	–	Pepsin, pH 2, 2 h	Dialysis tube (cut-off 12–14 kDa) containing 25 ml of water and an amount of sodium bicarbonate 37°C for 90 min		Bioavailability (μg/g) *White bean* Raw 0.045 Traditional 0.017 Microwave- RTE 0.46 *Chickpea* Raw 0.025 Traditional 0.026 Microwave 0.027 RTE 0.41 *Lentils* Raw 0.025 Traditional 0.017 Microwave 0.02 RTE 0.45	Sebastiá et al. (2001)
Effects of enhancers (ascorbic acid/amla fruit) on iron bioavailability of cooked samples from cereal, wheat and pulses	–	Pepsin, pH 2, 37°C, 2 h	Dialysis tube (cut off 10 kDa) containing 25 ml sodium bicarbonate solution, pancreatine-bile mixture pH 7 37°C for 2 h	ICP-ES	Bioavailability after the additions (None, Amla 10%, Amla 30%, Ascorbic acid 0.05% respectively) Rice: 9.52, 7.32, 4.77, 11.94 Ragi: 0.84, 1.02, 0.67, 1.23 Wheat: 1.88, 2.32, 3.20, 2.00 Jowar: 3.40, 3.40, 2.59, 6.13 Black gram dhal: 2.18, 1.26, 1.20, 2.77 Red gram dhal: 3/27, 0.65, 0.85, 4.32, 3.32 Green gram dhal: 3.32, 2.38, 1.05, 6.89 Bengal gram dhal: 5.89, 1.54, 1.50, 7.06 Rice + black gram dhal (2:1): 8.52, 4.18, 3.97, 12.94 Ragi + red gram dhal (4:1): 1.39, 0.88, 0.69, 2.04 Wheat + green gram dhal (4:1): 1.96, 0.64, 0.57, 2.20 Jowar + bengal gram dhal (4:1): 2.47, 1.30, 1.26, 2.38	Gowri et al. (2001)

(Continued)

TABLE 2 | Continued

Type of study/foods	Conditions (pH, incubation time, centrifugation, buffer)			Iron quantification	Findings	References
	Oral phase	Gastric phase	Intestinal phase			
Iron fortification and heat processing on iron bioavailability in finger millet flour and finger millet-based foods (dumpling and roti)	–	Pepsin, pH 2, 37°C, 2 h	Dialysis tube (cut off 10 kDa) containing 25 ml sodium bicarbonate solution, pancreatine-bile mixture pH 7 37°C for 3 h or longer until the pH of the digest reached 7. 5% nitric acid was added to the dialysate, centrifuged then filtered	AAS	Heat processing of the fortified and unfortified flour improved the bioavailability (mg/g) Unfortified flour: 0.23 mg/100 g to 0.50 mg/100 g Dumpling co-fortified with EDTA: 2.25 mg/100 g Roti co-fortified with EDTA: 2.39 mg/100 g Flour fortified with EDTA shows increase in bioavailability	Tripathi and Platel (2011)
Assessing iron bioavailability in plant and animal foods in Mexican diets and effects of inhibitors on the iron bioavailability		Pepsin, pH 2, 37°C, 2 h	Dialysis tube (cut off 8 kDa) containing 25 ml sodium bicarbonate solution, pancreatine-bile mixture pH 5 37°C for 2 h HCL was added to dialysate and adjusted the volume to 25 ml before measurement	Spectrophotometer at 530 nm	Bioavailability (mg/g) *Leafy vegetables* Raw 1.9–4.59 mg/100 g Cooked 1.98–4.45 mg/100 g *Cereals* Raw 2.62–2.93 mg/100 g Cooked 2.56–3.08 mg/100 g *Legume and tubers* Raw 2.11–3.56 mg/100 g Cooked 2.45–3.56 mg/100 g *Animal products* Raw 7.27–9.8 mg/100 g Cooked 7.30–9.79 mg/100 g	Sotelo et al. (2010)
Effects of soaking and germination on different grain fraction from finger millet	–	Pepsin, pH 2, 37°C, 2 h	Dialysis tube (cut off 8–12 kDa) containing 25 ml sodium bicarbonate solution, 37°C for 30 min then pancreatin-bile mixture was added and shaken for 2 h until the pH reached 7	AAS	Bioavailability (%) Bran rich fraction: 19.8–25.71% Endosperm rich fraction: 8.26–13.18%	Jha et al. (2015)
Fruits and vegetables consumed in Uganda	–	Pepsin, pH 2, 37°C, 2 h	Dialysis tube (cut off 10 kDa) containing 25 ml sodium bicarbonate solution, pancreatine-bile mixture pH 7 37°C for 2 h	FAAS	Bioavailability (%) *Wild fruits* Oywello (17.93), Oceyo (15.14), Kalara (22.91), Tongogwal Madito (2.65), Kano (15.61), Tugu (0.81) *Wild vegetables* Gwanya (9.80), Obuga lum (22.65), Oyado (10.15), Pot kalara (6.15), Otigo lum/nyim (18.37), Malakwang Odwonga (12.36), Boo ayom (12.51), Layika (27.65) and Ayuyu (13.83)	Tuyizere et al. (2021)

TABLE 3 | Iron bioavailability study using gastrointestinal models.

Type of study/foods	Conditions (pH, incubation time, centrifugation, buffer)			Iron quantification	Findings	References
	Oral phase	Gastric phase	Intestinal phase			
Effects of household process on the iron bioavailability from black bean samples (raw, pressure cooker, regular pan)	GI and dialysis membrane	10 g of ground sample were suspended in 60 ml of 20 mM glycine-HCl buffer, pH 2, 1.3 ml of pepsin (porcine), (1.6 g pepsin in 10 ml 20 mM glycine-HCl buffer, pH 2.0), 37°C, 2 h	pH 7.2 with 1 M $NaHCO_3$, pancreatine porcine 13 ml of a pancreatin porcine (0.4 g pancreatin in 100 ml of ultrapure water) a dialysis bag (cut of 10,000 Da with 2 ml water) was placed in the digestion system, 37°C, 2 h	Iron in the dialysate were analyzed by ICP-MS	Bioavailability (%) Regular pan with soaking water: 0.18% Pressure cooker with soaking water: 0.33% Regular pan without soaking water: 0.17% Pressure cooker without soaking water: 0.22%	Feitosa et al. (2018)
Investigating the effects of bio-fortification in iron bioavailability and bioavailability from cowpea cultivars and non-biofortified beans	200 mg. 200 µl of the 1% (w/v) α-amylase solution in $NaHCO_3$ buffer, pH 6.8 Enzymes sources were not indicated	3 ml of a 0.5% (w/v) pH 1.2 pepsin, 2 h at 37°C	3 mL of a 3% (w/v) pancreatin solution and 2.5% (w/v) bile salts in $NaHCO_3$ buffer pH 7.4, pH to 7.4. A dialysis membrane (14 kDa MWCO filled with $NaHCO_3$ pH 7.4) was inserted into the tube, 2 h at 37°C	ICP-MS Dialysate (solution from the dialysis membrane) Soluble (supernatant after centrifuged at 3,000 rpm)	Bioavailability (%) *Dialysate fraction* Biofortified cowpea cultivars: ARA 3.5%, TUM 3.3%, XIQ 3.8% Non-biofortified GUA 5.8% Common beans cultivars: CAR 1.2% WHI 2.8% GRE 1.5% BLA 0.9% *Soluble fraction* Biofortified ARA 52.3%, TUM 46.6%, XIQ 32.5% Non-biofortified GUA 54.3% Common beans cultivars: CAR 13.7% WHI 30.3% GRE 25.7% BLA 12.7%	Coelho et al. (2021)
[a]Effects of fermentation and cooking on iron bioavailability from finger millet sour porridge using INFOGEST static	STATIC INFOGEST		The conditions were similar except the use of dialysis bag during the intestinal phase containing NaCl and $NaHCO_3$	ICP-OES	Bioavailability Flour 7.7%/0.065 mg/100 g dm SFS 5.7%/0.056 mg/100 g dm SFP 6.1%/0.066 mg/100 g dm	Gabaza et al. (2018)
[a]Effects of coking on iron bioavailability in whole and dehulled Bambara groundnut	STATIC INFOGEST			ICP-OES	Bioavailability (%) 16–24% in whole and dehulled	Gwala et al. (2020)

(Continued)

TABLE 3 | Continued

Type of study/foods	Conditions (pH, incubation time, centrifugation, buffer)			Iron quantification	Findings	References
	Oral phase	Gastric phase	Intestinal phase			
[b]Effects of phytase on iron bioavailability in rapeseed, sunflower seed, whole wheat flour and white wheat flour using dynamic TIM model	Dynamic TIM			AAS	Bioavailability% *With phytase* Rapeseed 6.7 Sunflower seed 18.8 Whole wheat flour 38.3 White wheat flour 144 *Without phytase* Rapeseed 4 Sunflower seed 15.7 Whole wheat flour 35.2 White wheat flour 95.1	Larsson et al. (1997)

[a]*The conditions for studies that used static INFOGEST model was described in (Minekus et al., 2014) unless otherwise stated.* [b]*The conditions for Dynamic TIM was described in (Minekus et al., 1995).*

One of the general limitations of the dialysis technique is that large molecules such as haem iron or ferritin would be bioavailable but not dialysable as they could not diffuse through the membrane, but some smaller compounds like phenolic complexes or complexes with organic acids can diffuse through the membrane but generally would not be bioavailable. This limitation can be overcome by coupling this method with cell culture (Bohn et al., 2018).

All the studies presented above used a static model which did not simulate the gastric emptying or continuous changes in pH and secretion flow rates.

Gastrointestinal Model

Gastrointestinal models are *in-vitro* methods that are designed to simulate the human digestive system. There have been various models that differ in terms of the apparatus, enzyme concentrations and activity, digestion time, simulated digestion fluids, the ratio of sample to buffer etc.

A study by Larsson et al. (1997) adapted the TNO Gastro-Intestinal Model (TIM), a multicompartmental dynamic gastrointestinal model developed by Minekus et al. (1995) to investigate the effects of phytate on the iron absorption from wheat flour, whole wheat flour, rapeseed and sunflower seed and was then compared with the data obtained from human and animal studies. The addition of exogenous phytase improved the amount of dialyzed iron in all samples. This model consisted of four compartments to simulate the stomach, duodenum, jejunum and ileum. The model was equipped with a computer program to regulate the parameters such as pH for stomach and duodenum, secretion rates of gastric and duodenal juice into different compartments. The gastric and duodenal parts are equipped with pH electrodes to control the pH values for the stomach and duodenum. The advantage of this *in-vitro* dynamic model is that it resembles more closely the *in-vivo* physiological parameters simulating the dynamic condition of stomach and small intestine with peristaltic movements, absorption of nutrients, physiological emptying patterns and transit times. The bioavailability of minerals was measured from the proportion of compounds that dilutes across the hollow fiber system which contains a dialysis membrane with a molecular weight cutoff of 10 kDa connected to the jejunal and ileal compartments during the intestinal passage (Minekus, 2015).

A more recent method called the INFOGEST static model was developed by Minekus et al. (2014) and is receiving attention due to its general standardized protocol. This includes the use of enzyme sources, pH, ionic strength, electrolytes used, bile, dilution and digestion time. This static model uses a constant ratio of food to enzymes, electrolytes, and constant pH for each digestive phase and was developed recently to allow comparisons of food digestibility and micronutrient bioavailability between laboratories (Minekus et al., 2014; Brodkorb et al., 2019).

As mentioned previously, iron content does not always reflect the value of bioavailability due to some level of mineral chelation with inhibitors such as phytates and polyphenols. Gwala et al. (2020) used the INFOGEST model to evaluate the iron bioavailability in whole and dehulled Bambara groundnut. They found that the amount of bioavailable iron was about

14–20% of the total iron content in raw and cooked samples (**Table 3**).

Gabaza et al. (2018) examined the effect of fermentation and cooking on iron bioavailability of finger millet sour porridge produced at different households. The study used the static INFOGEST model as proposed by Minekus et al. (2014) with some modifications; the introduction of a dialysis bag into the digestion vessel with a molecular weight cutoff of 12 to 14 kDa containing a mixture of sodium chloride and sodium bicarbonate after 1.5 h of gastric phase. In this study, two different fractions of iron were measured. The first fraction was the fluid in the dialysis bag which was denoted as dialysable iron and the other fraction was the soluble part obtained from the digested mixture after centrifugation namely soluble non-dialysable iron fraction. Both fractions were used as the total soluble iron. The author found no significant improvement on the iron bioavailability of flour, spontaneous fermented slurries (SFS) and spontaneous fermented porridge (SFP) which were 7.7, 5.7 and 6.1% respectively (**Table 3**). The bioavailable iron content was 0.065, 0.056 and 0.066 mg/200 g dry matter for flour, SSF and SFP respectively. The author argued that the reason fermentation did not lead to any improvement on the iron bioavailability was due to traditional fermentation that was highly variable and unpredictable in household practice. In some cases, fermentation is normally aided by pre-processing such as decortication, soaking and germination. This type of study is again useful to provide baseline knowledge for future intervention and that the iron content and bioavailability from fermentation product may not be generalized.

Interestingly, a recent study by Muleya et al. (2021) used a modified static INFOGEST model by using an isotope labeling of reagent iron (^{57}Fe) to evaluate the contribution of iron in reagents used in the INFOGEST method in relation to iron in sample and the possible interference of reagent-derived iron with the bioavailability measurement in cereals and legumes. During *in-vitro* digestion, iron from samples and reagents enter a common pool that undergoes the same interactions that influence bioavailability. The stable isotope approach was compared with two normally used approaches to calculate iron bioavailability. The first approach used blank correction in order to obtain the iron bioavailability in the dialysate fraction while the second approach did not and the iron bioavailability was determined in all fractions. The author suggested that the use of isotopic labeling of reagent iron results in accurate and reliable iron bioavailability measurements particularly when analyzing samples with lower iron content than the iron in the reagents particularly in cereals and legumes.

There were studies that used different gastrointestinal models to the INFOGEST model to measure the iron bioavailability as demonstrated by Feitosa et al. (2018) and Coelho et al. (2021). Both studies used different concentration of digestive enzymes, duration of digestion, pH values and buffer concentrations in the different phase of digestion (**Table 3**). These two studies used the dialysis bag in the small intestinal phase.

Feitosa et al. (2018) explored the effects of household processing by two cooking methods, boiling in a regular pan and pressure cooking with or without the water the black beans were soaked in. The study found that the iron bioavailability from black beans. The study found that the iron bioavailability in both processes was low (0.18–0.33%) and beans cooked with soaking water in a pressure cooker resulted in the highest iron bioavailability (0.22%). The knowledge of applying the most efficient household procedures is important to ensure that the iron bioavailability is optimal after cooking.

Coelho et al. (2021) investigated the effects of bio-fortification on the iron bioavailability from bio-fortified cowpea cultivars BRS Xiquexique (XIQ), BRS Tumucumaque (TUM) and BRS Aracê (ARA) and non-biofortified BRS Guariba (GUA), common Black (BLA), White (WHI), Carioca (CAR) and Green (GRE) bean cultivars in raw and cooked form. The protocol consisted of three steps including oral, gastric and intestinal phase. This study expressed the iron concentration as a bio-accessible (soluble supernatant obtained after centrifugation) and bioavailability (dialysate) fractions. However, since bioavailability term has been used throughout the review, the bio-accessible fraction will be known as bioavailability from soluble fraction and the other fraction will be bioavailability from the dialysate. Bio-fortification did improve the iron bioavailability but not in all cowpea cultivars (**Table 3**). Non-bio-fortified GUA showed high bioavailability in both fractions. The iron bioavailability for cowpea cultivars were higher (32.5–54.3%) than common beans (12.7–30.9%) in bioavailability from soluble fraction. Similarly, from the fractions, cowpea cultivars (3.5–5.8%) showed higher iron bioavailability compared to common beans (0.9–2.8%). Cooking showed an increase in the bioavailability of dialysate fractions in TUM bio-fortified cowpea, green and black common beans. Cooking may have reduced the polyphenol content, however, the differential effects of cooking on different cultivars is unclear but it may be related to different polyphenol content in different cultivars. This *in-vitro* study is useful to demonstrate the effectiveness of crop breeding program before moving on to evaluate *in-vivo* the beneficial effects of the bio-fortified crop.

Cell Culture

This method is normally coupled with either solubility, dialysability or a gastrointestinal model. This assay measures iron uptake via ferritin formation in a monolayer of Caco-2 cells after the food sample is digested. Caco-2 cells are human epithelial cell line derived from a human colonic adenocarcinoma which behaves like intestinal cells. The advantage of these combined methods is that they can be used to study the mechanism of iron uptake and transport.

Yun et al. (2004) demonstrated the use of *in-vitro* digestion coupled with a Caco-2 cell model to replicate meals fed in human studies (egg meals with varying ascorbic acid concentration and wheat rolls with varying tannic acid concentration) to determine how the cell culture reflects to the iron bioavailability measured in human trials. Ferritin formation by the Caco-2 cells, which serves as a marker for cell iron uptake was used as an indicator of iron bioavailability. This study observed a strong correlation between this model and human trials. A significant correlation between iron uptake by Caco-2 cells from the meals and human absorption was observed ($R = 0.934$, $p = 0.012$ for an ascorbic acid added meal and $R = 0.927$, $p = 0.007$ for a tannic acid added meal).

In-vitro model coupled with Caco-2 cell was used to measure the iron bioavailability from eight common beans bean genotypes under three conditions: whole cooked beans containing polyphenols, cotyledon (beans without seed coats) without polyphenols and cotyledon without polyphenols with extrinsic ascorbate. The Caco-2 cell ferritin formation for the whole cooked beans ranged from 1.1 to 11.5 ng ferritin/mg protein. Higher ferritin formation was observed in the cooked cotyledon without polyphenol which ranged between 10.8 and 31.9 ng ferritin/mg protein. In the cooked cotyledon with added ascorbate, the ferritin formation ranged between 67.8 and 147.9 ng ferritin/mg protein (Ariza-Nieto et al., 2007). According to the author, the phosphorylated sugars chelate iron with higher avidity than non-phosphorylated forms. This complexation improves the iron solubility during digestion thus increasing the uptake by the cells. However, the increase in iron uptake is only observed at high concentration of sugar as the fructose only chelates iron above a threshold ratio which in this study used iron:sugar ratio of 1:200. As demonstrated in the study, the iron bioavailability varies among common bean genotypes suggesting that the iron bioavailability is genotype dependent and the removal of the seed coat and associated polyphenols improved the iron bioavailability.

This model can also be used to study the interactions between iron and enhancers or inhibitors. For example, it was recently demonstrated that the relative bioavailability of iron is higher in cabbage followed by broccoli, pepper, kale and spinach. This study also showed that the higher iron bioavailability in cabbage could result from the formation of complexes with fructose present in cabbage. In order to determine the effect of fructose on iron uptake in Caco-2 cell, fructose 1,6-biphosphate (F16BP) was added to iron and this led to an increase in ferritin formation in Caco-2 cells (Rodriguez-Ramiro et al., 2019).

Khoja et al. (2021) explored the iron bioavailability in fenugreek, boaba and moringa which have been used for medicinal purposes which could be potential sources of iron. The study used *in-vitro* digestion followed by iron uptake in Caco-2 cells to evaluate the iron bioavailability from these plant sources. The iron uptake by Caco-2 cells from fenugreek sprouts, fenugreek seeds, baobab fruit pulp and moringa leaves were about 20, 7, 3 and 10 ng ferritin/mg protein.

BENEFITS AND LIMITATION OF *in-vitro* METHODS AND RECOMMENDATIONS

Though human studies are still recognized as the gold standard for evaluating iron bioavailability, *in-vitro* methods appear to be an alternative method to estimate the iron bioavailability in plant-based foods prior to human studies. This is because *in-vitro* methods have been found to be reliable indicators of iron bioavailability with good reproducibility if all the experimental conditions are taken into account between different studies. For example Aragón et al. (2012) compared the validity of an iron dialysability method with *in-vivo* data to assess the iron bioavailability from staple biofortified food crops. The first recipe was to assess the effect of iron bioavailability with different concentration of ascorbic acid in a meal. The second recipe was to evaluate the effects of iron absorption promoters Na_2EDTA and the fortificants ferrous fumarate, ferrous sulfate and NaFeEDTA in tortillas. The *in-vitro* dialysability results showed a similar trend to the *in-vivo* results which used these recipes to feed the subjects and were highly and statistically correlated. The study also found that the results from the *in-vitro* method yielded more statistically significant differences between treatments than the *in-vivo*. This can be explained by reduced variability in the data compared to *in-vivo*.

An additional benefit is that there are no differences in baseline iron status such as can be seen in human studies which results in high variability in iron bioavailability as compared to *in-vitro* methods as observed in the study of (Aragón et al., 2012). As outlined above, there was great variation in the conditions of the *in-vitro* methods as discussed in this review. This demonstrates that the *in-vitro* models provide better control over conditions of the experiments which may lead to precise and reproducible results. It is also simple, rapid and low cost and many samples can be evaluated at one time.

There are also limitations of *in-vitro* methods that should be addressed. A study by (Mamiro et al., 2004) showed that reduction of phytate content of a complementary cereal-based food (from 1,150 to 660 mg/100 g) led to an increase in *in-vitro* iron solubility from 4.8 to 18.8% but showed no effect on hemoglobin status of infants of 6 to 12 months of age. So, an increase iron bioavailability measured by an *in-vitro* solubility method in processed food does not guarantee an increase in iron absorption and therefore in iron status of humans consuming phytate containing foods. These discrepancies between *in-vivo* and *in-vitro* studies may be attributed to the absence of simulation of the intestinal absorption and the physiological regulation mechanisms in the *in-vitro* digestion systems. This could also be explained by an adequate iron status of the infants at baseline.

It is still important to recognize that despite human studies being rather time consuming, expensive and complicated to perform, *in-vivo* measurement is necessary to assess the human factors affecting iron uptake. However, if the main interest is on the relative value of iron bioavailability from different plant sources and processing, *in-vitro* can be considered as a better option.

CONCLUSIONS

It is clear from this review that *in-vitro* methods are an important tool for preliminary screening that helps to assess the iron bioavailability in range of foods and staple crops, effect of processing conditions, assess the effects of inhibitors on iron bioavailability and other approaches such as fortification to improve iron bioavailability. Though, as mentioned, the results from *in-vitro* measurements have been shown to provide a useful estimate of *in-vivo* estimates of iron bio-accessibility or bioavailability. Whilst there have been great variations in *in-vitro* methodologies, they are useful for comparing and classifying foods according to the bioavailability. Clearly, there is a diverse use of *in-vitro* methods described in this review. This means that the use of *in-vitro* methods allows the possibility to control or modify the conditions of the experiment depending on the aim of the study. It also offers the possibility to optimally control the conditions which can lead to high accuracy. However, in order to compare the values or establish a food classification according to iron bioavailability, the standardization of the conditions of the assay is needed as demonstrated by the INFOGEST harmonized method. The *in-vitro* methods are also useful to predict the bioavailability of iron in commonly consumed foods to underpin the dietary recommendations for alleviating iron deficiency anemia.

AUTHOR CONTRIBUTIONS

NS wrote the first draft of the manuscript. DG and SA provided constructive feedback, contributed to manuscript revision, read, and approved the submitted version. All authors contributed to the article and approved the submitted version.

REFERENCES

Afify, A. E.-M. M., El-Beltagi, H. S., Abd El-Salam, S. M., and Omran, A. A. (2011). Bioavailability of iron, zinc, phytate and phytase activity during soaking and germination of white sorghum varieties. *PLoS ONE* 6:e25512. doi: 10.1371/journal.pone.0025512

Aragón, I. J., Ortiz, D., and Pachón, H. (2012). Comparison between *in vitro* and *in vivo* methods to screen iron bioavailability. *CyTA-J. Food* 10, 103–111. doi: 10.1080/19476337.2011.596283

Argyri, K., Birba, A., Miller, D., Komaitis, M., and Kapsokefalou, M. (2009). Predicting relative concentrations of bioavailable iron in foods using *in vitro* digestion: new developments. *Food Chem.* 113, 602–607. doi: 10.1016/j.foodchem.2008.07.089

Ariza-Nieto, M., Blair, M. W., Welch, R. M., and Glahn, R. P. (2007). Screening of iron bioavailability patterns in eight bean (Phaseolus vulgaris L.) genotypes using the Caco-2 cell *in vitro* model. *J. Agric. Food Chem.* 55, 7950–7956. doi: 10.1021/jf070023y

Blanco-Rojo, R., and Vaquero, M. P. (2019). Iron bioavailability from food fortification to precision nutrition. *Innov. Food Sci. Emerg. Technol.* 51, 126–138. doi: 10.1016/j.ifset.2018.04.015

Bohn, T., Carriere, F., Day, L., Deglaire, A., Egger, L., Freitas, D., et al. (2018). Correlation between *in vitro* and *in vivo* data on food digestion. What can we predict with static *in vitro* digestion models? *Crit. Rev. Food Sci. Nutr.* 58, 2239–2261. doi: 10.1080/10408398.2017.1315362

Brodkorb, A., Egger, L., Alminger, M., Alvito, P., Assunção, R., Ballance, S., et al. (2019). INFOGEST static *in vitro* simulation of gastrointestinal food digestion. *Nat. Protoc.* 14, 991–1014. doi: 10.1038/s41596-018-0119-1

Cilla, A., López-García, G., and Barberá, R. (2018). *In vitro* bioavailability of iron and calcium in cereals and derivatives: a review. *Food Rev. Int.* 34, 1–33. doi: 10.1080/87559129.2016.1210631

Coelho, R. C., Barsotti, R. C. F., Maltez, H. F., Júnior, C. A. L., and de Sousa Barbosa, H. (2021). Expanding information on the bioaccessibility and bioavailability of iron and zinc in biofortified cowpea seeds. *Food Chem.* 347:129027. doi: 10.1016/j.foodchem.2021.129027

Etcheverry, P., Grusak, M. A., and Fleige, L. E. (2012). Application of *in vitro* bioaccessibility and bioavailability methods for calcium, carotenoids, folate, iron, magnesium, polyphenols, zinc, and vitamins B6, B12, D, and E. *Front. Physiol.* 3:317. doi: 10.3389/fphys.2012.00317

Feitosa, S., Greiner, R., Meinhardt, A.-K., Müller, A., Almeida, D. T., and Posten, C. (2018). Effect of traditional household processes on iron, zinc and copper bioaccessibility in black bean (Phaseolus vulgaris L.). *Foods* 7:123. doi: 10.3390/foods7080123

Ferruzzi, M. G., Kruger, J., Mohamedshah, Z., Debelo, H., and Taylor, J. R. (2020). Insights from *in vitro* exploration of factors influencing iron, zinc and provitamin A carotenoid bioaccessibility and intestinal absorption from cereals. *J. Cereal Sci.* 96:103126. doi: 10.1016/j.jcs.2020.103126

Forbes, A. L., Arnaud, M., Chichester, C., Cook, J., Harrison, B., Hurrell, R., et al. (1989). Comparison of *in vitro*, animal, and clinical determinations of iron bioavailability: international nutritional anemia consultative group task force report on iron bioavailability. *Am. J. Clin. Nutr.* 49, 225–238. doi: 10.1093/ajcn/49.2.225

Gabaza, M., Shumoy, H., Louwagie, L., Muchuweti, M., Vandamme, P., Du Laing, G., et al. (2018). Traditional fermentation and cooking of finger millet: implications on mineral binders and subsequent bioaccessibility. *J. Food Compost. Anal.* 68, 87–94. doi: 10.1016/j.jfca.2017.05.011

Glahn, R. P., and Noh, H. (2021). Redefining bean iron biofortification: a review of the evidence for moving to a high Fe bioavailability approach. *Front. Sustain. Food Syst.* 5:215. doi: 10.3389/fsufs.2021.682130

Glahn, R. P., Tako, E., Cichy, K., and Wiesinger, J. (2016). The cotyledon cell wall and intracellular matrix are factors that limit iron bioavailability of the common bean (Phaseolus vulgaris). *Food Funct.* 7, 3193–3200. doi: 10.1039/C6FO00490C

Gowri, B., Platel, K., Prakash, J., and Srinivasan, K. (2001). Influence of amla fruits (Emblica officinalis) on the bio-availability of iron from staple cereals and pulses. *Nutr. Res.* 21, 1483–1492. doi: 10.1016/S0271-5317(01)00362-1

Gwala, S., Kyomugasho, C., Wainaina, I., Rousseau, S., Hendrickx, M., and Grauwet, T. (2020). Ageing, dehulling and cooking of Bambara groundnuts: consequences for mineral retention and *in vitro* bioaccessibility. *Food Funct.* 11, 2509–2521. doi: 10.1039/C9FO01731C

Hemalatha, S., Platel, K., and Srinivasan, K. (2007a). Influence of heat processing on the bioaccessibility of zinc and iron from cereals and pulses consumed in India. *J. Trace Elem. Med. Biol.* 21, 1–7. doi: 10.1016/j.jtemb.2006.10.002

Hemalatha, S., Platel, K., and Srinivasan, K. (2007b). Zinc and iron contents and their bioaccessibility in cereals and pulses consumed in India. *Food Chem.* 102, 1328–1336. doi: 10.1016/j.foodchem.2006.07.015

Hurrell, R., and Egli, I. (2010). Iron bioavailability and dietary reference values. *Am. J. Clin. Nutr.* 91, 1461-1467. doi: 10.3945/ajcn0.2010.28674F

ICMR-NIN (2020). *Recommended Dietary Allowances and Estimated Average Requirements Nutrient Requirements for Indians.* Hyderabad: Indian Council of Medical Research National Institute of Nutrition.

Jha, N., Krishnan, R., and Meera, M. (2015). Effect of different soaking conditions on inhibitory factors and bioaccessibility of iron and zinc in pearl millet. *J. Cereal Sci.* 66, 46–52. doi: 10.1016/j.jcs.2015.10.002

Kapsokefalou, M., and Miller, D. D. (1991). Effects of meat and selected food components on the valence of non-heme iron during *in vitro* digestion. *J. Food Sci.* 56, 352–355. doi: 10.1111/j.1365-2621.1991.tb05278.x

Khoja, K. K., Aslam, M. F., Sharp, P. A., and Latunde-Dada, G. O. (2021). *In vitro* bioaccessibility and bioavailability of iron from fenugreek, baobab and moringa. *Food Chem.* 335:127671. doi: 10.1016/j.foodchem.2020.127671

Kiers, J. L., Nout, R. M. J., and Rombouts, F. M. (2000). *In vitro* digestibility of processed and fermented soya bean, cowpea and maize. *J. Sci. Food Agric.* 80, 1325–1331. doi: 10.1002/1097-0010(200007)80:9<1325::AID-JSFA648>3.0.CO;2-K

Larsson, M., Minekus, M., and Havenaar, R. (1997). Estimation of the bioavailability of iron and phosphorus in cereals using a dynamic *in vitro* gastrointestinal model. *J. Sci. Food Agric.* 74, 99–106.

Lestienne, I., Besançon, P., Caporiccio, B., Lullien-Péllerin, V., and Tréche, S. (2005a). Iron and zinc *in vitro* availability in pearl millet flours (Pennisetum glaucum) with varying phytate, tannin, and fiber contents. *J. Agric. Food Chem.* 53, 3240–3247. doi: 10.1021/jf0480593

Lestienne, I., Caporiccio, B., Besançon, P., Rochette, I., and Trèche, S. (2005b). Relative contribution of phytates, fibers, and tannins to low iron and zinc *in vitro* solubility in pearl millet (Pennisetum glaucum) flour and grain fractions. *J. Agric. Food Chem.* 53, 8342–8348. doi: 10.1021/jf050741p

Longvah, T., Anantan, I., Bhaskarachary, K., Venkaiah, K., and Longvah, T. (2017). *Indian Food Composition Tables*. Indian Council of Medical Research Hyderabad.

Luo, Y., Xie, W., and Cui, Q. (2010). Effects of phytases and dehulling treatments on *in vitro* iron and zinc bioavailability in faba bean (Vicia faba L.) flour and legume fractions. *J. Food Sci.* 75, 191–198. doi: 10.1111/j.1750-3841.2009.01490.x

Luo, Y.-W., Xie, W.-H., Jin, X.-X., Wang, Q., and Zai, X.-M. (2013). Effects of germination and cooking for enhanced *in vitro* iron, calcium and zinc bioaccessibility from faba bean, azuki bean and mung bean sprouts. *CyTA-J. Food* 11, 318–323. doi: 10.1080/19476337.2012.757756

Luten, J., Crews, H., Flynn, A., Van Dael, P., Kastenmayer, P., Hurrell, R., et al. (1996). Interlaboratory trial on the determination of the *in vitro* iron dialysability from food. *J. Sci. Food Agric.* 72, 415–424.

Mamiro, P., Mwanri, A., Mamiro, D., Nyagaya, M., and Ntwenya, J. (2016). *In-vitro* bioavailability of selected minerals in dry and green shelled beans. *Afr. J. Agric. Res.* 11, 730–737. doi: 10.5897/AJAR2012.883

Mamiro, P., Van, J., Mwikya, S., and Huyghebaert, A. (2001). *In vitro* extractability of calcium, iron, and zinc in finger millet and kidney beans during processing. *J. Food Sci.* 66, 1271–1275. doi: 10.1111/j.1365-2621.2001.tb15200.x

Mamiro, P. S., Kolsteren, P. W., van Camp, J. H., Roberfroid, D. A., Tatala, S., and Opsomer, A. S. (2004). Processed complementary food does not improve growth or hemoglobin status of rural Tanzanian infants from 6–12 months of age in Kilosa district, Tanzania. *J. Nutr.* 134, 1084–1090. doi: 10.1093/jn/134.5.1084

Miller, D. D., Schricker, B. R., Rasmussen, R. R., and Van Campen, D. (1981). An *in vitro* method for estimation of iron availability from meals. *Am. J. Clin. Nutr.* 34, 2248–2256. doi: 10.1093/ajcn/34.10.2248

Minekus, M. (2015). "The TNO gastro-intestinal model (TIM)," in *The Impact of Food Bioactives on Health*, eds K. Verhoeckx, P. Cotter, I. López-Expósito, C. Kleiveland, T. Lea, A. Mackie, T. Requena, D. Swiatecka, and H. Wichers (Berlin: Springer), 37-46. doi: 10.1007/978-3-319-16104-4_5

Minekus, M., Alminger, M., Alvito, P., Ballance, S., Bohn, T., Bourlieu, C., et al. (2014). A standardised static *in vitro* digestion method suitable for food–an international consensus. *Food. Funct.* 5, 1113–1124. doi: 10.1039/C3FO60702J

Minekus, M., Marteau, P., Havenaar, R., and Huis in't Veld, J. H. H. (1995). A multicompartmental dynamic computer-controlled model simulating the stomach and small intestine. *Altern. Lab. Anim.* 23, 197–209. doi: 10.1177/026119299502300205

Muleya, M., Young, S. D., and Bailey, E. H. (2021). A stable isotope approach to accurately determine iron and zinc bioaccessibility in cereals and legumes based on a modified INFOGEST static *in vitro* digestion method. *Food Res. Int.* 139:109948. doi: 10.1016/j.foodres.2020.109948

Nair, K. M., and Iyengar, V. (2009). Iron content, bioavailability and factors affecting iron status of Indians. *Indian J. Med. Res.* 130, 634–645.

Pizarro, F., Olivares, M., Valenzuela, C., Brito, A., Weinborn, V., Flores, S., et al. (2016). The effect of proteins from animal source foods on heme iron bioavailability in humans. *Food Chem.* 196, 733–738. doi: 10.1016/j.foodchem.2015.10.012

Platel, K., and Srinivasan, K. (2016). Bioavailability of micronutrients from plant foods: an update. *Crit. Rev. Food Sci. Nutr.* 56, 1608–1619. doi: 10.1080/10408398.2013.781011

Pynaert, I., Armah, C., Fairweather-Tait, S., Kolsteren, P., Van Camp, J., and De Henauw, S. (2006). Iron solubility compared with *in vitro* digestion–Caco-2 cell culture method for the assessment of iron bioavailability in a processed and unprocessed complementary food for Tanzanian infants (6–12 months). *Br. J. Nutr.* 95, 721–726. doi: 10.1079/BJN20051722

Rodriguez-Ramiro, I., Dell'Aquila, C., Ward, J., Neal, A., Bruggraber, S., Shewry, P., et al. (2019). Estimation of the iron bioavailability in green vegetables using an *in vitro* digestion/Caco-2 cell model. *Food Chem.* 301:125292. doi: 10.1016/j.foodchem.2019.125292

Rousseau, S., Kyomugasho, C., Celus, M., Hendrickx, M. E., and Grauwet, T. (2020). Barriers impairing mineral bioaccessibility and bioavailability in plant-based foods and the perspectives for food processing. *Crit. Rev. Food Sci. Nutr.* 60, 826–843. doi: 10.1080/10408398.2018.1552243

Sahuquillo, A., Barberá, R., and Farré, R. (2003). Bioaccessibility of calcium, iron and zinc from three legume samples. *Nahrung* 47, 438–441. doi: 10.1002/food.200390097

Sebastiá, V., Barberá, R., Farré, R., and Lagarda, M. J. (2001). Effects of legume processing on calcium, iron and zinc contents and dialysabilities. *J. Sci. Food Agric.* 81, 1180–1185. doi: 10.1002/jsfa.927

Sotelo, A., González-Osnaya, L., Sánchez-Chinchillas, A., and Trejo, A. (2010). Role of oxate, phytate, tannins and cooking on iron bioavailability from foods commonly consumed in Mexico. *Int. J. Food Sci. Nutr.* 61, 29–39. doi: 10.3109/09637480903213649

Tripathi, B., and Platel, K. (2011). Iron fortification of finger millet (Eleucine coracana) flour with EDTA and folic acid as co-fortificants. *Food Chem.* 126, 537–542. doi: 10.1016/j.foodchem.2010.11.039

Tuyizere, J. D., Okidi, L., Elolu, S., and Ongeng, D. (2021). *In vitro* bioavailability-based assessment of the contribution of wild fruits and vegetables to household dietary iron requirements among rural households in a developing country setting: the case of acholi subregion of Uganda. *Food Sci. Nutr.* 9, 625–638. doi: 10.1002/fsn3.1977

Valencia, U. S., Ann-Sofie, S., and Jenny, R. (1999). Processing of quinoa (Chenopodium quinoa, Willd): effects on *in vitro* iron availability and phytate hydrolysis. *Int. J. Food Sci. Nutr.* 50, 203–211. doi: 10.1080/096374899101247

WHO (2020). *Who Guideline on Use of Ferritin Concentrations to Assess Iron Status in Individuals and Populations*. WHO.

Yun, S., Habicht, J.-P., Miller, D. D., and Glahn, R. P. (2004). An *in vitro* digestion/Caco-2 cell culture system accurately predicts the effects of ascorbic acid and polyphenolic compounds on iron bioavailability in humans. *J. Nutr.* 134, 2717–2721. doi: 10.1093/jn/134.10.2717

Conflict of Interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

frontiers in Sustainable Food Systems

DATA REPORT
published: 08 November 2021
doi: 10.3389/fsufs.2021.728120

Agricultural Productivity, Aging Farming Workforce, Sustainable Agriculture, and Well-Being: Household Survey Data From Central Thailand

Sylvia Szabo[1], Chidchanok Apipoonyanon[2], Malay Pramanik[3], Takuji W. Tsusaka[4] and Kevin Leeson[5]*

[1] Department of Social Welfare and Counselling, Dongguk University, Seoul, South Korea, [2] Department of Development and Sustainability, School of Environment, Resources and Development, Asian Institute of Technology (AIT), Pathum Thani, Thailand, [3] Tata Jamsetji School of Disaster Studies, Tata Institute of Social Sciences, Mumbai, India, [4] Organization for Advanced and Integrated Research, Kobe University, Kobe, Japan, [5] College of Sciences and Engineering, University of Tasmania, Hobart, TAS, Australia

Edited by:
Kathleen L. Hefferon, Cornell University, United States

Reviewed by:
Krishna Singh, Dr. Rajendra Prasad Central Agricultural University, India
Liz Carlisle, University of California, Santa Barbara, United States

***Correspondence:**
Takuji W. Tsusaka
takuji.tsusaka@gmail.com

Specialty section:
This article was submitted to Nutrition and Sustainable Diets, a section of the journal Frontiers in Sustainable Food Systems

Received: *20 June 2021*
Accepted: *28 September 2021*
Published: *08 November 2021*

Citation:
Szabo S, Apipoonyanon C, Pramanik M, Tsusaka TW and Leeson K (2021) Agricultural Productivity, Aging Farming Workforce, Sustainable Agriculture, and Well-Being: Household Survey Data From Central Thailand. Front. Sustain. Food Syst. 5:728120. doi: 10.3389/fsufs.2021.728120

Keywords: aging, agricultural development, agricultural productivity, agricultural sustainability, farmers' perception, farming workforce, nutritional diversification, Thailand

INTRODUCTION

Agriculture and food systems are critical for maintaining food and nutrition security, driving economic development, alleviating poverty, and preserving ecological functions and services on national and international agendas (Whitfield et al., 2018). Furthermore, the system intersects all the United Nations' (UN) agenda items for sustainable development goals (SDGs), highlighting a growing global concern for, and sometimes contentious debate, over food system sustainability (FAO, 2019). In the twenty-first century, agricultural and food systems have faced a complex set of local and global challenges (Whitfield et al., 2018). This includes poor diets, poverty, and environmental concerns related to water, land scarcity, and climate change as some of the major global problems (Diama et al., 2020). In order to address these issues, we need to incorporate dietary and on-farm diversity with a holistic solution and smart food approach (Diama et al., 2020).

It is generally accepted that agriculture and food systems must adapt to uncertain and changing climatic conditions by building resilience and food system sustainability (Whitfield et al., 2018; Diama et al., 2020). This requires an understanding of the dynamics and interactions of the system and how changes in agricultural practices have been shaped through learning and social interactions (Whitfield, 2015); the innovative ways that human beings adapt within changing environments (Reij and Waters-Bayer, 2001); and the multifaceted priorities and value systems of individual consumers and producers (Lusk and Briggeman, 2019). Population structure plays a significant role in understanding the key factors affecting agricultural production, including its volume and future direction (Guancheng et al., 2015). Population structure and population change can also help to identify choices regarding agricultural inputs and crop selection. Accordingly, the movement of labor from rural to urban areas during the urbanization process brings changes in agricultural form, type and pattern in rural areas (Knodel and Chayovan, 2009).

In Thailand, a high rate of rural to urban migration and declining family size plays a significant role in influencing the involvement of aging populations in agricultural production. For example, aging people are considered likely to encourage greater use of machinery to address labor shortage issues. This is a direct result of an increasingly aging population and mobility of the rural population

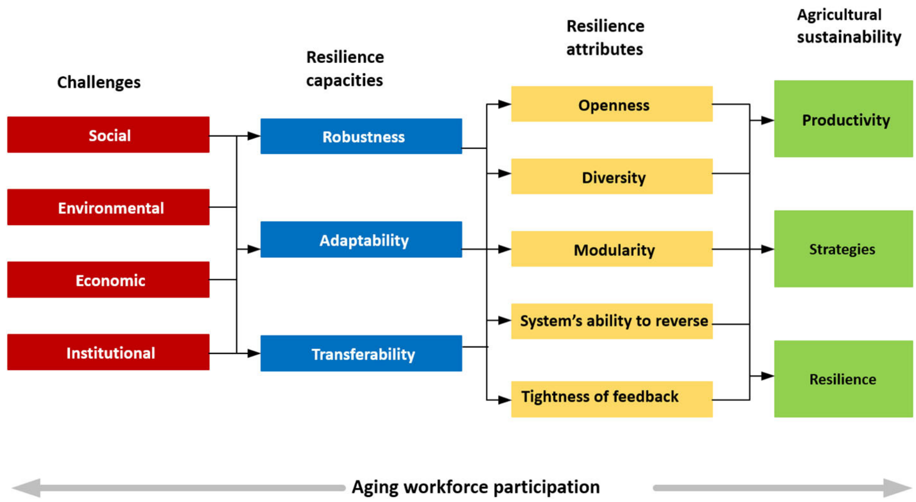

FIGURE 1 | Conceptual framework showing inter-linkages between different elements affecting the resiliency of agricultural systems.

TABLE 1 | Sample size estimated for the present survey.

District/Sub-district	Household (*N*)	Percentage (%)	Sample size (*n*)
Ban Sang District			
(1) Bang Taen	1,454	35	128
(2) Bang Pla Ra	833	20	73
Si Maha Phot District			
(3) Dong Krathong Yam	1,315	32	117
(4) Bang Kung	536	13	47
Total	4,138	100	365

Source: *Department of Provincial Administration (2021).*

in Thailand, which has reduced agricultural labor supplies and led to a rise in labor costs. Recent research by Rigg et al. (2020) has shown that young people who remain in rural areas gradually increase their earnings by undertaking non-farming work. Phongsiri et al. (2017) also indicate that young people are not interested in agriculture due to negative perceptions of low social status. This triggers significant involvement of the aging population in farming practices at every stage of production (e.g., pre-planting, growth-related, and post-growth), which can also reduce farming productivity (Seok et al., 2018).

Previous studies in Thailand have shown that aging farmers are often dependent on hired labor, which increases production costs (Formoso, 2016). In contrast, the aging rural population encourages restructuring of internal driving factors for agricultural production, including the use of technology, controlling farm accidents and minimizing health risks. Perceptions of these factors, albeit important, remain unexamined. The changes in labor input in the process of land-use transitions affect agricultural production in terms of labor quantity and quality. Therefore, the dominant influence is mainly through the supply of agricultural labor, agricultural land use, and the agricultural output structure. The effects of an aging population engagement are presented through large-scale production and socio-economic functions. **Figure 1** shows the conceptual framework of the present study, illustrating the inter-linkages between different elements which affect the resiliency of agricultural systems.

MATERIALS AND METHODS

Sample Selection

This study collected household data from four sub-districts in Prachinburi Province, Thailand (**Table 1**). First, we applied a multi-stage sampling strategy by separating total households into several groups of farm households, such as agriculture and aquaculture (**Figure 2**). In the first step, we adopted purposive sampling to maintain the homogeneous representation of every farming group, which included agricultural production (i.e., rain-fed and irrigated rice production) and pond aquaculture (fish and shrimp). We then applied the random sampling method to select farm households from every village with cooperating local fieldworkers at the Rice Center and the Bureau of Registration Administration (BORA) of the Department of Provincial Administration (DOPA).

To calculate the minimum suggested sample size, Yamane's method (Yamane, 1973) was used (see Equation 1). By doing this, we obtained a sample size of 365 households from the total population of 4,138 households in the province.

$$n = \frac{N}{1 + Ne^2} \quad (1)$$

Where n indicates the minimum sample size, N refers to the total population, and e indicates the acceptable margin of error (0.05 or 5%).

The distribution of sampled households across Prachinburi province was determined using the following

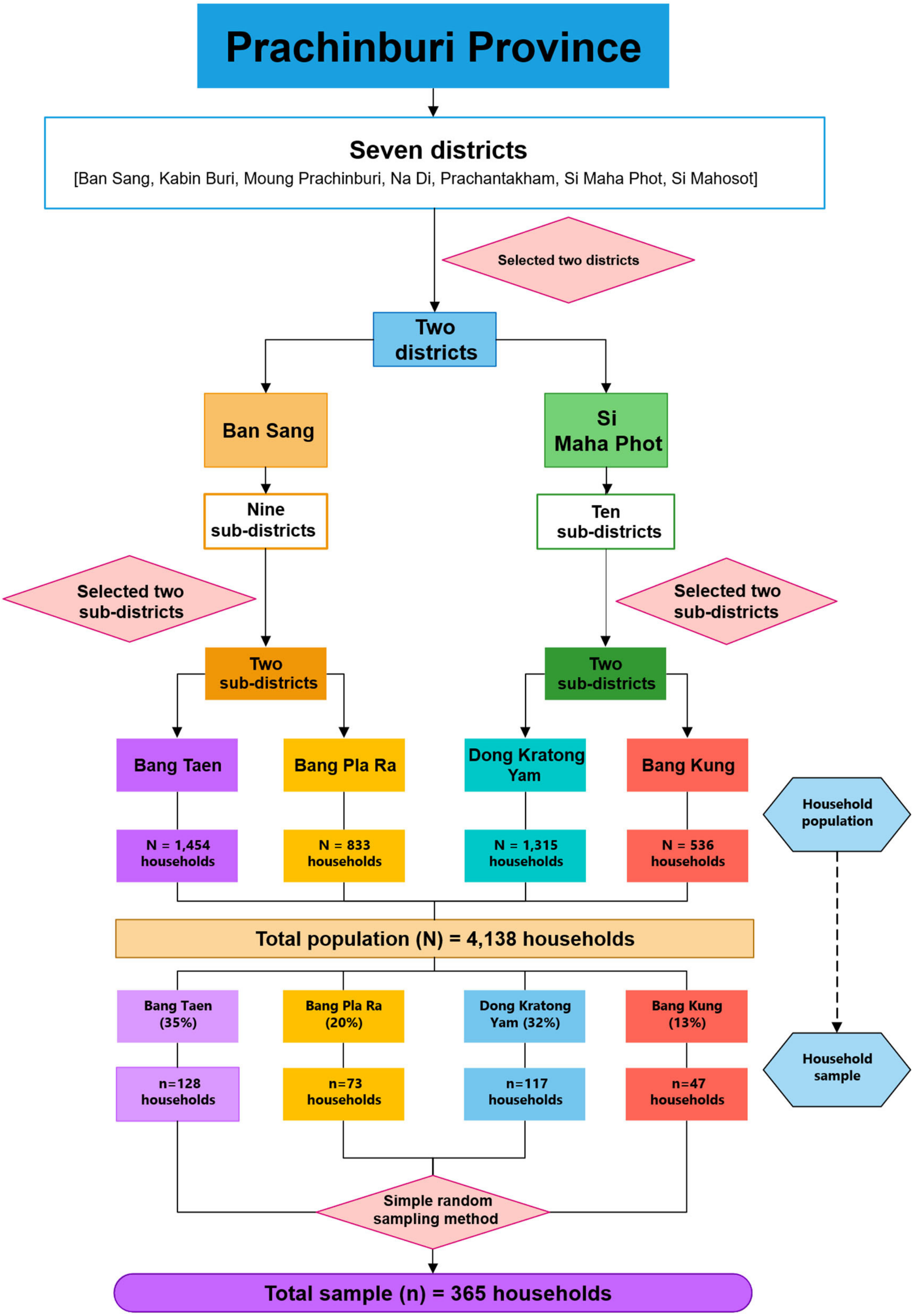

FIGURE 2 | Schematic diagram showing multi-stage sampling procedure for dividing the total households into different farming household groups.

method (Equation 2).

$$Ka = \frac{(N \times 365)}{Tp} \quad (2)$$

Where Ka refers to the number of households selected from four sub-districts, N indicates the population of the sub-districts, and Tp indicates the total population of the province.

Development of the Questionnaire

A structured draft questionnaire was developed to guide the interviewing process and then shared with local stakeholders and collaborators involved in the project. The questionnaire was divided into four parts: (1) household demographic characteristics; (2) the current situation of agricultural sustainability, production and productivity; (3) the livelihoods and well-being of aging farmers; and (4) strategies to improve agricultural productivity and sustainability. A pilot survey was carried out with a sample of 30 households within the study area. Following rigorous field-testing, the questionnaire was finalized for implementation.

Survey Administration and Analysis

The survey was administered to households in four sub-districts within the Prachinburi region from August to October 2018. The survey procedure was conducted in three steps: (1) the survey questionnaire was translated into Thai; (2) the questionnaire was distributed to 128 respondents in Bang Taen, 73 in Bang Pla Ra, 116 in Don Kratong Yam, and 47 in Bang Kung; and (3) the cover letter explained the aim and importance of the survey, the potential participation benefits, and the criteria for respondent selection. In addition, the cover letter assured all participants of complete confidentiality. Including research assistants, a total of 365 selected respondents were interviewed.

Key Variables

The explanatory variables were selected based on the existing literature. For example, several studies conducted in different geographical contexts examined the relationship between farmers' age and productivity (Poungchompu et al., 2012; Guancheng et al., 2015). These studies used variables measuring types of agricultural production, geophysical, social and climate-related barriers to greater agricultural productivity, mentoring, use of technology, retirement, health concerns, and risks of farm accidents of the aging workforce. Farm and farmers' characteristics (i.e., age, gender and education, household income, household size, farming experience) are typically used in analyses examining agricultural sustainability (Zou et al., 2018; Filloux et al., 2019). Selected variables and data coding are presented in **Table 2**. All variables and related coding are available in the dataset file (**Supplementary Material**).

TABLE 2 | Selected variables, their description, measurement and relevant reference literature.

Variables	Descriptions and Measurement	Relevant literature
Age	Age of respondents (1 = elderly, 0 = other)	Rigg et al. (2020)
Gender	Gender of respondents (1 = male; 0 = female)	Kideghesho and Msuya (2010)
Education	Education levels of respondents (1 = primary level, 0 = other)	Guancheng et al. (2015), Rigg et al. (2020)
Farm experience	Length of farming experience of respondents (years)	Anim (2011), Ntshangase et al. (2018)
Farm size	Size of farmland (hectares)	He (2013), Griffin et al. (2019)
Household poverty	Household where income is below the poverty line (USD $5.50 per day)	Griffin et al. (2019), Rigg et al. (2020)
Adult in family	Total number of adults in a family (number)	Ntshangase et al. (2018)
Total production	The total output of agricultural production (kilogram)	Dzuganov et al. (2020)
Climate change	Perceived the effect of drought or flood on the agricultural system (1 = a major problem, 0 = other)	Connor et al. (2020), Isaac et al. (2020)
Agricultural technology	Difficulty with agricultural technology (1 = a major problem, 0 = other)	Phongsiri et al. (2017), Philip et al. (2019), Dzuganov et al. (2020), Hoang (2020)
Farm investment	Difficulty with on-farm finance service support (1 = a major problem, 0 = other)	Abid (2014)
Farm labor	Difficulty with use of farm labor capacity (1 = a major problem, 0 = other)	Souvi et al. (2021)
Integrated farming system	Whether an integrated farming system is adopted by the farmer (1 = adopted, 0 = other)	Salaisook et al. (2020)
Organic farming system	Whether an organic farming system is adopted by the farmer (1 = adopted, 0 = other)	Karnasuta and Laoanantana (2021)
Vegetable garden area	Area planted to vegetable crops (rai = 0.16 ha)	Suwanmaneepong and Mankeb (2017)
Fish pond area	Area of pond for fish culture (rai = 0.16 ha)	Salaisook et al., 2020
Agricultural market	Difficulty with access and use of agricultural market information (1 = a major problem, 0 = other)	Hoang (2020), Thi and Bui (2021)
Irrigation service	Difficulty with the irrigation system and access to irrigation service (1 = a major problem, 0 = other)	Kapil et al. (2020)

POTENTIAL RESEARCH PATHWAYS

The dataset provides insights into the agricultural practices, sustainability, nutritional diversity, environmental and health benefits, barriers and opportunities in the Thai agricultural sector, with a focus on central Thailand. As Thailand is rapidly urbanizing and its population is experiencing rapid aging, examining ways of enhancing agricultural practices, diversifying production, and consumption behind traditional rice mono-cropping is of critical importance. Accordingly, engagement in the agricultural sector is becoming essential for policy advocacy.

Using the present dataset, researchers are presented with an opportunity to analyse the factors influencing sustainable agriculture, specifically agricultural productivity, aging farming workforce, and community well-being. Further analyses of this dataset can be undertaken by combining these data with other existing datasets. This also includes administrative data from Thailand's sub-district (tambon) offices as well as spatial data. The data can be used for comparative studies and constituting a baseline for further studies in this region. Linking with nutritional and health data can contribute to an understanding of the food security status of the aging agriculture workforce. This is also useful for mapping the health, nutrition, and its association with agricultural production throughout the region. The recent agriculture policy (Thailand 4.0) focuses on increasing yield per rai by reducing inefficiencies. The dataset will also help to understand the factors, roles, and potential issues of the aging workforce. It will help to initiate potential policies for agricultural sustainability through the involvement of an aging workforce.

DATA AVAILABILITY STATEMENT

The original contributions presented in the study are included in the article/**Supplementary Material**, further inquiries can be directed to the corresponding authors.

ETHICS STATEMENT

Ethical review and approval was not required for the study on human participants in accordance with the local legislation and institutional requirements. Written informed consent for participation was not required for this study in accordance with the national legislation and the institutional requirements.

AUTHOR CONTRIBUTIONS

SS and CA designed the study. CA collected the data. MP, CA, SS, TT, and KL drafted the article. All authors revised the article critically and approved the final version and agree to be accountable for all aspects of the work. All persons designated as authors qualify for authorship.

FUNDING

All authors acknowledge the financial support from the Deltas' Dealings with Uncertainty (DoUbT) research project funded by the French Agricultural Research Centre for International Development (CIRAD). Likewise, the authors acknowledge the funding from the UK Research and Innovation's Global Challenges Research Fund (UKRI GCRF) through the Trade, Development and the Environment Hub project (Project No. ES/S008160/1), led by the UN Environment Programme World Conservation Monitoring Centre (UNEP-WCMC).

SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fsufs.2021.728120/full#supplementary-material

REFERENCES

Abid, M. (2014). Farmers' perceptions of and adaptation strategies to climate change and their determinants; the case of Punjab province. *Pakistan*. *Earth Syst. Dyn*. 5, 1359–1406. doi: 10.5194/esdd-5-1359-2014

Anim, F. D. K. (2011). Factors affecting rural household farm labour supply in farming communities of South Africa. *J. Hum. Ecol*. 34, 23–28. doi: 10.1080/09709274.2011.11906365

Connor, M., Guia, A. H., De Quilloy, R., Nguyen, H., Van Gummert, M., and Sander, B. O. (2020). When climate change is not psychologically distant - Factors in fl uencing the acceptance of sustainable farming practices in the Mekong River Delta of Vietnam. *World Dev. Perspect*. 18:100204. doi: 10.1016/j.wdp.2020.100204

Department of Provincial Administration (2021). Phra Nakhon, Bangkok, Thailand. Available online at: http://stat.bora.dopa.go.th/stat/statnew/statTDD/views/showProvinceData.php (accessed April 22, 2021).

Diama, A., Anitha, S., Kane-Potaka, J., Htut, T. T., Jalagam, A., Kumar, P., et al. (2020). How the smart food concept can lead to the transformation of food systems and combat malnutrition: different approaches in africa, globally, and a case study from Myanmar with lessons learnt for creating behavior change in diets. *World Rev Nutr Diet*. 121, 149–158. doi: 10.1159/000507494

Dzuganov, B., Shekikhachev, Y. A., Sh, T., Chechenov, M., and Mishkhozhev, V. (2020). Status and prospects of technical equipment of small enterprises in agricultural production Status and prospects of technical equipment of small enterprises in agricultural production. *IOP Conf. Ser. Mater. Sci. Eng*. 919, 4–10. doi: 10.1088/1757-899X/919/3/032015

FAO (2019). *The State of Food and Agriculture 2019. Moving Forward on Food Loss and Waste Reduction*. Rome: FAO.

Filloux, T., Faysse, N., and Phiboon, K. (2019). Public policy to support young farmers in Thailand. *Outlook Agric*. 48, 292–299. doi: 10.1177/0030727019880187

Formoso, B. (2016). Are Thai Peasants still Farmers? The socio-economic transformation of two villages of Northeastern Thailand. *Rech. En Sci. Hum. sur l'Asie du Sud-Est* 28, 39–60. doi: 10.4000/moussons.3636

Griffin, B., Hartarska, V., and Denis, N. (2019). Retirement age farmers' exit and disinvestment from farming. *Int. J. Econ. Financ*. 11, 136–148. doi: 10.5539/ijef.v11n12p136

Guancheng, G., Wen, Q., and Jingjuan, Z. (2015). The impact of ageing agricultural labor population on farmland output: From the perspective of farmer preferences. *Math. Probl. Eng*. 4, 1–7. doi: 10.1155/2015/730618

He, X. (2013). Agricultural labor force aging research-based on surveys in rural Zhejiang Province. *Popul. Econ*. 2, 69–78.

Hoang, H. G. (2020). Determinants of the adoption of mobile phones for fruit marketing by Vietnamese farmers. *World Dev. Perspect*. 17:100178. doi: 10.1016/j.wdp.2020.100178

Isaac, N., Fromm, I., and Frimpong, B. N. (2020). Factors influencing the adoption of cocoa agroforestry systems in mitigating climate change in Ghana: the case of Sefwi Wiawso in Western Region. *Environ. Sustain. Clim. Chang*. 2, 20–23. doi: 10.3390/land10030266

Kapil, K., Adhikari, K. B., Shiva, C. D., and Marahatta, S. (2020). Factors determining farmers' strength of access to the irrigation system in Kaski district of Nepal. *Cogent. Food Agric*. 6, 1–10. doi: 10.1080/23311932.2020.1843751

Karnasuta, S., and Laoanantana, P. (2021). Organic farming model of paddy rice production with environmental efficiency in Thailand. *Turkish J. Comput. Math. Educ*. 12, 3066–3074.

Kideghesho, J. R., and Msuya, T. S. (2010). Gender and socio-economic factors influencing domestication of indigenous medicinal plants in the West Usambara Mountains, northern Tanzania. *Int. J. Biodivers. Sci. Ecosyst. Serv. Manag*. 6, 3–12. doi: 10.1080/17451590.2010.480946

Knodel, J., and Chayovan, N. (2009). Older persons in Thailand: a demographic, social and economic profile. *Ageing Int*. 33, 3–14. doi: 10.1007/s12126-009-9025-8

Lusk, J. L., and Briggeman, B. C. (2019). Food values. *Am. J. Agric. Econ*. 91, 184–196. doi: 10.1111/j.1467-8276.2008.01175.x

Ntshangase, N. L., Muroyiwa, B., and Sibanda, M. (2018). Farmers' perceptions and factors influencing the adoption of no-till conservation agriculture by small-scale farmers in Zashuke, KwaZulu-Natal Province. *Sustainability* 10:555. doi: 10.3390/su10020555

Philip, B., Daigneault, A., and Dawson, J. (2019). Age, values, farming objectives, past management decisions, and future intentions in New Zealand agriculture. *J. Environ. Manage*. 231, 110–120. doi: 10.1016/j.jenvman.2018.10.018

Phongsiri, M., Rigg, J., Salamanca, A., and Sripun, M. (2017). *Who Will Trend the Farm and Grow the Rice?* Bangkok Post. News. Available online at: https://www.bangkokpost.com/opinion/opinion/1384410/who-will-tend-the-farm-and-grow-the-rice-

Poungchompu, S., Tsuneo, K., and Poungchompu, P. (2012). Aspects of the aging farming population and food security in agriculture for Thailand and Japan. *Int. J. Environ. Rural Dev*. 3, 102–107.

Reij, C., and Waters-Bayer, A. (Eds.). (2001). *Farmer Innovation in Africa: A Source of Inspiration for Agricultural Development*. London: Earthscan.

Rigg, J., Phongsiri, M., Promphakping, B., Salamanca, A., and Sripun, M. (2020). Who will tend the farm? Interrogating the ageing Asian farmer. *J. Peasant Stud*. 47, 306–325. doi: 10.1080/03066150.2019.1572605

Salaisook, P., Faysse, N., and Tsusaka, T. W. (2020). Reasons for adoption of sustainable land management practices in a changing context: a mixed approach in Thailand. *Land Use Policy* 96:104676. doi: 10.1016/j.landusepol.2020.104676

Seok, J. H., Moon, H., Kim, G. S., and Reed, M. R. (2018). Is aging the important factor for sustainable agricultural development in Korea? Evidence from the relationship between aging and farm technical efficiency. *Sustain*. 10:2137. doi: 10.3390/su10072137

Souvi, K. N., Sun, C., Egbendewe-mondzozo, A., Kafui, K., and Alabi-doku, B. N. (2021). Analysis of the impacts of socio-economic factors on hiring an external labor force in tilapia farming in Southern Togo. *Aquac. Fish*. 6, 216–222. doi: 10.1016/j.aaf.2020.07.010

Suwanmaneepong, S., and Mankeb, P. (2017). Economic aspects of urban vegetable gardening in Bangkok Metropolitan, Thailand. *Int. J. Agric. Technol.* 13, 2161–2173. doi: 10.1016/j.ecolecon.2006.04.006

Thi, H., and Bui, M. (2021). Factors influencing farmers' decision to convert to organic tea cultivation in the mountainous areas of northern. *J. Org. Agric*. 11, 51–61. doi: 10.1007/s13165-020-00322-2

Whitfield, S. (2015). *Adapting to Climate Uncertainty in African Agriculture: Narratives and Knowledge Politics.* London: Routledge. doi: 10.4324/9781315725680

Whitfield, S., Challinor, A. J., and Rees, R. M. (2018). Frontiers in climate-smart food systems: outlining the research space. *Front. Sustain. Food Syst*. 2:2. doi: 10.3389/fsufs.2018.00002

Yamane, T. (1973). *Statistics: An Introductory Analysis*. *3rd Edn.* New York, NY: Harper and Row Publisher.

Zou, B., Mishra, A. K., and Luo, B. (2018). Aging population, farm succession, and farmland usage: evidence from rural China. *Land Use Policy* 77, 437–445. doi: 10.1016/j.landusepol.2018.06.001

Conflict of Interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

frontiers in Sustainable Food Systems

ORIGINAL RESEARCH
published: 26 November 2021
doi: 10.3389/fsufs.2021.731181

Evaluating the Potential of Protected Cultivation for Off-Season Leafy Vegetable Production: Prospects for Crop Productivity and Nutritional Improvement

Srinivasan Ramasamy[1], Mei-Ying Lin[1], Wan-Jen Wu[2], Hsin-I Wang[2] and Paola Sotelo-Cardona[1]*

[1] Safe and Sustainable Value Chains Flagship Program, World Vegetable Center, Tainan, Taiwan, [2] Healthy Diets Flagship Program, World Vegetable Center, Tainan, Taiwan

The effects of different protective structures on horticultural and nutritional yield of amaranth and water spinach were studied in three seasons of 2020–2021 in Taiwan. The number of people that can receive recommended dietary intake of iron and β-Carotene from vegetables grown under different production conditions was also estimated. The yield of white and red amaranths was consistently better (7.68–19.70 t/ha) under pink poly-net house in all the seasons, but the yield of water spinach was consistently better under white poly-net house (16.25–20.88 t/ha). Spider mite (fall & spring) and aphid (winter) infestation was mostly observed on all crops under poly-net houses. Neoxanthin, lutein and β-carotene were almost two-fold higher in red amaranth harvested from poly-net houses than open field. Based on the RDI values, β-Carotene supply to both men and women (14+) was consistently higher in all crops produced under pink ploy-net houses in all seasons, except for white amaranth produced under white poly-net house during winter. Its supply to 64,788 more men and 83,298 more women was estimated for red amaranth harvested from pink poly-net house than other production conditions. α-carotene was 2–3 fold higher in amaranths and water spinach harvested from poly-net houses than open field. The iron content of the amaranths was lower in poly-net houses (234.50–574.04 g/ha) than open field (645.42–881.67 g/ha) in the fall, but its supply from pink poly-net house was comparable with open field in the winter. However, pink poly-net house was the highest iron supplier from water spinach (323.90 g/ha) in the winter, which was estimated to provide iron to 19,450–22,939 more men and women than other production conditions. Both poly-net houses were the sole supplier of iron through amaranths in the spring, with pink poly-net house supplying iron to 2,000–5,000 more men and women. Thus, protected cultivation not only leads to more marketable yields but also results in higher quantities of health promoting nutrients. Hence, pink poly-net house may be considered to produce more nutritious vegetables, especially during the off-season to bridge the gaps in the seasonal variations in vegetable consumption, besides providing better income opportunities to the smallholder farmers.

Keywords: colored net structures, off-season production, nutritious vegetables, iron, carotenoids, amaranth, water spinach

OPEN ACCESS

Edited by:
Stefania Grando, Consultant, Ascoli Piceno, Italy

Reviewed by:
Chayon Goswami, Bangladesh Agricultural University, Bangladesh
Alexandru Rusu, Biozoon Food Innovations GmbH, Germany

***Correspondence:**
Srinivasan Ramasamy
srini.ramasamy@worldveg.org

Specialty section:
This article was submitted to Nutrition and Sustainable Diets, a section of the journal Frontiers in Sustainable Food Systems

Received: *26 June 2021*
Accepted: *01 November 2021*
Published: *26 November 2021*

Citation:
Ramasamy S, Lin M-Y, Wu W-J, Wang H-I and Sotelo-Cardona P (2021) Evaluating the Potential of Protected Cultivation for Off-Season Leafy Vegetable Production: Prospects for Crop Productivity and Nutritional Improvement. Front. Sustain. Food Syst. 5:731181. doi: 10.3389/fsufs.2021.731181

INTRODUCTION

Leafy vegetables are an important component in the farming systems as well as in the diets of people in Asia and Africa. Various leafy vegetables such as leafy brassicas (Chinese cabbage, pak-choi, kale, mustard, etc.), amaranth, water spinach, Malabar spinach, jute mallow, chayote, spider plant and African nightshade are grown in different parts of Asia and Africa, and they are mostly considered as "traditional" vegetables, since they are a part of alimentary traditions and cultural identity (Towns and Shackleton, 2018). These vegetables play an important role among the smallholder farmers, since they are a source of food and nutritional security, besides serving as income generating high value crops. For instance, Cambodian farmers were able to generate a revenue of US$ 4,776/ha from Chinese kale cultivation, whereas Vietnamese farmers earned US$ 5,070/ha from water spinach cultivation (Genova et al., 2010). Vegetable production has led to 3–14 times higher profits per hectare than in rice farming in Cambodia and Vietnam, while profits per labor-day are double (Joosten et al., 2015). The leafy vegetables are repeat-cycle crops, and the average length of growing period is 7–8 weeks, though farmers often re-sow their field after harvest or use staggered sowing to extend the harvest period (Schreinemachers et al., 2017). Thus, leafy vegetables are an important source of income for smallholder farmers.

Leafy vegetables are also an important source of nutrients. The leafy vegetables including amaranth and Chinese kale supply vitamins (especially vitamins A, C, folate), minerals such as calcium, potassium, iron, phosphorus, zinc, copper and manganese and dietary fiber to the human diet (Makobo et al., 2010; Ebert et al., 2011; Fowler, 2011). Water spinach is rich in protein, calcium, pro-vitamin A and vitamin C (Westphal, 1994). In addition, water spinach is a key contributor of lutein/zeaxanthin (Pan et al., 2018). However, the production of leafy vegetables is highly seasonal. For instance, amaranth is the predominant leafy vegetable in the summer months in Taiwan. Although water spinach is also grown during the summer months, leafy brassicas are mostly produced during the cooler months (Wang and Ebert, 2012).

Production of leafy vegetables is constrained by abiotic and biotic factors. Typhoons, for example, which regularly hit Taiwan during summer months, are often associated with heavy rainfall of up to 3,000 mm and subsequent flooding. Such extreme weather conditions not only lead to heavy yield losses in leafy vegetables, but also escalates their prices in the market (Wang and Ebert, 2012). Prices may soar to 3–5 times the normal level (Lee and Yang, 1999), which reduce their consumption during the summer months. Biotic factors such as pests and diseases also adversely affect the productivity of leafy vegetables. The leaf webber, *Spoladea recurvalis* F. (Lepidoptera: Crambidae) causes significant yield losses on amaranth in Asia (Hsu and Srinivasan, 2012) and Africa (Smith et al., 2018). White rust of amaranth, caused by *Albugo bliti* (Biv.) Kuntze, is a serious problem during the hot and humid conditions in Taiwan as well as in Southeast Asia (Grubben and Van Sloten, 1981; National Research Council, 1984), which causes almost 100% infection, considerably reducing the commercial value of the crop. Spotted tortoise beetle, *Aspidomorpha miliaris* F. (Coleoptera: Chrysomelidae), Convolvulus hawk-moth, *Agrius convolvuli* L. (Lepidoptera: Sphingidae), sweet potato stem borer, *Omphisa anastomosalis* Guenée (Lepidoptera: Crambidae) and common armyworm, *Spodoptera litura* F. (Lepidoptera: Noctuidae) are the major pests of water spinach (Muniappan et al., 2012).

Most leafy vegetables can be successfully grown under protective structures year-round. The leafy vegetables grown under protective structures using plug seedlings have been demonstrated to be grown faster, and harvested earlier with fewer pest problems compared to conventional production using direct seeding (Lee and Yang, 1999). Production under protective structures not only increases the total annual crop yield per unit area, but also improves the quality, besides extending their production period (Nordey et al., 2017). In recent years, use of color shade nets was found to protect the crops from adverse environmental conditions, improve the quality of vegetables and maintain post-harvest quality for an extended period (Ilić et al., 2018). But there is no evidence yet on how these shades affect yield or nutritional content of leafy greens. Hence, the objective of the current study is to determine the effects of different protective structures on the horticultural and nutritional yield of amaranth and water spinach across the seasons in Taiwan.

MATERIALS AND METHODS

Field Trials

Location and Seasons

The study was conducted at the World Vegetable Center, Shanhua, Tainan, Taiwan (23°08′29″N, 120°19′15″E) at a mean elevation of 9 m above the sea level. The trials were conducted following a complete randomized block design (CRBD), with three blocks. Three field trials were conducted during fall season (Sept 23–Oct 28, 2020), winter season (Dec 09 2020–Feb 8 2021), and spring season (March 24–May 5, 2021).

Treatments and Data Collection

Three leafy vegetables, *viz*., amaranth (*Amaranthus tricolor*, cv. white amaranth and cv. red amaranth) and water spinach (*Ipomoea aquatica* cv. kangkong) were compared under white poly-net house (clear color pattern, allowing full sunlight spectrum), pink poly-net house (magenta color pattern, Blue: Green: Red: Far Red percentages B:G:R:FR = 40:32:60:127, and 80% density knitted shade net) and open field conditions. Nets were manufactured by SpectralX, LeBio International Technology Corp. Ltd, Tainan, Taiwan. Each poly-net house was 7 x 12 x 4-m (W:L:H). Hence, each production system was considered as a treatment, and four replications were maintained for each treatment. The seeds were obtained from Hsinyusen seed company (Yunlin County, Taiwan). The crops were sown on raised bed (9.2-m long and 1-m wide) in each replication and managed following the customary production practices, including surface irrigation at weekly intervals and manual weeding three times in the season. About 25 kg of organic fertilizer (compost) (Chung Rong Industrial Company, Tainan, Taiwan) was applied to each bed. The incidence of pests and diseases were recorded from each replication. Spider mite occurred in the fall and spring seasons, whereas aphid occurred in the winter. The spider mite damage was rated using a 0–5 scale (Nihoul et al., 1991) and aphid damage was rated using a 1–5 scale: 1 (<10 aphids), 2 (11–50 aphids), 3 (51–100 aphids), 4 (101–500 aphids) and 5 (>500 aphids). At harvest, the vegetables were sorted and graded as marketable and unmarketable, and the yield of each category was recorded. The climatic conditions (temperature and relative humidity) in each replication were recorded throughout the season. Besides horticultural yield, nutritional analysis including dry matter, anti-oxidant activity, iron, and carotenoids (violaxanthin, neoxanthin, lutein, α-Carotene and β-Carotene) was carried out in all the crops and treatments. At harvest, pooled plant samples were collected from each treatment and used for the nutritional analysis. Two biological replications were used for each crop, and the mean value for each nutritional compound was used to estimate the total nutritional yield from the unit area (one hectare).

Nutritional Analysis

Iron

AOAC method no. 975.03 was used for iron determination (AOAC, 1990). Briefly, 0.2 g of dried sample powder from each treatment was mixed with 5 ml of 36 N sulfuric acid in the digestion flask. The samples were then kept aside overnight. The elemental analysis was continued by heating the digestion tubes at 300°C for 2 h. The contents were then cooled to about 150°C, and 2–3 ml of 30% hydrogen peroxide (H2O2) was added. The tubes were placed in the digester at 300°C for 1h to make the mixture transparent. The mixture was then cooled to about 40°C, and diluted with 50 ml distilled water. The iron content was determined in each sample using inductively coupled plasma–optical emission spectrometry (ICP-OES) instrument (8000 ICP-OES, PerkinElmer, Waltham, MA, USA). The standards were also prepared to make the calibration curve.

Antioxidant Activity

Leafy vegetable samples were analyzed for antioxidant activity (AOA) by ARP method (Arnao et al., 2001). This method measures the capacity of different components to scavenge the ABTS radical cation as compared to the standard antioxidant Trolox (0–4 mM) in a dose response curve. 0.1 g of freeze-dried powder with 9.9 ml of methanol was added in a centrifuge tube. The mixture was shaken for 4 h at high speed, and centrifuged at 6,000 rpm for 10 min. The supernatant was transferred into vials and stored at −70°C until analyzed. The reaction mixture contained 10 ml of 20 mM ABTS / 50 mM sodium phosphate buffer (pH 7.5) with 0.5 ml of HRP stock solution and 90 ml of ethanol. The mixture was then centrifuged at 12,000 rpm for 5 min and the supernatant was collected. Twenty microliters of antioxidant sample with appropriate dilution in water or methanol was added to the 2 mL of the reaction medium. The decrease in absorbance, which was proportional to the ABTS quenched, was determined after 5 min by spectrophotometer (U-2001, HITACHI, Tokyo, Japan) at 730 nm. The AOA of a sample for the ARP assay was measured within the linear relationship of concentration vs. optical density decrease, and presented as Trolox equivalent (TE) in μmol/g vegetable sample (fresh weight basis).

Dry Matter

Dry matter was determined from the weight difference of 1.0 g of fine powder before and after placing in an oven (DN 63, Yamato, Tokyo, Japan) at 135°C for 2 h.

Carotenoids

The carotene content was determined using the high-performance liquid chromatography (HPLC) method (Rodriguez-Amaya and Kimura, 2004). Briefly, 0.1 g of freeze-dried powder was mixed thoroughly with 0.5 ml of distilled water and 4.5 ml of acetone in glass vial, and the mixture was shaken for 30 min. Two milliliters of supernatant were pipetted into 10 ml test tube, and then dried using N_2 gas at 36°C for 20 min. To the dried sample, 100 μl of tetrahydrofuran (THF) and 1,900 μl Methanol was added and mixed well. The solution was then filtered through a 0.22 μm membrane and the final solution of 2 ml was injected into HPLC vials by using glass syringes enclosed with 0.22 μm pore size, and 13 mm diameter syringe filter. Separation and identification of carotenoids was performed using a HPLC system (Waters 2695, Milford, MA, USA) equipped with an auto-sampler, a photodiode array detector (Waters 996) monitoring at wavelength between 210 and 700 nm. The static phase was a C 30 Column (YMC™ Carotenoid 3.0 μm, 4.6 ×150 mm). The running conditions were set at 30°C using a gradient at 1.3 mL/min from 0 to 1% THF in methanol at 0–15 min, 1–25% THF in methanol at 15–25 min, 25–70% THF in methanol at 25–50 min, and the final 100% THF at 50–60 min. Identification of sample carotenoids was performed by comparing retention time and light absorption spectra (350–700 nm) of known standards. The peak areas were calibrated against known amounts of standards.

Estimating the Number of People That Can Receive Recommended Dietary iIntake of Iron and β-Carotene From Amaranth and Water Spinach Grown Under Different Production Conditions

Based on the nutritional yield per hectare, the number of people that can receive recommended dietary intake (RDI) of iron and β-Carotene from white amaranth, red amaranth and water spinach were estimated. We have attempted to pursue this perspective in the current study, since most of the existing studies attempt to understand the impact of agronomic practices on the yield but not on the nutrients. We chose only iron and β-carotene, although α-carotene was also a pro-vitamin A carotenoid. The revised bio-efficacy of α-carotene in a mixed diet is 1: 24 (Institute of Medicine, 2001), and hence it has been estimated to be 16.8–21.6 mg/day α-carotene that would fulfill the RDI for vitamin A of healthy adults. Since the α-carotene content in the amaranth and water spinach was comparatively lower than β-carotene, we did not include it in the estimation. Violaxanthin, neoxanthin and lutein are the epoxy carotenoids, which might be degraded by to the acidic conditions in the stomach (Asai et al., 2008; Britton et al., 2009; Maoka and Etoh, 2010), and hence we also did not include them in the estimation. The widely accepted

TABLE 1 | Analyses for marketable yield and nutritional content of white amaranth under colored poly-net house, white poly-net house, and open field conditions in Taiwan during 2020-–2021.

Source	df	Yield		Dry matter		Fe		Violaxanthin		Neoxanthin		Lutein		α-Carotene		ß-Carotene		AOA	
		F	Pr > F	F	Pr > F	F	Pr > F	F	Pr > F	F	Pr > F	F	Pr > F	F	Pr > F	F	Pr > F	F	Pr > F
Model	17	30.32	<0.0001	30.55	<0.0001	114.53	<0.0001	45.92	<0.0001	29.23	<0.0001	28.98	<0.0001	32.92	<0.0001	28.03	<0.0001	29.38	<0.0001
Season	2	88.46	<0.0001	90.84	<0.0001	225.10	<0.0001	134.51	<0.0001	76.20	<0.0001	75.72	<0.0001	45.23	<0.0001	70.23	<0.0001	81.03	<0.0001
Treatment	2	43.99	<0.0001	40.42	<0.0001	83.80	<0.0001	2.57	0.1100	67.31	<0.0001	67.49	<0.0001	155.55	<0.0001	78.09	<0.0001	56.92	<0.0001
Season ∗ Treatment	4	16.61	***<0.0001***	18.02	***<0.0001***	193.11	***<0.0001***	57.93	***<0.0001***	9.72	***0.0004***	9.35	***0.0005***	2.92	***0.0571***	6.40	***0.0033***	12.13	***0.0001***

*The bold values indicate the statistical significance for Season*Treatment.*

TABLE 2 | Analyses for marketable yield and nutritional content of red amaranth under colored poly-net house, white poly-net house, and open field conditions in Taiwan during 2020–2021.

Source	df	Yield		Dry matter		Fe		Violaxanthin		Neoxanthin		Lutein		α-Carotene		ß-Carotene		AOA	
		F	Pr > F	F	Pr > F	F	Pr > F	F	Pr > F	F	Pr > F	F	Pr > F	F	Pr > F	F	Pr > F	F	Pr > F
Model	17	10.20	<0.0001	10.23	<0.0001	13.10	<0.0001	10.71	<0.0001	10.53	<0.0001	10.69	<0.0001	13.64	<0.0001	10.45	<0.0001	10.22	<0.0001
Season	2	77.92	<0.0001	72.59	<0.0001	111.77	<0.0001	89.24	<0.0001	66.97	<0.0001	63.88	<0.0001	42.74	<0.0001	66.78	<0.0001	73.10	<0.0001
Treatment	2	7.34	0.0060	12.56	*0.0006*	8.89	0.0028	1.35	0.2890	20.26	*<0.0001*	23.63	*<0.0001*	61.58	*<0.0001*	19.05	*<0.0001*	12.09	*0.0007*
Season ∗ Treatment	4	3.24	***0.0419***	2.41	0.0951	11.46	***0.0002***	5.75	***0.0052***	1.88	0.1662	1.63	0.2186	2.26	0.1107	1.79	0.1842	2.48	0.0886

*The bold values indicate the statistical significance for Season*Treatment.*

TABLE 3 | Analyses for marketable yield and nutritional content of water spinach under colored poly-net house, white poly-net house, and open field conditions in Taiwan during 2020–2021.

Source	df	Yield		Dry matter		Fe		Violaxanthin		Neoxanthin		Lutein		α-Carotene		ß-Carotene		AOA	
		F	Pr > F	F	Pr > F	F	Pr > F	F	Pr > F	F	Pr > F	F	Pr > F	F	Pr > F	F	Pr > F	F	Pr > F
Model	17	12.24	<0.0001	13.01	<0.0001	33.67	<0.0001	14.32	<0.0001	19.01	<0.0001	17.86	<0.0001	31.29	<0.0001	17.04	<0.0001	18.64	<0.0001
Season	2	35.02	<0.0001	38.30	<0.0001	46.05	<0.0001	32.33	<0.0001	26.38	0.0002	27.34	0.0001	17.95	0.0007	31.09	<0.0001	46.14	<0.0001
Treatment	2	12.40	0.0005	6.13	0.0099	140.96	<0.0001	38.34	0.1100	96.26	<0.0001	83.34	<0.0001	218.97	<0.0001	63.79	<0.0001	20.42	<0.0001
Season ∗Treatment	4	22.13	***<0.0001***	25.18	***<0.0001***	35.11	***<0.0001***	18.19	***<0.0001***	12.38	***<0.0001***	13.22	***0.0005***	6.36	***0.0026***	16.90	***<0.0001***	34.88	***<0.0001***

*The bold values indicate the statistical significance for Season*Treatment.*

RDI value of 8 mg/day (for men 19+ and women 51+) and 18 mg/day (for women 19–50) for iron was used in the estimation. The revised bio-efficacy of β-carotene in a mixed diet is 1:12, whereas it is 1:24 for other pro-vitamin A carotenoids (Institute of Medicine, 2001). The widely accepted recommended dietary allowance (RDA) for vitamin A is 900 µg RAE for men (14+) and 700 µg RAE for women (14+), except the pregnant and lactating women. Thus, it has been estimated to be 10.8 mg/day β-carotene that would fulfill the RDA for vitamin A (900 µg) for healthy men and 8.4 mg/day for healthy women (700 µg) (Böhm et al., 2020). Hence, these RDI/RDA values for iron and β-carotene were used to estimate the number of people that can receive these nutrients from the crop harvests in the current study.

Data Analysis

The data was analyzed using ANOVA with the procedure Proc GLM of SAS version 9.4 (SAS Institute, Cary, NC, USA). The significant differences were identified and means were separated by Tukey's HSD test (differences were considered significant at $\alpha = 0.05$). Data on spider mite infestation did not follow normal distribution (even with data transformation). Therefore, a non-parametrical analysis was conducted. Each season was independently analyzed/crop, using the NPAR1WAY Procedure in SAS. Distribution of Wilcoxon Scores for spider mite percentages was analyzed with a Kruskal-Wallis Test. Later, a pairwise Two-Sided Multiple Comparison Analysis Dwass-Steel-Critchlow-Fligner Method was conducted to get differences between specific treatments per season/crop. For aphid infestation, categorical data was analyzed using the CATMOD procedure in SAS and the analysis of variance and the Analysis of Weighted Least Squares Estimates was done using a Chi-Square test for the treatment parameter. Data on diseases was transformed ASIN[SQRT(x)] for normality and UNIVARIATE Procedure was conducted to confirmed data had a fitted normal distribution following a Shapiro-Wilk test for normality. Combined analysis was conducted to evaluate differences among seasons in each crop. Non-transformed data is presented in the results section.

RESULTS

Marketable Yield of Amaranth and Water Spinach

Interaction effects (Treatment*Season) showed significant difference for marketable yield (**Tables 1–3**). White amaranth yield was significantly higher under pink poly-net house (19.70 t/ha) in the fall season, followed by open field (19.30 t/ha) and the white poly-net house (17.88 t/ha), which were on par with each other (**Figure 1A**). The yield was generally lower in the winter season compared to the fall season. In winter, both the pink poly-net house (15.10 t/ha) and the white poly-net house (15.30 t/ha) recorded similar yield, but it was significantly lower in the open field conditions (5.70 t/ha). Among the three seasons, spring recorded the lowest marketable yield in all the three production conditions. Like winter, both the pink poly-net house (9.75 t/ha) and the white poly-net house (9.83 t/ha) recorded similar yield, but nothing was harvested from the open field conditions.

The yield of red amaranth was also significantly higher under pink poly-net house (16.95 t/ha) in the fall season, followed by open field (13.50 t/ha) and the white poly-net house (13.45 t/ha), which were on par with each other (**Figure 1B**). Unlike white amaranth, the yield of red amaranth was slightly higher in the winter season compared to the fall season. In winter also, the poly-net houses recorded significantly higher yield (up to 18.17 t/ha), followed by the open field conditions (16.90 t/ha). Spring recorded the lowest marketable yield in all the three production conditions. Both the pink poly-net house (7.68 t/ha) and the white poly-net house (8.37 t/ha) recorded similar yield, but nothing was harvested from the open field conditions.

In the fall season, both open field (17.84 t/ha) and the white poly-net house (16.25 t/ha) recorded higher yield of water spinach than the pink poly-net house (14.80 t/ha) (**Figure 1C**). However, in the winter, this crop yielded significantly higher only under the white poly-net house conditions (19.70 t/ha), followed by pink poly-net house (16.07 t/ha), but the harvest from the open field was much lower (6.93 t/ha). In contrary to the amaranth crops, water spinach yielded significantly higher during the spring season. The yield was similar in all the three production conditions and ranged 20.55–20.88 t/ha.

Pests and Diseases

Spider mite (*Tetranychus* spp.) appeared to be the predominant pest on all the three crops in the poly-net houses, irrespective of their color in the fall and spring seasons (**Figure 2**). However, open-field water spinach was also infested by the mites in the fall season. Aphids were recorded as the major pest in the winter crop. About 77.5% of the red amaranth (Chi-square = 431.38, $P < 0.0001$) and 100% of the white amaranth (Chi-square = 88.40, $P < 0.0001$) in white poly-net house had reached the damage score of 5, whereas it was 75% in both red amaranth and white amaranth plants in the pink poly-net house condition (**Figure 3**). Although 30% of the red amaranth in open field condition were mildly (1–2 score) infested by aphids, the white amaranth was completely free from aphid infestation. Similarly, a vast majority of water spinach plants (72.5%) were free from aphids (Chi-square = 352.16, $P < 0.0001$), but about 87.5% of plants in the white poly-net house and about 75% of plants in the pink poly-net house were infested by aphids. There was no disease infecting the water spinach. However, white and red amaranths were infected by *Pythium* and *Rhizoctonia*. Interaction effects (Treatment*Season) showed significant difference for disease incidence on white amaranth, but not on red amaranth (**Table 4**). The disease was comparatively severe on white amaranth than on red amaranth (**Table 5**), but more or less similar in all the three production conditions, with the maximum incidence of 7.55% during the fall season.

Nutritional Content

Interaction effects (Treatment*Season) showed significant difference for all the nutritional parameters of white amaranth (**Table 1**). The dry matter content was corresponding to the yield of the crop under different production conditions (**Table 6**).

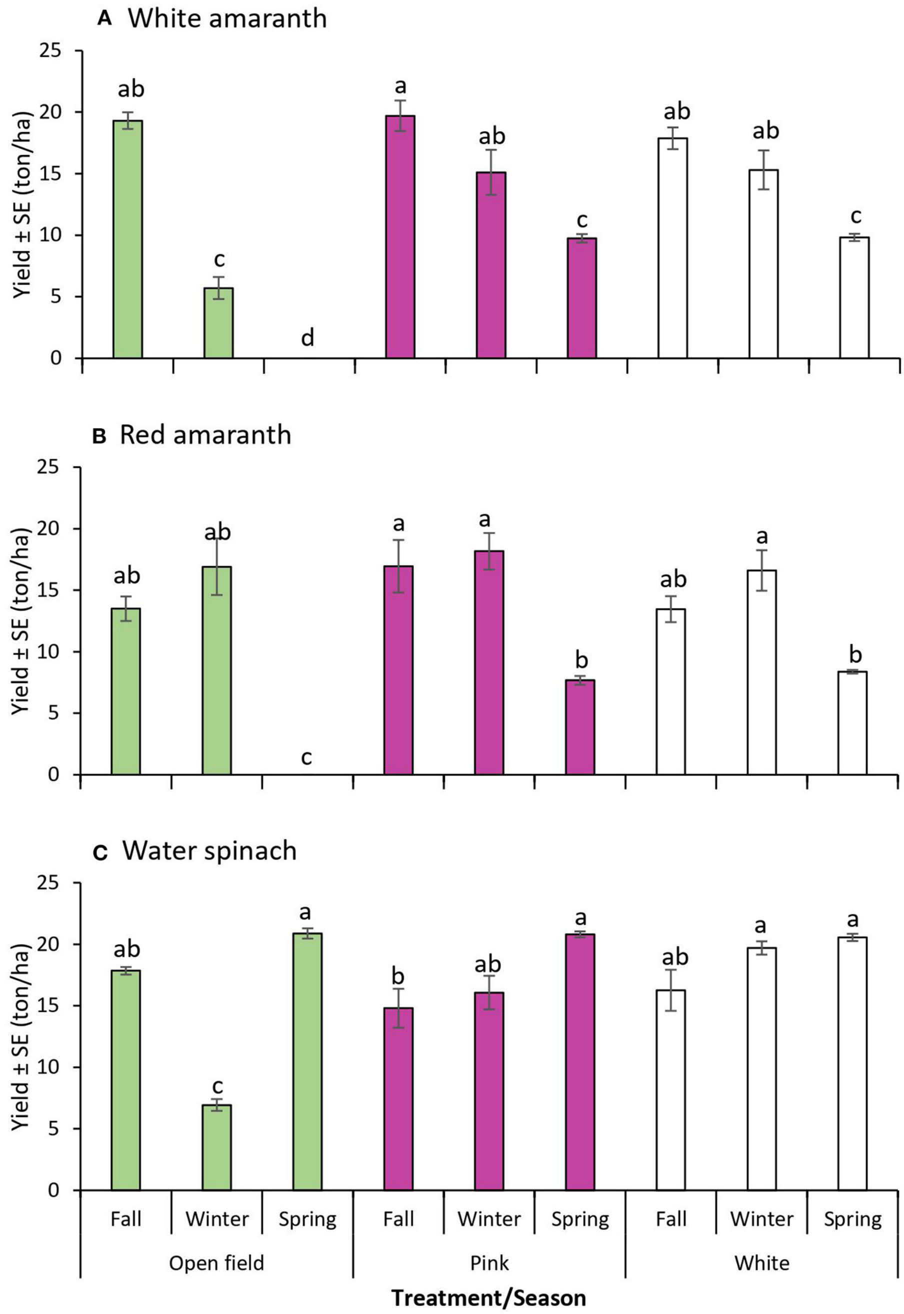

FIGURE 1 | Yield ± SE (ton/ha) of leafy vegetables grown under different production conditions. **(A)** White amaranth, **(B)** Red amaranth, **(C)** Water spinach. Means represent average of four replications in each treatment/season. Means followed by the same letter(s) in a crop are not significantly different ($p < 0.05$).

The iron content was significantly higher during the fall season in the white amaranth harvested from the open field (881.67 g/ha), followed by pink poly-net house (336.48 g/ha) and white poly-net house (234.50 g/ha). However, the open field (260.39 g/ha) and the pink poly-net house (257.91 g/ha) supplied more iron than the white poly-net house (200.72 g/ha) in the winter season. Since no crop was harvested from the open field during the spring season, there was no iron supply from the open field condition. However, both the poly-net houses yielded similar amount of iron (128.89–166.53 g/ha) during the spring. Although open field production of white amaranth supplied better violaxanthin, neoxanthin and lutein in the fall season, it fell behind the poly-net houses in the winter crop. Supply of these compounds from the pink poly-net house was

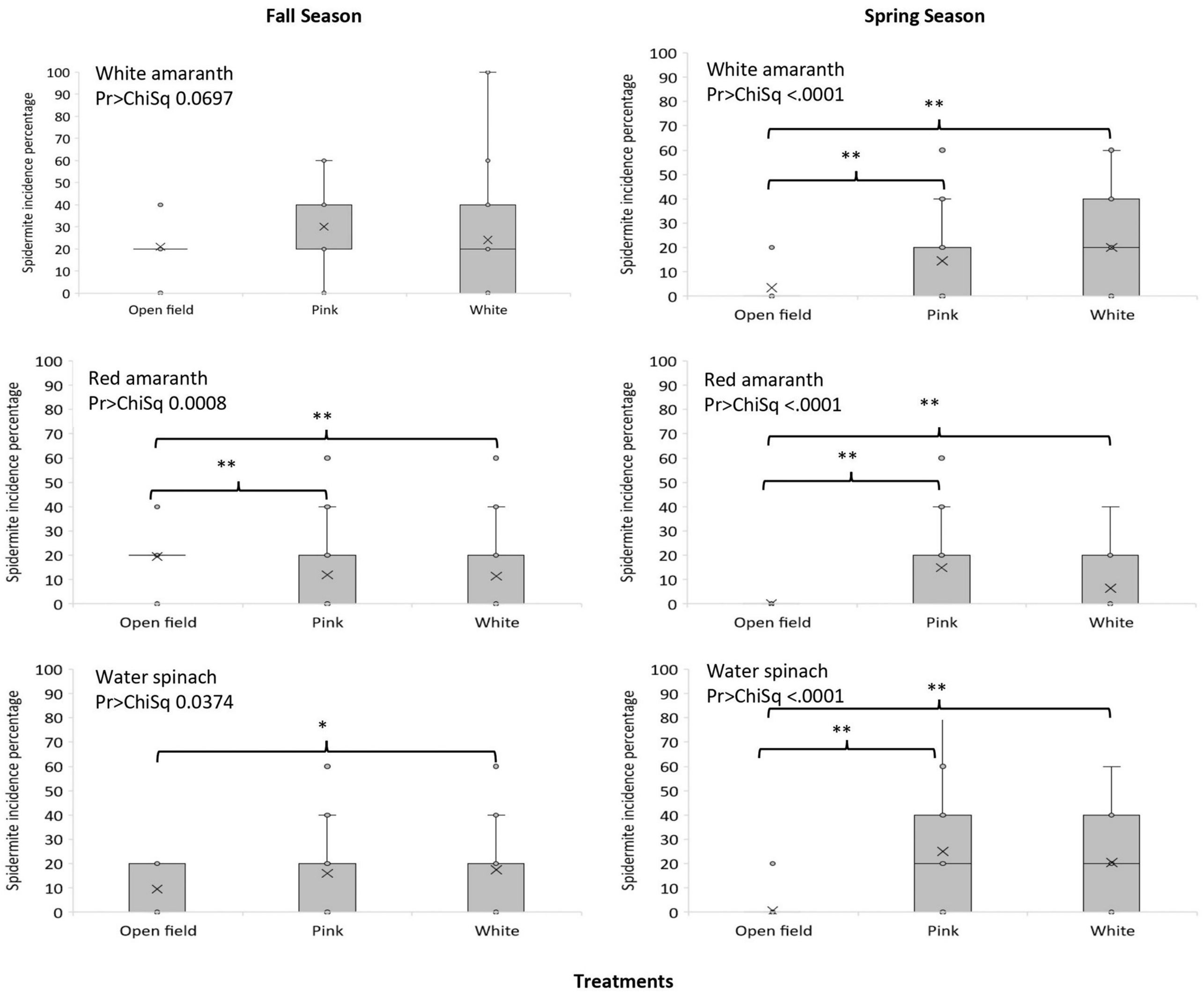

FIGURE 2 | Mean (±SD) incidence of spider mite (*Tetranychus* spp.) on leafy vegetables grown under different production conditions. **(A)** White amaranth, **(B)** Red amaranth, **(C)** Water spinach. Means represent average of four replications in each treatment/season. *significant; **highly significant.

significantly higher than from the white poly-net house in both fall and winter crops. Similarly, both the poly-net houses provided significantly higher amount of α-Carotene (18.90–47.18 g/ha) and β-Carotene (362.32–732.06 g/ha) than the open field crops (0–16.06 g/ha and 0–556.29 g/ha) during these seasons. Consistently, better anti-oxidant activity was recorded for both the poly-net houses (91.68–190.24 mole/ha) than the open field (0–165.43 mole/ha) in the fall and winter seasons. However, all the nutritional parameters were significantly higher in both the poly-net houses than in the open field during the spring season.

Interaction effects (Treatment*Season) showed significant difference only for iron and violaxanthin content of red amaranth (**Table 2**). The iron content was significantly higher in red amaranth produced from open field (645.42 g/ha), compared to the white poly-net house (352.14 g/ha) during the fall season (**Table 7**). However, it was intermediate in the crop produced from the pink poly-net house (574.04 g/ha). Similar trend was also recorded in the winter season. The violaxanthin supply was almost on par in all the treatments in both fall (798.47–954.43 g/ha) and winter (985.47–1178.38 g/ha) seasons. In red amaranth as well, there was no supply of iron and violaxanthin from open field production during spring, but both the poly-net houses provided equal amounts (432.31–496.74 g/ha). In addition, dry matter content, carotenoids and anti-oxidant activity of red amaranth only responded to treatment effect (**Table 8**). Moreover, pink poly-net house recorded significantly higher amount of all the other nutritional compounds (**Table 8**). White poly-net house also recorded on par values for most of the parameters, except for neoxanthin, lutein and α-Carotene. All these nutritional compounds were significantly lower in red amaranth harvested from the open field, compared to the poly-net houses.

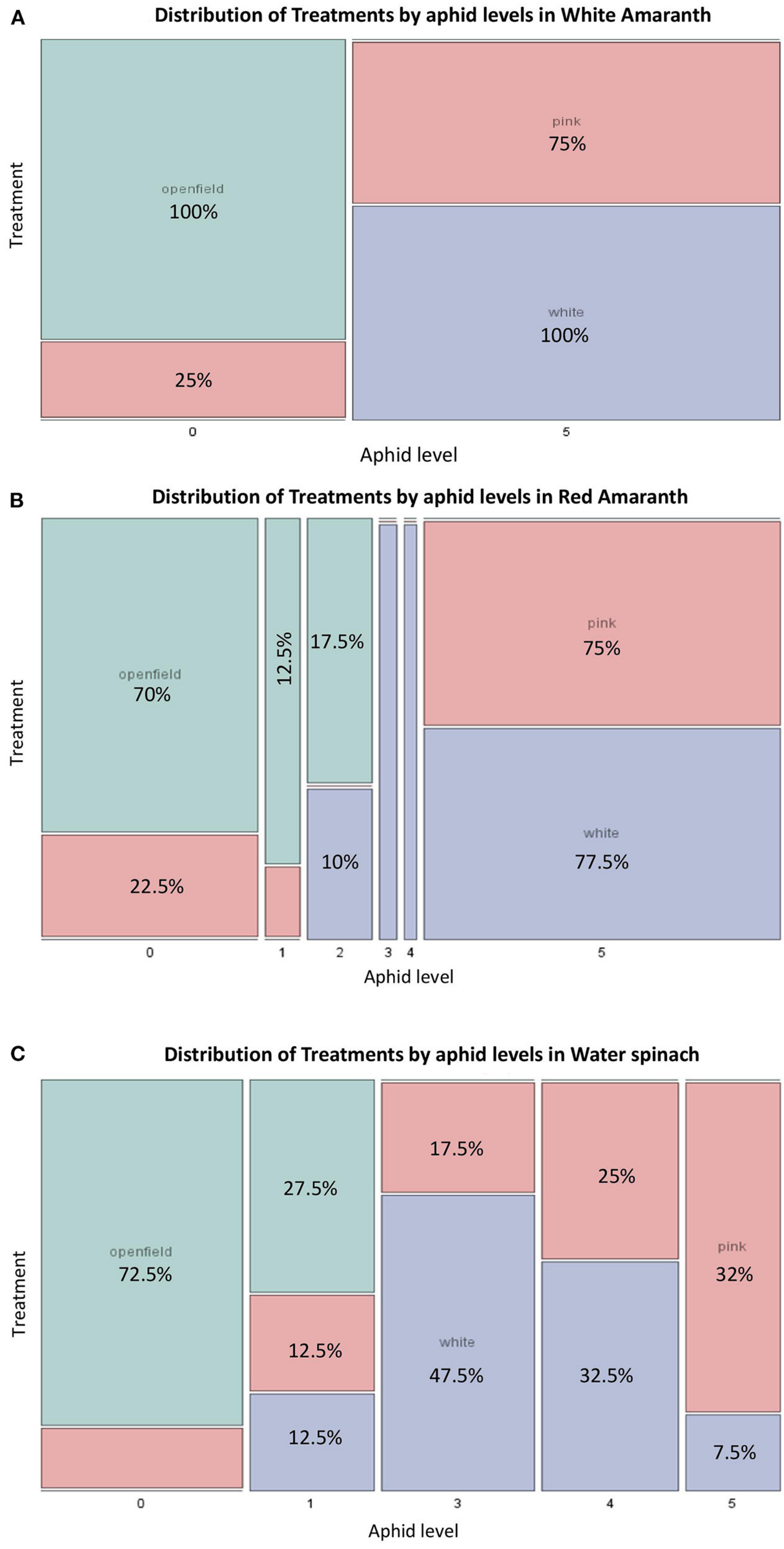

FIGURE 3 | Mean incidence of aphids on leafy vegetables grown under different production conditions. **(A)** White amaranth, **(B)** Red amaranth, **(C)** Water spinach. Means represent average of four replications in each treatment/season.

TABLE 4 | Analyses for disease incidence on white amaranth and red amaranth under colored poly-net house, white poly-net house, and open field conditions in Taiwan during 2020–2021.

Source	df	White Amaranth		Red Amaranth	
		F	Pr > F	F	Pr > F
Model	17	43.12	<0.0001	1.02	0.48
Season	2	21.48	0.0004	8.58	0.0082
Treatment	2	97.44	<0.0001	3.42	0.0578
Season*Treatment	4	49.09	<0.0001	1.20	0.3504

TABLE 5 | Disease incidence (%) on white amaranth and red amaranth under colored poly-net house, white poly-net house, and open field conditions in Taiwan during 2020–2021.

Season	Treatment	White Amaranth		Red Amaranth	
		N	Disease percentage ± SE	N	Disease percentage ± SE
Fall	Open field	4	6.37 ± 1.23 ab	4	1.80 ± 0.22
	Pink	4	7.55 ± 1.14 a	4	1.94 ± 0.15
	White	4	7.55 ± 1.14 a	4	2.24 ± 1.04
Winter	Open field	4	0 e	4	0.00
	Pink	4	3.96 ± 0.63 cd	4	2.37 ± 0.90
	White	4	4.63 ± 0.53 bc	4	1.32 ± 0.79
Spring	Open field	4	2.86 ± 0.24 d	4	0.83 ± 0.10
	Pink	4	3.04 ± 0.13 d	4	1.92 ± 0.07
	White	4	3.40 ± 0.05 cd	4	1.84 ± 0.26

Means followed by the same letter(s) in a column are not significantly different ($p < 0.05$).

Interaction effects (Treatment*Season) showed significant difference for all the nutritional parameters of water spinach (**Table 3**). The dry matter content was significantly higher in the water spinach harvested from the open field during fall season (2,128.6 kg/ha), but it was the lowest in the winter season (826.2 kg/ha) (**Table 9**). In spring, the dry matter content was significantly higher in the plants harvested from pink poly-net house (2,520.7 kg/ha), followed by the open field (2,490.6 kg/ha). The iron content was significantly higher during the fall season in the water spinach harvested from the open field (361.7 g/ha), followed by pink poly-net house (298.4 g/ha), but only the pink poly-net house supplied more iron (323.9 g/ha) than the other two production conditions (140.4–168.3 g/ha) in the winter season. Both the open field condition (423.2 g/ha) and the pink poly-net house (419.3 g/ha) yielded significantly higher amount of iron during the spring. Consistently higher number of carotenoids were obtained from the water spinach harvested under the pink poly-net house in all the seasons, and it was significantly higher during the spring than the other seasons. Significantly higher anti-oxidant activity was recorded for the open field in the fall (336.3 mole/ha) and spring (393.4 mole/ha) seasons, which was on par with the pink poly-net house (344.5 mole/ha) in the spring.

TABLE 6 | Dry matter content, iron, carotenoids and anti-oxidant activity of white amaranth under colored poly-net house, white poly-net house, and open field conditions in Taiwan during 2020–2021.

Season	Treatment	Dry matter (kg/ha)	Fe (g/ha)	Violaxanthin (g/ha)	Neoxanthin (g/ha)	Lutein (g/ha)	α-Carotene (g/ha)	β-Carotene (g/ha)	AOA, (mole/ha)
Fall	Open field	2,455.48 ± 85.66 a	881.67 ± 30.76 a	533.26 ± 18.60 a	222.04 ± 7.75 ab	831.75 ± 29.02 ab	16.06 ± 0.56 d	556.29 ± 19.40 b	165.43 ± 5.77 ab
	Pink	2,393.01 ± 150.83 a	336.48 ± 21.21 b	342.67 ± 21.60 b	280.90 ± 17.70 a	1,049.44 ± 66.14 a	47.18 ± 2.97 a	732.06 ± 46.14 a	190.24 ± 11.99 a
	White	2,246.90 ± 110.06 a	234.50 ± 11.59 cd	310.09 ± 15.33 bc	234.98 ± 11.61 ab	899.50 ± 44.46 ab	34.39 ± 1.70 b	700.61 ± 34.63 ab	166.80 ± 8.25 ab
Winter	Open field	725.19 ± 114.50 c	260.39 ± 41.11 bc	157.49 ± 24.87 d	65.58 ± 10.35 de	245.65 ± 38.79 de	4.74 ± 0.75 e	164.29 ± 25.94 d	48.85 ± 7.71 d
	Pink	1,834.25 ± 222.77 a	257.91 ± 31.32 bc	262.66 ± 31.90 bc	215.31 ± 26.15 ab	804.39 ± 97.70 ab	36.16 ± 4.39 b	561.13 ± 68.15 ab	145.82 ± 17.71 ab
	White	1,923.22 ± 198.61 a	200.72 ± 20.73 cde	265.42 ± 27.41 c	201.13 ± 20.77 b	769.92 ± 79.51 b	29.44 ± 3.04 bc	599.69 ± 61.93 ab	142.77 ± 14.73 b
Spring	Open field	0 c	0 e	0 e	0 e	0 e	0 e	0 d	0 d
	Pink	1,184.36 ± 41.64 b	166.53 ± 5.85 de	169.60 ± 5.96 d	139.03 ± 4.89 c	519.39 ± 18.26 c	23.35 ± 0.82 cd	362.32 ± 12.74 c	94.15 ± 3.31 c
	White	1,235.01 ± 37.14 b	128.89 ± 3.88 de	170.44 ± 5.13 d	129.16 ± 3.88 cd	494.41 ± 14.87 cd	18.90 ± 0.57 d	385.09 ± 11.58 c	91.68 ± 2.76 c

Means followed by the same letter(s) in a column are not significantly different ($p < 0.05$).

Number of People That Can Receive Recommended Dietary Intake of Iron and β-Carotene

Based on the widely accepted RDI of iron, open field produced white amaranth from a hectare was estimated to supply iron to 68,149-80,896 more men (19+ years old) and women (51+ years old) than the crop produced under poly-net houses during the fall season (**Table 10**). In the winter, the white amaranth produced from the open field conditions as well as the pink poly-net houses was estimated to supply iron to almost equal number (>7,000 people) of men and women (51+). In addition, water spinach produced under the pink poly-net houses during winter was estimated to provide iron to 19,450–22,939 more men (19+) and women (51+) than the other production conditions. In the spring, the white and red amaranths produced under poly net-houses supplied the iron, and the pink poly-net house was estimated to supply to 2,000–5,000 more men and women than the white poly-net house. In case of women (19–50 years), the same trend in the results was also recorded for all the crops, seasons and production conditions. β-Carotene supply to both men (14+) and women (14+) was consistently higher in all the crops produced under the pink ploy-net houses in all the seasons, except for the white amaranths produced under white poly-net house during the winter. The maximum supply from the red amaranth produced under pink poly-net houses reached a value of 64,788 more men and 83,298 more women than the other production conditions in all the seasons (**Table 10**). Thus, the pink poly-net house was found to be the most suitable production condition for supplying β-Carotene to a maximum number of people.

TABLE 7 | Iron and violaxanthin content of red amaranth under colored poly-net house, white poly-net house, and open field conditions in Taiwan during 2020–2021.

Season	Treatment	Fe (g/ha)	Violaxanthin (g/ha)
Fall	Open field	645.42 ± 47.61 ab	941.31 ± 69.44 a
	Pink	574.04 ± 72.43 abc	954.43 ± 120.43 a
	White	352.14 ± 27.55 cd	798.47 ± 62.48 ab
Winter	Open field	807.97 ± 109.96 a	1,178.38 ± 160.37 a
	Pink	615.25 ± 50.39 abc	1,022.94 ± 83.79 a
	White	434.61 ± 43.02 bcd	985.47 ± 97.55 a
Spring	Open field	0 e	0 c
	Pink	260.01 ±11.89 d	432.31 ± 19.78 b
	White	219.07 ± 3.84 d	496.74 ± 8.72 b

Means followed by the same letter(s) in a column are not significantly different ($p < 0.05$).

DISCUSSION

In this study, we investigated the effect of different colored poly-net house compared to open field conditions in terms of yield, dry matter content, iron, carotenoids and anti-oxidant activity for three leafy vegetables, *viz*., white amaranth, red amaranth, and water spinach, over three seasons (fall, winter and spring). In addition, the overall effect of pests and diseases was also evaluated under the above-mentioned conditions. Finally, the number of people who could receive selected micro-nutrients from the crops harvested under different production conditions was estimated based on the RDI values. This is quite important to understand if sufficient quantity of nutrient-dense vegetables can be grown in a relatively small area, considering the context of fast shrinking farmlands. To our knowledge, there are not many scientific information available on this perspective.

Generally, the amaranth yield was higher in the fall season, which could be due to the higher prevailing temperature during this season, ranging between 25.7 and 27.2°C (**Table 11**). An increased temperature (from 28 to 32°C) was found to promote both root and leaf growth in edible amaranth (*A. tricolor* cv. White leaf) in Taiwan (Hwang et al., 2018). Amaranth is a C4 plant, and an earlier study had demonstrated that photorespiration losses in C4 plants were limited and hence C4 plants had higher net photosynthetic rates at higher temperatures compared to C3 plants (Long, 1999). Hence, the higher temperature during the fall season was found to have a positive effect on the growth of this C4 crop. Besides temperature, the light conditions inside the poly-net houses were believed to have provided the optimum microclimate for growth and development of amaranth crops. For instance, the blue irradiance (400–500 nm) inside the pink net house was about 40%, whereas it was about 25% in the white poly-net house and open field conditions. It was already demonstrated that the blue (400–450 nm) polyethylene shade condition produced taller plant height, a greater number of leaves, biomass yield and bioactive compounds in red amaranth, compared with other conditions of films (Khandaker et al., 2010). This might be due to the fact that some plant species are more sensitive to blue light (Casal, 1994). Thus, the combination of higher temperature and blue light in the poly-net houses could have contributed for the better yield. Although both white and red amaranths are C4 plants, the yield of red amaranth was slightly higher in the winter season compared to the fall season, whereas it was not

TABLE 8 | Dry matter content, carotenoids and anti-oxidant activity of red amaranth under colored poly-net house, white poly-net house, and open field conditions in Taiwan during 2020–2021.

Treatment	Dry matter (kg/ha)	Neoxanthin (g/ha)	Lutein (g/ha)	α-Carotene (g/ha)	β-Carotene (g/ha)	AOA (mole/ha)
Open field	1,461.99 ± 376.39 b	484.94 ± 124.85 c	1,380.93 ± 355.52 c	54.95 ± 14.15 c	723.69 ± 186.31 b	134.09 ± 34.52 b
Pink	2,630.93 ± 307.10 a	1,015.99 ± 118.59 a	3,085.79 ± 360.19 a	241.31 ± 28.17 a	1,487.38 ± 173.62 a	238.84 ± 27.88 a
White	2,290.67 ± 211.18 a	815.31 ± 75.17 b	2,468.60 ± 227.59 b	166.28 ± 15.33 b	1,243.68 ±114.66 a	208.33 ± 19.21 a

Means followed by the same letter(s) in a column are not significantly different ($p < 0.05$).

TABLE 9 | Dry matter content, iron, carotenoids and anti-oxidant activity of water spinach under colored poly-net house, white poly-net house, and open field conditions in Taiwan during 2020–2021.

Season	Treatment	Dry matter (kg/ha)	Fe (g/ha)	Violaxanthin (g/ha)	Neoxanthin (g/ha)	Lutein (g/ha)	α-Carotene (g/ha)	β-Carotene (g/ha)	AOA (mole/ha)
Fall	Open field	2,128.6 ± 37.3 abc	361.7 ± 6.3 ab	502.2 ± 8.8 bc	402.3 ± 7.0 d	1,142.0 ± 20.0 c	17.1 ± 0.3 de	562.3 ± 9.8 bc	336.3 ± 5.9 ab
	Pink	1,793.5 ± 192.2 cd	298.4 ± 32.0 b	541.1 ± 58.0 bc	581.2 ± 62.3 bc	1,559.8 ± 167.1 b	49.0 ± 5.2 b	660.6 ± 70.8 bc	245.1 ± 26.3 c
	White	1,594.3 ± 163.7 d	138.8 ± 14.2 c	409.9 ± 42.1 c	379.3 ± 38.9 d	1,055.0 ± 108.3 c	28.8 ± 3.0 cd	436.5 ± 44.8 d	195.2 ± 20.0 cd
Winter	Open field	826.2 ± 56.9 e	140.4 ± 9.7 c	194.9 ± 13.4 d	156.1 ± 10.7 e	443.2 ± 30.5 d	6.7 ± 0.5 e	218.2 ± 15.0 e	130.5 ± 9.0 d
	Pink	1,947.0 ± 166.9 bcd	323.9 ± 27.8 b	587.4 ± 50.4 b	631.0 ± 54.1 b	1,693.3 ± 145.2 b	53.2 ± 4.6 b	717.2 ± 61.5 b	266.1 ± 22.8 bc
	White	1,932.7 ± 53.4 cd	168.3 ± 4.6 c	497.0 ± 13.7 bc	459.8 ± 12.7 cd	1,279.0 ± 35.4 bc	34.9 ± 1.0 c	529.2 ± 14.6 cd	236.6 ± 6.5 c
Spring	Open field	2,490.6 ± 50.5 ab	423.2 ± 8.6 a	587.6 ± 11.9 b	470.7 ± 9.5 bcd	1,336.1 ± 27.1 bc	20.1 ± 0.4 d	657.9 ± 13.3 bc	393.4 ± 8.0 a
	Pink	2,520.7 ± 30.1 a	419.3 ± 5.0 a	760.5 ± 9.1 a	816.9 ± 9.7 a	2,192.1 ± 26.2 a	68.9 ± 0.8 a	928.4 ± 11.1 a	344.5 ± 4.1 a
	White	2,016.1 ± 28.5 bcd	175.6 ± 2.5 c	518.4 ± 7.3 bc	479.6 ± 6.8 bc	1,334.2 ± 18.8 bc	36.4 ± 0.5 c	552.0 ± 7.8 bcd	246.8 ± 3.5 c

Means followed by the same letter(s) in a column are not significantly different ($p < 0.05$).

TABLE 10 | Number of people that can receive recommended daily intake of iron and β-Carotene from white amaranth, red amaranth and water spinach grown under colored poly-net house, white poly-net house, and open field conditions in Taiwan during 2020–2021.

Season	Treatment	Iron						β-Carotene					
		White amaranth		Red amaranth		Water spinach		White amaranth		Red amaranth		Water spinach	
		Men (>19) & Women (>51)	Women (19–50)	Men (>19) & Women (>51)	Women (19–50)	Men (>19) & Women (>51)	Women (19–50)	Men (>14)	Women (>14)	Men (>14)	Women (>14)	Men (>14)	Women (>14)
Fall	Open field	110,209	48,982	80,678	35,857	45,218	20,097	51,508	66,225	103,031	132,469	52,064	66,939
	Pink	42,060	18,693	71,755	31,891	37,298	16,577	67,783	87,150	167,819	215,767	61,169	78,645
	White	29,313	13,028	44,018	19,563	17,355	7,713	64,871	83,406	120,948	155,505	40,416	51,963
Winter	Open field	32,549	14,466	100,996	44,887	17,551	7,801	15,212	19,558	128,981	165,832	20,208	25,982
	Pink	32,239	14,328	76,906	34,181	40,490	17,996	51,956	66,801	179,865	231,255	66,404	85,376
	White	25,090	11,151	54,326	24,145	21,040	9,351	55,527	71,392	149,275	191,925	48,997	62,996
Spring	Open field	0	0	0	0	52,906	23,514	0	0	0	0	60,917	78,321
	Pink	20,816	9,252	32,501	14,445	52,419	23,297	33,548	43,133	76,014	97,732	85,967	110,529
	White	16,111	7,161	27,384	12,171	21,948	9,754	35,656	45,844	75,244	96,743	51,111	65,714

the case for white amaranth. Hence, besides temperature and light conditions, other factors may also contribute to these yield differences across the seasons. For instance, the photosynthetic pigments of higher plants include chlorophyll and carotenoids. The carotenoids content of the red amaranth were higher than the white amaranth, and most of the carotenoids in the white amaranth were lower in the winter season than in the fall. Hence, future studies should elucidate the role of photosynthetic pigments on yield under different production systems across the growing seasons. In addition, comparatively lower yield in the spring season, despite the warmer temperature could have been attributed to the higher spider mite infestation during this season. The growth and performance of water spinach was relatively better in the poly-net houses in all the seasons compared to the open field conditions, in which the lowest yield was recorded in the winter. Water spinach has originated from tropical regions, with high tolerance to heat and wet (Liou, 1981). The optimal growth conditions are temperatures between 20 and 27°C and the humidity of above 75% (Pinker et al., 2007). Since the winter temperature was quite low (**Table 11**), especially in the open field conditions, the yield might have declined significantly.

It is not surprising to record the spider mite infestation during the fall and spring season, in which the temperature is relatively warmer than the cold winter in Taiwan (**Table 11**). Most spider mites prefer warm and dry conditions. Although spider mite populations are found on crops in winter months in Taiwan (Ho, 2000), we did not find mite infestation on both the amaranth varieties and water spinach during the winter season. This is also due to the fact that aphids emerged as a major pest during the winter months and hence they outcompete spider mites. Aphids have been recorded as a serious pest of amaranth (Ebert et al., 2011). In fact, aphids occurred highly inside the poly-net houses. Several earlier studies have documented the aphid outbreaks inside the net house conditions (Talekar et al., 2003; Majumdar and Powell, 2011). A warm weather inside the poly-net houses during the winter months and the absence of natural enemies inside the protective structures could have led to the proliferation of aphid infestation in the current study. The major entry point for aphids and mites is through the nylon net. Most net houses were built with 40-mesh netting. The 40-mesh or coarser mesh nets failed to exclude the thrips, whitefly and aphid in several countries (Talekar et al., 2003; Harmanto, 2006). The poly-net houses used in the current study have been built with 32-mesh netting only. Hence, they were not able to prevent the entry of aphids and spider mites. The most promising way to prevent the entry of small-sized insects and mites in net houses is to use nets with finer mesh. Nets with 50-mesh or 60-mesh size to exclude thrips, whitefly, and aphids have been suggested (Polston and Lapidot, 2007; Shahak et al., 2008; Palada and Wu, 2009). However, finer mesh size reduces the ventilation rate and increases the relative humidity inside the net houses (Harmanto et al., 2006), which could favor the incidence of diseases, although it was not the case in the current study. It is interesting to note that the crop yields in both the poly-net houses were similar or higher than the open field production conditions despite the higher incidences of spider mite or aphids inside the poly-net houses. Hence, proper control options to manage aphids and spider mite on leafy vegetables in poly-net house conditions should be considered so that the crop productivity can be further increased.

Among the three production conditions compared in the current study, most of the carotenoids consistently occurred in higher quantities in the white amaranth crop produced under pink poly-net houses. Neoxanthin, lutein, and β-carotene were slightly higher in the red amaranth grown under the poly-net houses. Similarly, α-carotene was also higher in the amaranth crops grown under the poly-net houses. A recent study conducted under open field conditions in the same location documented higher amounts of violaxanthin, neoxanthin and lutein (Nordey et al., 2021) than the white amaranth in the current study, but the α- and β-carotenes were similar in both the studies. However, all the carotenoids in the previous study were lower than the quantities in the red amaranth in our study. It should be noted that the previous study involved amaranth accessions from different species (*Amaranthus hypochondriacus*, *A. cruentus, A. dubius* and *A. blitum*), whereas the varieties in the current study belonged to *A. tricolor*. In line with this, other papers on amaranth studying the carotenoid profiles corroborated our results, where higher carotenoids in the red amaranth have been observed compared to other genotypes (Khanam and Oba, 2013; Sarker and Oba, 2020). Hence, the quantity of carotenoids not only differed among the species of amaranth, but also varied among the varieties within a species. In addition, these variations could be altered by the production conditions, as we observed in the case of red amaranth under poly-net house conditions compared to the open field conditions. Hence, protected cultivation conditions not only lead to more marketable yields but also result in higher quantities of health promoting nutrients.

The quantities of the carotenoids such as lutein and β-carotene observed in water spinach in the current study were similar to the earlier findings (Khoo et al., 2011; Chandra-Hioe et al., 2017), and the α-carotene was 10–25-fold higher than an earlier report (Khoo et al., 2011). As observed in the case of amaranth crops, α-carotene in water spinach was also two to three-fold higher in the crops harvested from the poly-net houses than the open field condition. The iron content of the amaranth varieties produced from the open field conditions was comparable with the previous study (Nordey et al., 2021), but surprisingly it was lower in both the varieties harvested from the poly-net houses. However, the iron supply from the pink poly-net house was comparable with the open field in the winter for both the amaranth crops but pink poly-net house was the highest iron supplier from water spinach in the winter. The poly-net houses were the sole supplier of iron through amaranth crops in the spring, although the niche was shared by open field with the pink poly-net house for the water spinach. It is important to note that only poly-net houses enabled the production of amaranth crops in the spring season, and the water spinach in the winter season.

While comparing pink poly-net house with the white poly-net house, the supply of carotenoids and iron was comparatively better from the pink poly-net house in most of the seasons for all the three crops. A previous study from Japan also demonstrated that red amaranth grown under blue shade polyethylene

TABLE 11 | Climatic conditions [temperature (°C), (Mean ± SD; Min-Max range), and relative humidity (%RH), (Mean ± SD; Min-Max range)] during fall season (Sept 23–Oct 28, 2020), winter season (Dec 9 2020–Feb 8 2021), and spring season (March 24–May 5, 2021).

Treatment	Fall Season		Winter Season		Spring Season	
	T (°C)	%RH	T (°C)	%RH	T (°C)	%RH
Open field	25.7 ± 1.5 (20.8–33.9)	81.5 ± 4.1 (58.8–97.2)	16.1 ± 2.9 (11.0–23.5)	83.8 ± 6.5 (57.6–98.1)	23.2 ± 2.0 (17.9–30.5)	80.7 ± 6.2 (56.2–97.4)
Pink	27.1 ± 4.6 (19.4–37.1)	70.0 ± 13.4 (40.1–90.2)	18.2 ± 5.2 (6.00–33.0)	68.7 ± 14.5 (25.0–90.5)	25.6 ± 5.1 (13.4–37.4)	63.6 ± 14.7 (34.7–87.4)
White	27.2 ± 4.5 (19.5–37.4)	72.3 ± 13.1 (43.4–92.4)	18.4 ± 5.2 (6.6–33.2)	72.0 ± 14.6 (27.5–94.3)	25.8 ± 5.0 (13.9–37.4)	68.6 ± 14.0 (39.9–90.3)

produced more biomass yield with health beneficiary bioactive compounds betacyanins, polyphenol and antioxidant activity during the low temperature regime in spring season (Khandaker et al., 2010). A recent study had assessed the effects of various ratios of combined red, blue, and amber light-emitting diodes on the expression of carotenoid biosynthetic genes and carotenoid accumulation in brassicas (Alrifai et al., 2021). It found that total and individual carotenoids were increased significantly under dose-dependent increasing amber–blue light and decreasing red in most brassica microgreens. According to this study, lipophilic 2,2-diphenyl-1-picrylhydrazyl and ferric reducing antioxidant power antioxidant activities were significantly increased under higher amber and blue light fractions, while oxygen radical absorbance capacity was generally decreased. Hence, the higher blue irradiance inside the pink net house than in the white poly-net house and open field conditions could have involved in the regulatory mechanism of carotenoid biosynthesis in the current study, which requires further validation in future studies.

Based on the RDI values of iron and β-carotene, the number of people who could receive these nutrients from the crops harvested under different production conditions was estimated in the current study. During the off-season (winter for water spinach and spring for amaranths), pink poly-net house was estimated to supply more iron to people than the other production conditions. In all the seasons, the poly-net houses provided more β-carotene than the open field produced crops. Hence, sufficient quantities of iron and β-carotene can be obtained from the leafy vegetables, particularly during the off-season using less cultivation area under protective structures. This is quite important in countries like Taiwan, where the overall share of agricultural land decreased during the last two decades (Chen et al., 2019). However, it should be noted that we made these theoretical estimates based on the RDI values, without considering the bioavailability. Hence, these results should be interpreted in terms of nutrient supply from different crop production conditions, but the actual contribution of these nutrients based on their bioavailability to improving health conditions should be investigated in further studies.

Thus, protected cultivation has demonstrated the supply of nutritious vegetables, especially during the off-season. Since the investment in the construction and maintenance of both the white and pink poly-net houses is similar, use of pink poly-net houses may be considered to produce more nutritious vegetables. Supply of vegetables during the off-season is quite important to bridge the gaps in the seasonal variations in vegetable consumption. In addition, off-season vegetable production can provide better income opportunities to the smallholder farmers. If properly constructed and maintained, protective structures will last longer and reduce the incidence of pests and diseases thus reducing the use of harmful chemical pesticides in vegetable production systems. Thus, protected cultivation is expected to build economic and environmental resilience to the smallholder vegetable producers, while supplying nutritious vegetables to the consumers, especially in the off-season. However, the impacts of different types of protected cultivation on the horticultural and nutritional yield are location- and crop-specific, which can be piloted in new locations before scaling out among smallholder farmers in Asia and Africa for better nutrition and incomes.

DATA AVAILABILITY STATEMENT

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

AUTHOR CONTRIBUTIONS

SR and PS-C: conceptualization and formal analysis. SR: funding acquisition and writing–original draft. SR, M-YL, W-JW, H-IW, and PS-C: methodology and investigation. M-YL, W-JW, H-IW, and PS-C: writing–review and editing. All authors have read and agreed to the published version of the manuscript.

FUNDING

Funding for this research was provided by the World Vegetable Center and by long-term strategic donors to the World Vegetable Center: Taiwan, the Foreign, the Commonwealth & Development Office (FCDO) from the UK government, United States Agency for International Development (USAID), Australian Centre for International Agricultural Research (ACIAR), Germany, Thailand, Philippines, Korea, and Japan.

ACKNOWLEDGMENTS

The authors express their gratitude to the field staff of Safe and Sustainable Value Chains Flagship Program for their continuous support for the fieldwork.

REFERENCES

Alrifai, O., Hao, X., Liu, R., Lu, Z., Marcone, M. F., and Tsao, R. (2021). LED-induced carotenoid synthesis and related gene expression in Brassica microgreens. *J. Agric. Food Chem.* 69, 4674–4685. doi: 10.1021/acs.jafc.1c00200

AOAC (1990). Official methods of Analyses. Method No. 975.03 metal in plants, AAS method, 15th ed. Arlington: Association of Official Analytical Chemists, Inc.

Arnao, M. B., Cano, A., and Acosta, M. (2001). The hydrophilic and lipophilic contribution to total antioxidant activity. *Food Chem.* 73, 239–244. doi: 10.1016/S0308-8146(00)00324-1

Asai, A., Yonekura, L., and Nagao, A. (2008). Low bioavailability of dietary epoxyxanthophylls in humans. *Br. J. Nutr.* 100, 273–277. doi: 10.1017/S0007114507895468

Böhm, V., Lietz, G., Olmedilla-Alonso, B., Phelan, D., Reboul, E., Bánati, D., et al. (2020). From carotenoid intake to carotenoid blood and tissue concentrations – implications for dietary intake recommendations. *Nutr. Rev.* 79, 544–573. doi: 10.1093/nutrit/nuaa008Fnyo

Britton, G., Liaaen-Jensen, S., and Pfander, H. (2009). *Carotenoids volume 5: nutrition and health.* Birkhäuser: Basel, Switzerland. doi: 10.1007/978-3-7643-7501-0

Casal, J. J. (1994). Stem extension growth responses to blue light require Pfr in tomato seedlings but are not reduced by low phytochrome levels of the aurea mutant. *Physiol. Plant.* 91, 263–267. doi: 10.1111/j.1399-3054.1994.tb00428.x

Chandra-Hioe, M. V., Rahman, H. H., and Arcot, J. (2017). Lutein and β-Carotene in Selected Asian Leafy Vegetables. *J. Food Chem. Nanotechnol.* 3, 93-97. doi: 10.17756/jfcn.2017-043

Chen, Y. Y., Huang, W., Wang, W. H., Juang, J. Y., Hong, J. S., Kato, T., et al. (2019). Reconstructing Taiwan's land cover changes between 1904 and 2015 from historical maps and satellite images. *Sci. Rep.* 9,3643. doi: 10.1038/s41598-019-40063-1

Ebert, A. W., Wu, T. H., and Wang, S. T. (2011). *Vegetable amaranth (Amaranthus L.). International Cooperators' Guide. AVRDC Publication Number: 11-754.* Shanhua, Tainan, Taiwan: AVRDC-The World Vegetable Center. Available online at: http://203.64.245.61/web_crops/indigenous/cooperators%20guide_amaranth_s_web.pdf

Fowler, D. (2011). Nutritional Value of Common Fruits & Vegetables Grown in Cambodia. Cambodia: MCC Prey Veng. Available online at: https://thehomeaway.files.wordpress.com/2011/09/nutritional-value-of-cambodian-crops.pdf

Genova, C., Weinberger, K., and Acedo, J.r., A.L. (2010). *An Assessment of Market Opportunities for Leafy Vegetables in the Upland Areas of Cambodia, Laos, and Vietnam. Final Project Report, RETA 6376. AVRDC Publication No. 10-734.* Taiwan: AVRDC – The World Vegetable Center. Available online at: https://avrdc.org/wpfb-file/eb0128-pdf/

Grubben, G. J. H., and Van Sloten, D. H. (1981). *Genetic resources of amaranths.* Rome: IBPGR.

Harmanto, Tantau, H., and Salokhe, V. M. (2006). Influence of insect screens with different mesh sizes on ventilation rate and microclimate of greenhouses in the humid tropics. Agricultural Engineering International: the CIGR Ejournal. Available online at: https://ecommons.cornell.edu/handle/1813/10512

Harmanto, T. H. J. (2006). *Evaluation of net greenhouses for tomato production in the tropics.* (Ph.D. dissertation) Hannover: University. Available online at: https://www.repo.uni-hannover.de/handle/123456789/6751

Ho, C. C. (2000). Spider-mite problems and control in Taiwan. *Exp. Appl. Acarol.* 24, 453–462. doi: 10.1023/A:1006443619632

Hsu, Y.-C., and Srinivasan, R. (2012). Desert horse purslane weed as an alternative host for amaranth leaf webber, *Hymenia recurvalis* in Taiwan. *Formos. Entomol.* 32, 297–302. Available online at: http://entsocjournal.yabee.com.tw/AlldataPos/JournalPos/Vol32/No3/TESFE.2012018.PDF

Hwang, S.-G., Chao, H.-C., and Lin, H.-L. (2018). Differential responses of pak choi and edible amaranth to an elevated temperature. *Hort. Sci.* 53, 195–199. doi: 10.21273/HORTSCI12667-17

Ilić, Z. S., Milenković, L., Šunić, L., and Manojlović, M. (2018). Color shade nets improve vegetables quality at harvest and maintain quality during storage. *Contemp. Agric.* 67, 9–19. doi: 10.2478/contagri-2018-0002

Institute of Medicine (2001). *Dietary Reference Intakes for Vitamin A, Vitamin K, Arsenic, Boron, Chromium, Copper, Iodine, Iron, Manganese, Molybdenum, Nickel, Silicon, Vanadium, and Zinc.* Washington, DC: Institute of Medicine.

Joosten, F. J., Dijkxhoorn, Y., Sertse, Y., and Ruben, R. (2015). *How does the fruit and vegetable sector contribute to food and nutrition security? LEI Nota 2015–2076, June 2015.* Wageningen: LEI Wageningen UR. pp. 57. Available online at: https://edepot.wur.nl/348737

Khanam, U. K. S., and Oba, S. (2013). Bioactive substances in leaves of two amaranth species, *Amaranthus lividus* and *A. hypochondriacus*. *Canadian J. Plant Sci.* 93, 47–58. doi: 10.4141/cjps2012-117

Khandaker, L., Masum Akond, A. S. M. G., Ali, M. B., and Oba, S. (2010). Biomass yield and accumulations of bioactive compounds in red amaranth (*Amaranthus tricolor* L.) grown under different colored shade polyethylene in spring season. *Sci. Hortic.* 123, 289–294. doi: 10.1016/j.scienta.2009.09.012

Khoo, H.-E., Nagendra, P. K., Kong, K.-W., Jiang, Y., and Ismail, A. (2011). Carotenoids and their isomers: color pigments in fruits and vegetables. *Molecules.* 16, 1710–1738. doi: 10.3390/molecules16021710

Lee, W.-S., and Yang, S.-R. (1999). Using a plug system to produce hygienic vegetables. Food and Fertilizer Technology Center for the Asian and Pacific Region. Taipei. Available online at: https://www.fftc.org.tw/htmlarea_file/library/20110809105010/eb477.pdf

Liou, T. (1981). Studies on seed production techniques of water convolvulus (Ipomoea Aquatica Forsk.)-II. Effect of planting dates on the seed production potentiality of different cultivars of water convolvulus grown under paddy and upland fields. *J. Agric. Res. China.* 30, 385–394.

Long, S. P. (1999). "Environmental responses," in *the C4 Plant Biology*, eds. R.F. Sage and R.K. Monson (San Diego, CA: Academic Press) 215–249. doi: 10.1016/B978-012614440-6/50008-2

Majumdar, A., and Powell, M. (2011). Net house vegetable production: pest management success and challenges. *J. NACAA.* 4. Available online at: https://www.nacaa.com/journal/index.php?jid=87

Makobo, N. D., Shoko, M. D., and Mtaita, T. A. (2010). Nutrient content of amaranth (*Amaranthus cruentus* L.) under different processing and preservation methods. *World J. Agric. Sci.* 6, 639–643.

Maoka, T., and Etoh, H. (2010). "Some biological functions of carotenoids in Japanese food", in *Functional Foods of the East John Shi, Chi-Tang Ho,* eds Fereidoon Shahidi. (Boca Raton: CRC Press), 85–97.

Muniappan, R., Shepard, B. M., Carner, G. R., and Ooi, P. A. C. (2012). *Arthropod pests of horticultural crops in tropical Asia.* Wallingford, Oxfordshire: CABI. doi: 10.1079/9781845939519.0000

National Research Council. (1984). Amaranth: Modern prospects for an ancient crop. Washington, DC: The National Academies Press. 74 p.

Nihoul, P., Van Impe, G., and Hance, T. (1991). Characterizing indices of damage to tomato by the two-spotted spider mite, *Tetranychus urticae* Koch (Acari: Tetranychidae) to achieve biological control. *J. Hortic. Sci.* 66, 643–648. doi: 10.1080/00221589.1991.11516195

Nordey, T., Basset-Mens, C., De Bon, H., Martin, T., Déletré, E., Simon, S., et al. (2017). Protected cultivation of vegetable crops in sub-Saharan Africa: limits and prospects for smallholders. A review. *Agron. Sustain. Dev.* 37, 53. doi: 10.1007/s13593-017-0460-8

Nyonje, W. A., Schafleitner, R., Abukutsa-Onyango, M., Yang, R.-Y., Makokha, A., and Owino, W. (2021). Precision phenotyping and association between morphological traits and nutritional content in Vegetable Amaranth (*Amaranthus* spp.). *J. Agric. Food Res.* 5:100165. doi: 10.1016/j.jafr.2021.100165

Palada, M. C., and Wu, D. L. (2009). Grafting sweet peppers for production in the hot-wet season. International Cooperator's Guide, AVRDC Publication Number:09-722-e. Available online at: http://203.64.245.61/fulltext_pdf/FLYER/f0002.pdf

Pan, W.-H., Yeh, N.-H., Yang, R.-Y., Lin, W.-H., Wu, W.-C., Yeh, W.-T., et al. (2018). Vegetable, fruit, and phytonutrient consumption patterns in Taiwan. *J. Food Drug Anal.* 26: 145–153. doi: 10.1016/j.jfda.2016.12.015

Pinker, I., Bubner, U., and Böhme, M. (2007). Selection of water spinach (*Ipomoea aquatica* Forssk.) genotypes for protected cultivation in temperate regions. *Acta. Hortic.* 752, 441–446. doi: 10.17660/ActaHortic.2007.752.80

Polston, J. E., and Lapidot, M. (2007). "Management of tomato yellow leaf curl virus: US and Israel perspectives", in *Tomato Yellow Leaf Curl Virus Disease:*

Management, Molecular Biology, Breeding for Resistance, ed. H. *Czosnek* (The Netherlands; Springer), 251–262. doi: 10.1007/978-1-4020-4769-5_15

Rodriguez-Amaya, D. B., and Kimura, M. (2004). *Harvest plus handbook for carotenoids analysis. HarvestPlus Technical Monograph 2*. Washington, DC and Cali: International Food Policy Research Institute (IFPRI) and International Center for Tropical Agriculture (CIAT). Available online at: http://ebrary.ifpri.org/utils/getfile/collection/p15738coll2/id/125148/filename/125149.pdf

Sarker, U., and Oba, S. (2020). Leaf pigmentation, its profiles and radical scavenging activity in selected *Amaranthus tricolor* leafy vegetables. *Sci. Rep*. 10, 18617. doi: 10.1038/s41598-020-66376-0

Schreinemachers, P., Chen, H. P., Nguyen, T. T. L., Buntong, B., Bouapaoe, L., Gautam, S., et al. (2017). Too much to handle? Pesticide dependence of smallholder vegetable farmers in Southeast Asia. *Sci. Total Environ*. 593–594, 470–477. doi: 10.1016/j.scitotenv.2017.03.181

Shahak, Y., Ratner, K., Zur, N., Offir,Y., Matan, E., Yehezkel, H., et al. (2008). Photoselective netting: An emerging approach in protected agriculture. *Acta Hortic*. 807, 79–84. doi: 10.17660/ActaHortic.2009.807.7

Smith, J. D., Dinssa, F. F., Anderson, R. S., Su, F.-C., and Srinivasan, R. (2018). Identification of major insect pests of Amaranthus spp. and germplasm screening for insect resistance in Tanzania. *Int. J. Trop. Insect Sci*. 38, 261–273. doi: 10.1017/S1742758418000115

Talekar, N. S., Su, F. C., and Lin, M. Y. (2003). How to produce safer leafy vegetables in net houses and net tunnels. Shanhua, Tainan, Taiwan: Asian Vegetable Research and Development Center. 18pp.

Towns, A. M., and Shackleton, C. (2018). Traditional, indigenous, or leafy? A definition, typology, and way forward for African vegetables. *Econ Bot*. 72, 461–477. doi: 10.1007/s12231-019-09448-1

Wang, S. T., and Ebert, A. W. (2012). Breeding of leafy amaranth for adaptation to climate change. In: Proceedings from SEAVEG 2012: Regional Symposium on High Value Vegetables in Southeast Asia: Production, Supply and Demand, 24-26 January 2012. Chiang Mai, Thailand. *pp*. 36–43.

Westphal, E. (1994). "Ipomoea aquatica" in *Plant resources of South-East Asia, No 8 Vegetables, Bongor, Indonesia*, eds. S. Siemonsma and K. Piluek (Wageningen, Netherlands: Pudoc), 181–184.

Conflict of Interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's Note: All claims expressed in this article are solely those of the authors and do not necessarily represent those of their affiliated organizations, or those of the publisher, the editors and the reviewers. Any product that may be evaluated in this article, or claim that may be made by its manufacturer, is not guaranteed or endorsed by the publisher.

frontiers in Sustainable Food Systems

ORIGINAL RESEARCH
published: 11 January 2022
doi: 10.3389/fsufs.2021.751349

Impact of Crop Diversification on Household Food and Nutrition Security in Southern and Central Mali

Adama Douyon[1,2], Omonlola Nadine Worou[1], Agathe Diama[1], Felix Badolo[1], Richard Kibarou Denou[1,2], Sidi Touré[1], Amadou Sidibé[2], Baloua Nebie[1] and Ramadjita Tabo[1]*

[1] *West and Central Africa Regional Hub, International Crops Research Institute for the Semi-Arid Tropics, Bamako, Mali,* [2] *Department of Economic and Social Sciences, Institut Polytechnique Rural de Formation et de Recherche Appliquée, Koulikoro, Mali*

Many African countries, including Mali, depend on the production of a single or a limited range of crops for national food security. In Mali, this heavy reliance on a range of basic commodities or staple crops, or even just one, exacerbates multiple risks to agricultural production, rural livelihoods, and nutrition. With this in mind, the smart food campaign was initiated to strengthen the resilience and nutritional situation of households and peasant communities where the diet is mainly cereal-based and remains very undiversified and poor in essential micronutrients. As part of the campaign, our study aims to analyze the impact of agricultural diversification on food consumption and household nutritional security. The analysis uses survey data from 332 individuals randomly selected. Multinomial logistic regression and the Simpson diversity index were used to determine the index and estimate the determinants of crop diversification. The consumption score index weighted by consumption frequency and anthropometric indices (for children) were used to assess the nutritional status of households. The results show four types of strategies of diversification: 7.55% are cereals only, 5.66% combine millet–sorghum–groundnut, 41.51% combine millet–sorghum–groundnut–cowpea, and 45.28% combine millet–sorghum–groundnut–cowpea–maize. The estimation of the regression model shows that socioeconomic factors have a positive influence. With a consumption score index of 34 in the villages and 40.5 in Bamako, based on eight food groups, we find that the quality of food is insufficient in rural areas, but it is acceptable in the urban center of Bamako. Analysis of the nutritional status of children aged 6–48 months reveals that 30% of the surveyed population is in a situation of nutritional insecurity (all forms combined). To help improve crop diversification and the nutritional quality of foods, we suggest, among other things, subsidies and public spending to facilitate access to inputs that allow the acquisition of a wider range of inputs and services, intensification of nutrition awareness, and education programs to maximize the incentive to consume nutritious foods from self-production and market purchases. Finally, we propose to facilitate access to technologies promoting food diversification and improving food and nutritional security, particularly in rural areas.

Keywords: crop diversification, food consumption, nutritional security, smart-food, household, Mali

OPEN ACCESS

Edited by:
Mehanathan Muthamilarasan, University of Hyderabad, India

Reviewed by:
A. R. Anuja, Central Marine Fisheries Research Institute (ICAR), India
J. Lydia Pramitha, Tamil Nadu Agricultural University, India

***Correspondence:**
Omonlola Nadine Worou
n.worou@cgiar.org

Specialty section:
This article was submitted to Nutrition and Sustainable Diets, a section of the journal Frontiers in Sustainable Food Systems

Received: *01 August 2021*
Accepted: *08 November 2021*
Published: *11 January 2022*

Citation:
Douyon A, Worou ON, Diama A, Badolo F, Denou RK, Touré S, Sidibé A, Nebie B and Tabo R (2022) Impact of Crop Diversification on Household Food and Nutrition Security in Southern and Central Mali. Front. Sustain. Food Syst. 5:751349. doi: 10.3389/fsufs.2021.751349

INTRODUCTION

The agricultural sector is emerging as the engine for growth and food security in Mali. The sector contributes up to 36% to the gross domestic product (GDP) and occupies 80% of the population (Maiga et al., 2019). Agricultural production varies by region, but the Southern and Central areas are generally the most productive, especially when it comes to cereals. The main cereals produced and consumed in the country are millet, sorghum, rice, and maize (Tounkara et al., 2019). Production metrics showed a sharp increase of those staple and cereal crops from year 2000, reflecting the potential for agriculture to achieve self-sufficiency in food production (World Bank, 2018; Kouressy et al., 2019). In contrast, the number of people facing chronic and persistent food insecurity and malnutrition has been steadily increasing in the past decade (Sanga et al., 2021). Segregated data showed that 54% of women 15–49 years of age with one child under 5 years suffer from anemia and that the national GDP in Mali is decreased by 2.7% due to vitamin and mineral deficiencies (Kennedy et al., 2009). FAO (1996) expressed that food insecurity exists when all human beings do not have, at all times, physical and economic access to sufficient, healthy, and nutritious food to enable them to meet their energy needs and food preferences to lead a healthy and active life. However, the notion of nutritional insecurity encompasses and goes beyond that of food insecurity. The prevalence of food insecurity and malnutrition is of concern especially when the availability and accessibility of nutritionally adequate foods are limited and/or uncertain. The consequences in Mali include low nutritional intake, the prevalence of stunting is of 27% for young children 0–23 months, and the perseverance of undernutrition among 18.5% women, particularly in rural areas (Konate et al., 2020).

Many drivers influence the linkage between agricultural productivity with food and nutrition security. Empirical evidence confirms the role of agriculture for the improvement of incomes and food, which provides two capital dimensions of food security: the availability and accessibility of food and reduction of malnutrition (FAO et al., 2021). Agricultural production in Mali is concentrated on few basic products, exacerbating the challenges of food insecurity, while the narrow staple production is reflected in food consumption with the dominant crops accounting for a significant portion of the caloric intake. Much of food produced locally is based on the proportion of crops in the different climatic zones (for example, rice and maize are cultivated in regions with high rainfall and irrigated areas, millet and sorghum in areas of medium and low precipitation). Moreover, since spatially distributed factors (climate, crops grown, infrastructure, market development, etc.) influence production or nutrition (Tefft et al., 2000), Joseph and Wodon (2008) saw in Mali important differences in the weight of the various cereals in the overall consumption basket of the population, as well as differences between various types of households in their consumption patterns.

In the prospect of malnutrition alleviation, on the one hand, crop diversification has been promoted by different nutrition and food security interventions. Adjimoti and Kwadzo (2018) found that crop diversification has a positive effect on household food security status, and for Ijaz et al. (2019), it is a dynamic tool to ensure the food security in a sustainable way. It has great potential to strengthen the resilience and nutritional situation of households and farming communities (World Bank, 2018; Heumesser and Kray, 2019), and the diversity of crops grown through dietary diversity can improve household food security. The factors leading to diversification decisions are numerous, among which we can cite the following: an increase in farmers' incomes, reduction of the risks of food shortages and deficiencies, response to changing consumer demands or modification of policy government, the response to external shocks, and, more recently, as a consequence of global warming. In rural remote areas where household access to food depends largely on its production, crop diversification provides farmers with the different crops that they cannot access either because of the cost or because of the poor infrastructure constraints.

On the other hand, many approaches have concentrated on macroeconomic solutions and concepts of national food security. For instance, the availability of overall food stocks was emphasized, taking into account local production, imports, and exports and usually concentrating on staple foods. Planning along these lines did help to assure adequate national supplies but did not always fully address problems of dietary diversity, food quality and safety, post-harvest losses, processing and marketing, or access to food by all individuals and households (FAO, 1992). In this line, the concept of Smartfood mainstreaming "traditional" Smart Foods back as staples across Africa and Asia has been initiated. Smart Foods are food items that fulfill the criteria of being good for you, the planet, and the farmer. Sorghum and millet were selected as the first Smart Foods, and a participatory fun-filled approach was adopted to create awareness, to develop culturally acceptable products, and to bring about behavior change to improve adoption, dietary diversity, and nutritional status (Diama et al., 2020). In drylands, the smart food approach is innovating that millet and sorghum which are nutritious crops (Barikmo et al., 2007; Chande et al., 2021) can be better consumed through improved local dishes; thus, the household's daily energy requirements, particularly those of women and children, may be raised. As baseline research of such social marketing intervention of the smart food approach, we had the main concern for assessing how diversification of crops has positive effects on food consumption and the nutritional situation of households. In fact, the approach is providing a paradigm supporting the trends that Malian farmers, while often willing to try new crops, are typically loath to give up sorghum/millet cultivation since it represents the major part of their effort to provide self-sufficiency to their families (Foltz, 2010).

For this study, it has been postulated that the households and the consumers of millet and sorghum, identified and characterized in rural areas and particularly in the urban center

Abbreviations: FCS, food consumption score; GDP, gross domestic product; MLR, multinomial logistic regression; UE-APSAN-Mali, Enhancing Crop Productivity and Climate Resilience for Food and Nutrition Security; SDI, Simpson diversity index; S, strategy; WFP, World Food Program.

of Bamako, have different needs. Therefore, the study focuses on three main objectives: (1) evaluate the food consumption score in households and the nutritional situation among children; (2) estimate the determinants of crop diversification; and (3) evaluate the effect of crop diversification and consumption of Smart Foods and household nutritional security.

MATERIALS AND METHODS

Study Area Description

The study area concerned the regions of Kayes, Koulikoro, Sikasso, Ségou, and district of Bamako. The cercles of Kati (Bankoumana), Kita (Bendougouba), Dioila (Wakoro), Segou (Pelengana et Cinzana-gare), and Koutiala (M'pessoba et N'golonianasso) are taken into account in the regions (**Figure 1**). These are study areas of the UE-APSAN project (plus Bamako) with mandated and focus intervention on millet, sorghum, groundnut, and cowpea crop production and consumption analysis. The description of regions is presented as follows.

Bamako is the capital and the most urbanized city of Mali. With an important port on the Niger River and a commercial center radiating over the entire sub region, the city is also the main administrative center of the country and has 2,529,300 inhabitants in 2020 with a high urban growth rate. Bamako is administratively considered as "a district" and divided into six communes headed by elected mayors. Agriculture is mainly limited to vegetable gardening, fishing, and livestock which are poorly developed. The district of Bamako concentrates 70% of industrial companies. The tertiary sector is the most developed. Bamako is retained in surveys because most of Mali's agricultural production is transferred there and it is where the major consumer markets are located.

The Kati cercle is a territorial collectivity of Mali in the Koulikoro region. In the Koulikoro region, agriculture remains the main activity and employs more than 80% of the population. The cultivated crops are mainly cereals, which are used in the diet of the populations. Crops are millet, sorghum, maize, rice, fonio, and cowpea. These include also industrial or cash crops such as cotton, sesame, and groundnut.

The Kita cercle is a territorial collectivity of Mali in the Kayes region. It has 33 municipalities. Agriculture, trade, and livestock breeding are the main economic activities. The main cultivated crops are cereals, cotton, and groundnut. Livestock breeding represents income diversification activity (there is persistent existence of small herds of cattle or sheep/goats in almost every family).

The Dioila cercle is also in the region of Koulikoro. The cercle is traversed by three rivers—the Bagoé, the Banifing, and the Baoulé—and their multiple affluents. Likewise, in the cercle of Kati, agricultural activities are developed and practiced by the majority of the population. The agricultural system adopted in the cercle can be summed up in 1 year of cotton cultivation, followed by 2 years of cereal cultivation. The latter are sometimes replaced by legumes. Traditionally, this rotation was followed by a fallow period of several years, but pressure on good land now makes this practice disappear.

Segou located at 240 km from Bamako is mainly located in the Sahelian zone, with semiarid climate (average annual rainfall: 513 mm). Segou is traversed by Niger River (over 292 km) and the Bani River. The population is estimated at 2,336,255 inhabitants. Women represent 50.5% of the population. With its 127,000 ha of irrigated land, Segou strongly contributes to Mali's food security. Indeed, the Office of Niger (the largest hydraulic structure) represents about 60% of Mali's rice needs, which is equivalent to just under one million tons. However, Segou is not just a rice granary, because the region is also a major producer of maize, millet, and sorghum followed by groundnut and cowpeas and has a relatively large herd.

The Koutiala cercle is located in the Sikasso region and is characterized by Sudano-Sahelian climate. The Koutiala cercle has an economy essentially based on agro-pastoral care. It is the area of cotton cultivation, and this culture gives Koutiala the name of "capital of white gold" in Mali. Cotton production is supported by technical services, NGOs, etc., through agricultural credit, training, marketing, etc. The main cereal crops are millet, sorghum, maize, and rice. Other cash crops are cowpeas and groundnuts. The Koutiala cercle exports livestock to Burkina Faso and Côte d'Ivoire, in particular cattle, goats, and sheep.

Data Collection

The methodological approach initially consisted of collecting all the information available on household food and nutritional security. Subsequently, a survey was carried out to verify the hypotheses. The bibliographic review also enabled us to better define the theme on the one hand and to take stock of the results of research and studies relating to the subject on the other hand.

Within the framework of this study, the data collection related to secondary field data through tools including questionnaires which were administered to the surveyed population. The questionnaires were specific to the different categories of actors. Data collection took place over the period from March to April 2021 in the areas mentioned above. The data mainly concern agricultural production; food consumption (including Smart Food) and other household uses; anthropometric indices for children from 6 to 48 months; and information on micronutrient deficiencies.

Sampling

The sampling procedure relating to the collection of primary data retained in the framework of this study is random selection. Initially, following the coverage area of the UE-APSAN-Mali project, a total of 182 households were drawn randomly from the list of seven villages: Bankoumana, Bendougouba, Wakoro, Pelengana, Cinzana-gare, M'pessoba, and N'golonianasso. Additionally, we surveyed six mothers of children under 5 in each village. Then, a sample of 150 individuals was drawn using the random sampling method from six municipalities of the district of Bamako, including 110 households. **Table 1** describes the sampling of study.

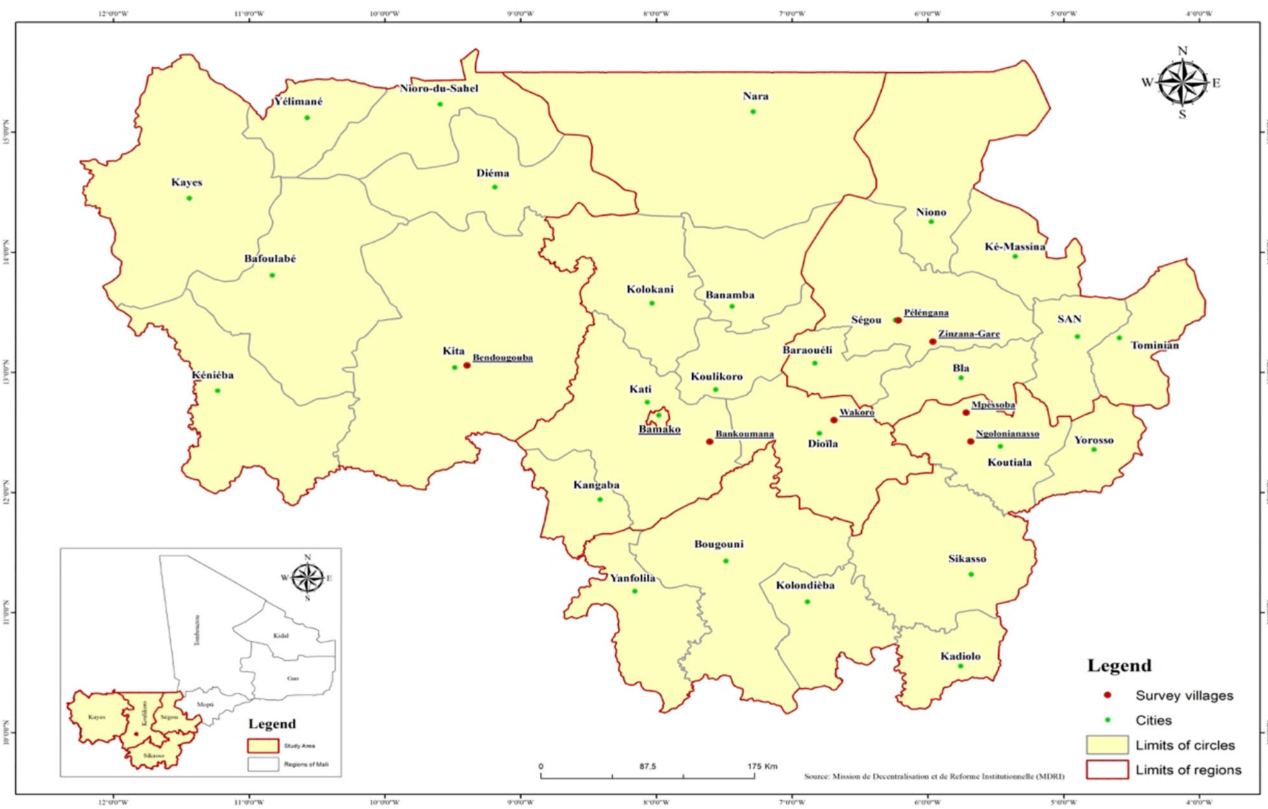

FIGURE 1 | Study areas mapping in Central and Southern Mali.

TABLE 1 | Description of the sampling size.

Categories	7 villages (Bankoumana, Bendougouba, Wakoro, Pelengana, Cinzana-gare, M'pessoba, and N'golonianasso)	6 urban municipalities (all of 6 municipalities of Bamako district)	Together
Children	42	30	72
Producers	53	–	53
Consumers	47	100	147
Transformers	10	10	20
Distributors	30	10	40
Totals	**182**	**150**	**332**

Data Analysis

Descriptive Statistics

Descriptive statistics were used to characterize respondents on the basis of age, education level, gender, and marital status. Percentages were used for estimating weight perception of the population.

The Simpson Diversity Index

Regarding the Simpson diversity index (SDI), it is between 0 and 1 where 0 denotes specialization and 1 extremely diverse. The general formula of this index is as follows:

$$SDI = 1 - \frac{\sum Ni(Ni-1)}{N(Ni-1)}$$

Ni: number of individuals of the given species.

N: total number of individuals.

The SDI is a relative abundance index: it takes into account wealth (number of crops produced) and distribution (distribution of quantities produced). Its value increases when the number of species increases and/or when the quantities produced per species are close to each other. We chose to calculate it from the production in kilograms of dry matter.

Food Diversity Measurement

We estimated the likelihood effects of diversification strategies (the dependent variables) from the independent variable which is the total production of the farm. From a qualitative reminder of consumption and consumption frequency during the last 7 days, we calculated the score consumption of food households as a proxy for the coverage of needs micronutrients. This score was calculated according to the mathematical method named the Food Consumption Score (FSC). The FSC is inspired by the World Food Program (WFP), using the frequency of consumption of the eight food groups consumed during the last seven (07) days preceding the survey by households. Those eight food groups comprise cereals and tubers, legumes, vegetables, fruits, meats and fish, milk, sugar, and oil.

The FCS is calculated as follows:

$$\text{FCS} = A_{\text{cereals}}X_{\text{cereals}} + A_{\text{legumes}}X_{\text{legumes}} + A_{\text{vegetables}}X_{\text{vegetables}} + A_{\text{fruits}}X_{\text{fruits}} + A_{\text{animals}}X_{\text{animals}} + A_{\text{sugar}}X_{\text{sugar}} + A_{\text{milk}}X_{\text{milk}} + A_{\text{oil}}X_{\text{oil}}$$

with

AI = weight assigned to the food group

XI = number of days of consumption relative to each food group ($\leq$7 days).

Thus, the conventional thresholds defined by the WFP to determine the three food consumption groups were used:

- Poor food consumption: FCS < 21 corresponds to a situation of severe food insecurity;
- Food consumption at the limit of acceptability, 21.5 < FCS < 35, which corresponds to a situation of moderate food insecurity;
- Acceptable food consumption: FCS > 35 which corresponds to a food security situation.

For the particular case of nutritional status of children under 5 years of age, we used children's height, weight, age, and brachial perimeter with *Z*-scores to estimate the nutritional status of children under 5. The *Z*-score is calculated as the following:

$$(\text{Measure} - \text{mean})/\text{standard deviation (of height, weight, age, and brachial perimeter)}$$

From the World Health Organization's interpretation of *z*-score: <-3 *z*-scores: children in insecurity situation.

Multinomial Logistic Regression

In this study, multinomial logistic regression (MLR) was used. This method is suitable for the analysis of category-dependent variables when producers have only one choice among a set of diversification strategies (Greene, 2002). According to the theory of the random utility model (RUM), it is estimated that the producer opts to maximize his income by comparing the income generated by an alternative strategy. However, the income tended is a latent variable determined by the characteristics of the agricultural exploitation and the error term.

$$\text{Thus, Uij}^* = \text{X}\beta\text{jij} + \varepsilon\text{ij}$$
$$\text{Uij : Independent variable}$$
$$\text{X}\beta\text{jij : Dependent variable}$$
$$\text{Eij : Error}$$

It denotes the probability of choice of the producer whether to combine millet plus sorghum only (S1) or to combine millet, sorghum, and groundnut (S2); combine millet, sorghum, groundnuts, and cowpea (S3); or combine millet, sorghum, groundnut, cowpea, and maize (S4). According to Greene (2002), the model is given as the following:

$$P_{ij} = \frac{Exp\ (X_i\ \beta_i)}{\sum_{j=1}^{4} Exp\ (X_i\ \beta_i\ )}$$

To solve the normalization problem, the model is written as follows:

$$P_{ij} = \frac{Exp\ (X_i\ \beta_i)}{1 + \sum_{j=1}^{4} Exp\ (X_i\ \beta_i\ )}$$

Thus, the model estimates the coefficient by the maximum probability.

The explanations used for our analysis are as follows:

X_1: age of producers; X_2: gender of producers; X_3: education level;
X_4: household size; X_5: possession work animals; X_6: possession poultry;
X_7: total area sown; X_8: the cost of agricultural inputs.

The MLR of the dependent variable diversification strategy on the explanatory variables made it possible to obtain the following results:

- The Chi^2 associated with the log ratio of our model has a probability of <5%, which means that the model is generally of good quality.
- The parameters for which the estimated coefficient is statistically significant at the 5% level are those in fluent adoption of a strategy of diversification of crops while the other variables do not affect or have less influential choice adoption of a crop diversification strategy.

Linear Regression

In this study, the likelihood effects of diversification strategies (the dependent variable) from the independent variable, which is the total production of the farm, were estimated. We applied this general equation to the *n* observations of Y and the corresponding values of X as follows:

$$Y_i = b_0 + b_1 X_i + \varepsilon_i fori = 1, ..., n$$

For each individual *i*, the random variable ε_i represents the error made, that is to say the difference between the value of *Y* observed and the value $b_0 + b_1 X_i$ given by the linear relation. Variables used for this analysis are diversification strategy (as dependent variable) and the global production (as independent variable).

RESULTS

Socioeconomic Characteristics and Production Patterns

The age of the investigated people ranges from 18 to over 60 for adults and from 6 to 48 months for those under 5 (**Figure 2A**). We therefore grouped the respondents into five age groups: the first <5 years old representing 24%; the second, 18–30 years old, 31%; the third, 31–50 years old, 32%; the fourth, 51–65 years old, which represents 12%; and only 1% which constitutes the last bracket (65 years and over). More than 60% are aged 50 or less, which indicates that the population of the study area is predominantly young and constitutes a labor force for agriculture. Admittedly, agricultural production activities involve very few women, but in our case the survey also concerned women who are sometimes well-imbued with the family food situation. As a result, participation share of women represents 43% against 57% for men (**Figure 2B**). Nevertheless, households are generally headed by men with no education in the majority or limited to primary school. Meanwhile, about a third (27%) of respondents are illiterate (**Figure 2C**). We find, however, that 49% attended school (15% of which reached higher education). According to the marital status, the married surveyed people are dominant in our study area with 89% against 11% for the unmarried (**Figure 2D**).

Within this population, analysis of the combination with a diversity index of 0.63 shows that the producers practice the diversification of crops of which 7.55% only make cereals, 5.66% combine millet–sorghum–groundnuts, 41.51% combine millet–sorghum–groundnut–cowpea, and 45.28% combine millet–sorghum–groundnut–cowpea–maize (**Table 2**). Overall, survey reveals that majority of farms practice mixed agriculture, and cereals are the staple crops intended for self-consumption. Livelihoods remain dependent to agricultural food productions and sale of agricultural products in rural areas.

Nutritional Situation of Households and Children

With consumers, **Table 3** shows the contribution share of cereals and legumes, taken individually, in the daily menus in rural and

TABLE 2 | Different strategies of diversification crops (S corresponds to the name of strategy).

Diversification strategies	Frequencies	Percent	cum
S1 (2 cereals)	4	7.55	7.55
S2 (2 cereals; 1 legume)	3	5.66	13.21
S3 (2 cereals; 2 legumes)	22	41.51	54.72
S4 (3 cereals; 2 legumes)	24	45.28	100.00
Total	53		100.00

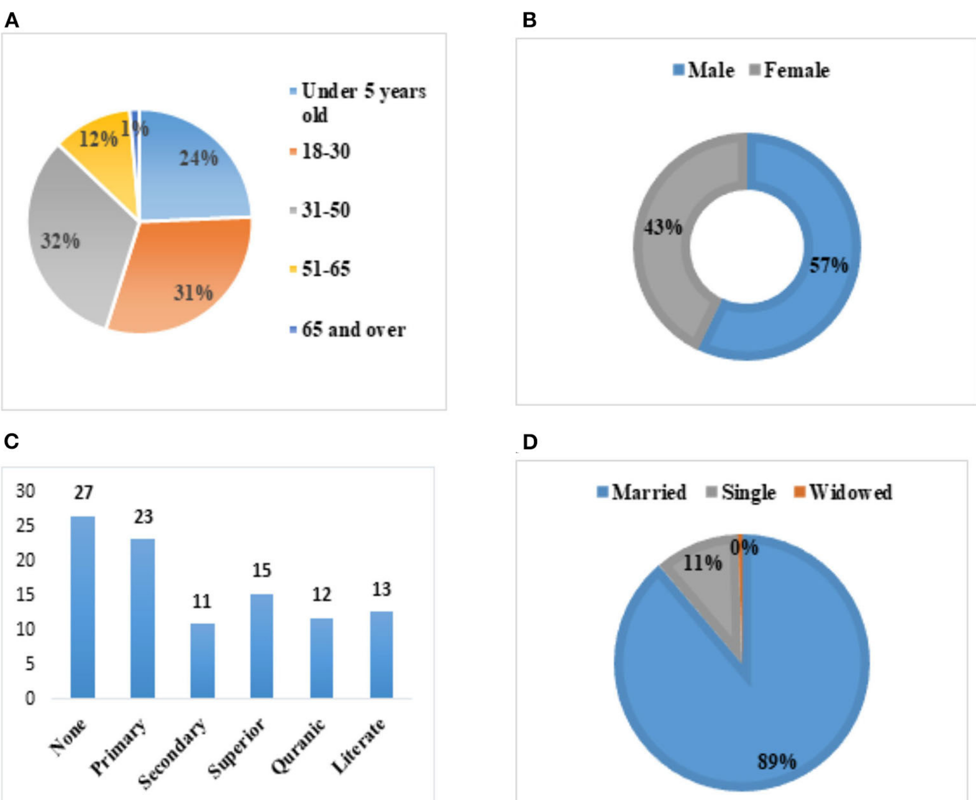

FIGURE 2 | Social characteristics of surveyed households. **(A)** Age group of respondents. **(B)** Gender of respondents. **(C)** Respondent's education level (%). **(D)** Marital status of respondents.

TABLE 3 | Contribution of cereals and legumes in daily menus.

Frequencies (in %)	Rural environment					Urban				
	Mil	Sorghum	Groundnut	Cowpea	Rice	Mil	Sorghum	Groundnut	Cowpea	Rice
Daily	51	55	32	6	0	26	2	8	2	87
2–3 times/week	38	24	48	50	45	44	24	66	21	13
Once a week	11	21	21	44	55	17	15	20	43	0
Once/month	0	0	0	0	0	11	16	1	27	0
1–2 times/year	0	0	0	0	0	2	6	2	4	0
Never	0	0	0	0	0	0	37	2	2	0
Food source	Own production: 80%; Own production and donation: 20%					Purchase: 89% and donation: 11%				

urban households in the surveyed areas. With respect to rural environment, millet and sorghum contribute to 51 and 55% of daily household diets, respectively, followed by groundnut by 32%. While in urban areas and particularly in the urban center of Bamako, 26% for millet and 2% for sorghum are consumed in the daily diets of city dwellers. In the same city, it is reported that rice crop provides the major staple food, as it is consumed at least every day by 87% households. In parallel, FCS of 34 in rural areas was depicted against 40.5 in Bamako, underlining that quality of food is poorer in rural areas as compared to the urban areas (**Figure 3**). This allows also to classify the rural populations in moderate food insecurity and that the urban populations are in a food security.

Foods from targeted crops of this study come from purchase (89%) in urban areas and self-consumption (80%) in rural areas. Another contribution in the diet is provided by imported and processed products made of maize, millet, sorghum, groundnut, and cowpea. The analysis of consumer perception of millet and sorghum (**Table 4**) showed that millet and sorghum are highly valued by households because 90% of households claim that these two cereals are very healthy for human consumption and only 10% have less appreciation. Households highlighted that these crops are not only rich in micronutrients but also good for babies and pregnant women.

Surveys with processors of cereals and legumes show that processing options are undertaken and responding to traditional diets of Malian consumers. Processed products are cited to be as follows: flour for Tô, Couscous of Millet, Monicourou, Dêguê, Tiakry, Laro, Djouka, etc. These products are distributed by food stores and supermarkets, particularly in the urban center of Bamako.

Analysis of the nutritional status of children aged 6–48 months reveals that 30% of children are in a situation of nutritional insecurity (all forms combined) (**Figure 4**). However, it emerges from the analysis of dietary diversity and anthropometric indices of children that the FCS is 34 in rural areas and 40 in the urban center of Bamako (**Table 5**). In addition, children of educated mothers had a better nutritional situation than that of children of uneducated mothers (**Figure 5**).

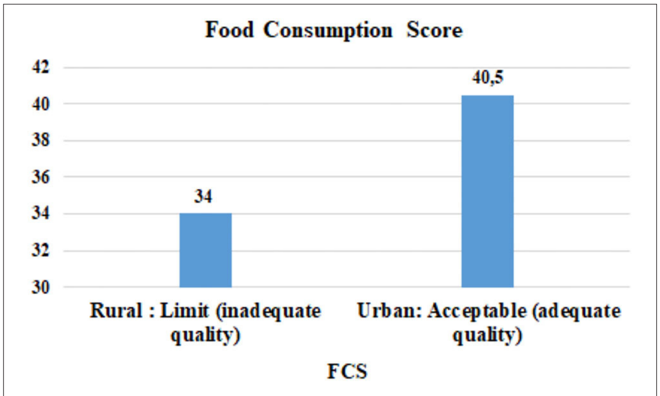

FIGURE 3 | Food Consumption Score (FCS) in rural vs. urban area.

TABLE 4 | Perception of millet and sorghum consumers.

Perception (in %)	Millet and sorghum
Very healthy and healthy	90
A little healthy	8
I do not know	2

Determinants and Effects of Crop Diversification and Its Importance in Production

The analysis of the determinants of choice of crop diversification strategies reveals that two (2) of the eight explanatory variables retained (age of producers, gender of producers, education level, household size, possession work animals, possession poultry, total area sown, and cost of agricultural inputs) are statistically significant at 5% (**Table 6**). These are the following:

- Level of education: the level of education influences the probability of choosing a diversification strategy, the more the level of education increases, and the more the propensity of the household to adopt S3 of diversification increase.
- Household size: this variable has a statistically significant surplus value at the 5% threshold. Thus, we can conclude that

this variable indicates a level of significance explaining the probability of choosing strategy S3 compared to strategies S1 and S2 if the size of the household increases.

The surplus or *p*-value of the explanatory variable (total production) is 0.013 (**Table 7**). This value represents the individual significance of the explanatory variables. In our case, it is below the 5% threshold, explaining that the adoption of crop diversification strategies has a positive effect on the overall production of the farm.

DISCUSSION

The findings of our study show wide farmer practices of crop diversification. These results are consistent with regional analyses across the Sahelian countries in West Africa (Félix et al., 2018; Abberton et al., 2021). Crop diversification is an important principle of nutrition sensitivity to improve dietary diversity and food production in farm. It is evidenced that diversification may be a route from agriculture to nutrition, as it can directly affect the amount and types of food available for consumption in smallholder households. Indeed, in our context, improving access and stock to food alone does not guarantee better nutritional outcomes as expressed by the FCS that lay down rural households under moderate food security. This diagnosis confirms the conclusion of Youssouf (2017) that there is higher prevalence of food insecurity in rural areas compared to urban areas. Since sorghum and millet together account for 73% of the land area devoted to cereal production and 51% of the cereals produced in Mali (Foltz, 2010), the heavy reliance on a low range of commodities or staple crops, or even just one, exacerbates the multiple risks to rural livelihoods and nutrition arising from agricultural production which is threatened by various climate-related stressors and market volatility. In fact, Adams (1992) explained that the prevalence and severity of household food insecurity are strongly related to rainfall.

Overall consumer perception on diversification using foods made from millet, sorghum, groundnuts, and cowpeas aligns with the recognition that they can help to correct certain deficiencies and reduce certain diseases. Consumers of millet and sorghum are mostly in rural areas while urban areas are characterized by increased consumption and preference of rice in their diet. Kearney (2010) highlighted that the undergoing rapid transition in urban areas is experiencing nutritional transition in underdeveloped countries, and this is partly due to policies of trade liberalization over the past two decades with an increasing number of obesity and cardiovascular accident. Another valuable paradox to consider in the analysis, according to Bocoum et al. (2014), is that some poor households are managing to cover their caloric requirements by eating cheap calories and some non-poor households not doing so because they consume expensive calories and/or face constraints such as the obligation to share meals with visitors and high expenditure on healthcare or transportation.

For youth, our study shows that the prevalence of food insecurity is 30%, this result being superimposed on that of 26.6% as reported by Republique du Mali (2019); in fact, inadequate and insufficient nutrition, along with many other factors, contributes to poor nutritional status in children aged 6–48 months, from which nearly a third are with stunted growth. Children under 5 in urban areas had a better nutritional status compared to those in rural areas. Urban living is associated with better diet as an urban environment provides better monetary opportunity and food accessibility. However, other parameters such as access to healthcare and childcare practices and access to drinking water may influence nutritional results. Meanwhile, education of girls

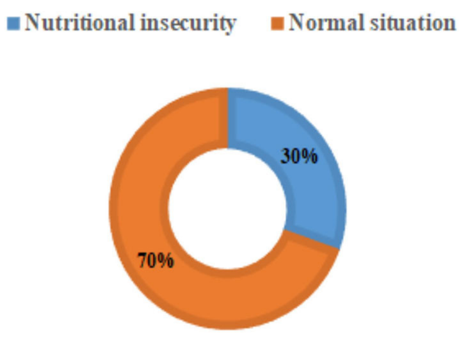

FIGURE 4 | Nutritional situation of children under 5 years old (%).

TABLE 5 | Food consumption score in rural and urban areas.

Food groups	Weighting	Villages frequency	Weighting × frequency	Bamako frequency	Weighting × frequency
Cereals and tubers	2	6	12	5	10
Legumes	3	3	9	2	6
Vegetables	1	2	2	3	3
Fruits	1	1	1	1	1
Meats and fish	4	1	4	2	8
Milk	4	1	4	2	8
Sugar	0.5	2	1	5	2.5
Oil	0.5	2	1	4	2
Totals			34		40.5

and women enables them to make more informed choices in matters of nutrition and health of their children (the higher the knowledge about the importance of a diversified diet, the better the nutritional status of children). Hirvonen (2016) supported that the difference in dietary diversity among children between rural and urban areas could be due to differences in parents' level of education, access to health services, and health between the two environments. As shown in our data, a high rate of illiteracy among women suggests a low level of knowledge on good feeding and care practices for young children. The survey reveals a low level of education, comparable to that reported by INSTAT AFRISTAT (2019) (34% for the whole country).

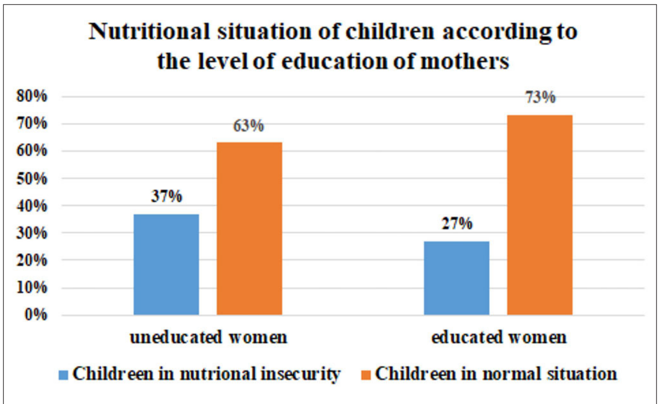

FIGURE 5 | Children's nutritional situation according the mother's level of education.

Multinomial logistic regression results underline socioeconomic factors that positively influence crop diversification. Notably, level of education, size of the household, and agricultural production per person positively influence the commitment of producers to the diversification of crops. Here again, having a certain level of education increases the probability for the producer to engage in the production of several crops (cereals and pulses) compared to producers only making cereals. This confirmed the findings of Idrisa et al. (2008) and Makombe et al. (2010). In fact, Mango et al. (2018) attributed that education can effectively improve prospects of the farming households to diversify their livelihoods through participation in off-farm formal employment activities as well as the access to and use of information while developing the capacity of farmers to enhance food security. Likewise, in cities,

TABLE 7 | Variability of agricultural production when producers adopt crop diversification strategies.

Diversification strategies	Coef.	Std. err.	$p > \|t\|$
Total production	0. 0000722	0.0000279	0.013
Number of obs. = 53			
$F_{(1,51)} = 6.70$			
Prob > F = 0.0125			
R-squared = 0.11			
Adj R-squared = 0.09			
Root MSE = 0.83			

TABLE 6 | Determinants of crop diversification using multinomial logistic regression results (S1 = cereals, S2 = 2, cereals, 1 legume, S3 = 2 cereals, 2 legumes, S4 = 3 cereals, 2 legumes).

Independent variables	Diversification strategies	Strategy (S1)			Strategy (S2)			Strategy (S3)		
		Coef.	Std. err.	*p*-values	Coef.	Std. err.	*p*-values	Coef.	Std. err.	*p*-values
Age of producers		0.48	2223.93	1.000	0.84	1692.24	1.000	0.029	0.03	0.39
Gender of producers		7.45	147292.1	1.000	14.87	66326.41	1.000	−22.44	25154.44	0.99
Education level		−7.57	19789.85	1.000	−6.56	24988.46	1.000	−1.40	0.54	0.01*
Household size		−0.16	1204.92	1.000	−0.57	2827.73	1.000	−0.11	0.053	0.04*
Possession work animals		−0.16	14309.25	1.000	0.64	1415.32	1.000	−0.036	0.034	0.29
Possession poultry		0.21	2212.73	1.000	−0.51	1472.27	1.000	0.048	0.063	0.57
Total area sown		−3.72	2212.73	1.000	−0.14	1125.93	1.000	0.048	0.063	0.45
Cost of agricultural inputs		0.00	0.37	0.999	−0.00	0.322	0.999	−0.00	8.46	0.12
Strategy (S4) (base outcome)										
Number of obs. = 53										
LR Chi^2 (24) = 59.64										
Prob. > Chi^2 = 0.0001										
Pseudo R^2 = 0.66										

there are more heads of households with an acceptable level of education. The significance of household size is explained by the capacity of an agricultural household to undertake several agricultural activities. Household stratification in Mali according to the capacity to sustain a secure, adequate, and viable diet revealed the food-secure to be large and wealthy households with sufficient resources to diversify production and to invest in agriculture and social networks of exchange (Adams, 1992).

Our study limitation is that the producer sample size is relatively small compared to the population concerned in this study and could probably pose a problem of representativeness of our data. It will be recalled, however, that the main goal sought was to understand the mechanisms of crop diversification, which is one of the factors in increasing productivity and improving the living conditions of the Malian population, rather than having a statistical representativeness. In Mango et al. (2018), with a comparable sample of 271 individuals, the authors have shown that diversification of crops represent a viable option in smallholder agriculture and it can ensure the establishment of resilient farming systems that can significantly contribute to household food security. The two studies are in favor that the diversification of crops improves agricultural production but could have increased its availability for household consumption, for better dietary intake of protein and energy and consequently nutritional outcomes. In addition to this, diversification is done by some growers to mitigate the adverse effects of climate change on crop yields. The positive impact of the diversification of crops on the living conditions of small farmers encourages for the intensification and promotion of crop diversification by policy toward the fight against persistent threat of malnutrition and food insecurity. Since it is often the poorest, low-resource farmers who grow millet and sorghum, there are significant poverty benefits from better millet and sorghum technology (Foltz, 2010) with a consequence that small changes in the productivity of those lands can have large impacts on overall food security of the nation.

CONCLUSION

Crop diversification is practiced by majority of smallholder farmers surveyed, but households in both urban and rural areas consume a limited range of crops and children are nutritionally insecure. There is a potential for smart food crop initiatives to support malnutrition and food insecurity in both areas since households have a sound perception of nutritional assets of those crops in their daily diet. This approach will have to focus its intervention on awareness and education programs for maximizing the incentive to consume nutritious foods from self-production and market purchases. In terms of food policy, much effort has been established by the Malian government's flagship on Scaling Up Nutrition to underline advocacy actions for nutrition based on the results of analyses, policies, and laws. Our results suggest that such policies need to approach a sustainable and holistic manner of crop diversification by offering an enabling environment that considers the improvement of education; reduction of extreme poverty; human health; the boosting of agricultural production with higher access to inputs; increased access to agricultural market infrastructure and climate smart technologies, business, and agricultural extension services; and support of processors for making nutritious and healthy products available in the local market.

DATA AVAILABILITY STATEMENT

The original contributions presented in the study are included in the article/supplementary materials, further inquiries can be directed to the corresponding author.

ETHICS STATEMENT

Written informed consent was obtained from the individual(s), and minor(s)' legal guardian/next of kin, for the publication of any potentially identifiable images or data included in this article.

AUTHOR CONTRIBUTIONS

All authors listed have made a substantial, direct, and intellectual contribution to the work and approved it for publication.

FUNDING

This study was funded by the International Crops Research Institute for the Semi-Arid Tropics through the Project Grant No FOOD/2019/407715 Améliorer la productivité des cultures et la resilience au Climat pour la Sécurité Alimentaire et Nutritionnelle au Mali (UE_APSAN-Mali) project.

ACKNOWLEDGMENTS

We thank Dr. Govoeyi Benoit for assistance in restructuring the methodological section. This work was supported by the European Union through project: Améliorer la productivité des cultures et la resilience au Climat pour la Sécurité Alimentaire et Nutritionnelle au Mali (UE-APSAN-Mali).

REFERENCES

Abberton, M., Abdoulaye, T., Ademonla, D. A., Aseidu, R., Ayantunde, A., Bayala, J., et al. (2021). *Priority Interventions for Transformational Change in the Sahel*. CCAFS Working Paper no. 353. Wageningen, Netherlands: CGIAR Research Program on Climate Change, Agriculture and Food Security (CCAFS).

Adams, A. M. (1992). *Seasonal Food Insecurity in the Sahel: Nutritional, Social and Economic Risk Among Bamana Agriculturalists in Mali*. Ph.D. thesis, London School of Hygiene and Tropical Medicine.

Adjimoti, G. O., and Kwadzo, G. T. M. (2018). Crop diversification and household food security status: evidence from rural Benin. *Agric. Food Secur.* 7, 82. doi: 10.1186/s40066-018-0233-x

Barikmo, I., Ouattara, F., and Oshaug, A. (2007). Differences in micronutrients content found in cereals from various parts of Mali. *J. Food Compos. Anal.* 20, 681–687. doi: 10.1016/j.jfca.2007.04.002

Bocoum, I., Dury, S., Egg, J., Herrera, J., and Prevel, Y. M. (2014). Does monetary poverty reflect caloric intake?. *Food Sec.* 6, 113–130. doi: 10.1007/s12571-013-0318-0

Chande, M., Muhimbula, H., Mremi, R., Muzanila, Y. C., Kumwenda, N. C., Msuya, J., et al. (2021). Drivers of millet consumption among school aged children in Central Tanzania. *Front. Sustain. Food Syst.* 5, 694160. doi: 10.3389/fsufs.2021.694160

Diama, A., Anitha, S., Kane-Potaka, J., Htut, T. T., Jalagam, A., Kumar, P., et al. (2020). How the smart food concept can lead to the transformation of food systems and combat malnutrition: different approaches in Africa, globally, and a case study from Myanmar with lessons learnt for creating behavior change in diets. *World Rev. Nutr. Diet.* 121, 149–158. doi: 10.1159/000507494

FAO (1992). *Food, Nutrition and Agriculture - 04 - Food Security*. *FAO Repository*. Available online at: https://www.fao.org/3/U8050t/u8050t01.htm (accessed October 13, 2021).

FAO (1996). *The Rome Declaration on World Food Security and Plan of Action of the World Food Summit*. Available online at: https://www.fao.org/3/w3613f/w3613f00.htm (accessed July 7, 2021).

FAO, IFAD, UNICEF, WFP, and WHO. (2021). The State of Food Security and Nutrition in the World 2021. *Transforming food systems for food security, improved nutrition and affordable healthy diets for all*. Rome: FAO. doi: 10.4060/cb4474en

Félix, G. F., Diedhiou, I., Le Garff, M., Timmermann, C., Clermont-Dauphin, C., Cournac, L., et al. (2018). Use and management of biodiversity by smallholder farmers in semi-arid West Africa. *Glob. Food Secur.* 18, 76–85. doi: 10.1016/j.gfs.2018.08.005

Foltz, J. (2010). *Opportunities and Investment Strategies to Improve Food Security and Reduce Poverty in Mali through the Diffusion of Improved Agricultural Technologies*. USAID-Mali AEG Group Report.

Greene, W. H. (2002). *Econometric Analysis*. Upper Saddle River, NJ: Prentice Hall. [Harmonis, C. (2019). Main Results and Problems. p. 4.]

Heumesser, C. H., and Kray, H. K. (2019). *Productive Diversification in African Agriculture and its Effects on Resilience and Nutrition*. World Bank. p. 244.

Hirvonen, K. (2016). Rural–urban differences in children's dietary diversity in Ethiopia: a Poisson decomposition analysis. *Econ. Lett.* 147, 12–15. doi: 10.1016/j.econlet.2016.08.003

Idrisa, Y. L., Gwary, M. M., and Shehu, H. (2008). Analysis of food security status among farming households in Jere Local Government of Borno State, Nigeria. *J. Trop. Agric. Food Environ. Ext.* 3, 199–205. doi: 10.4314/as.v7i3.45552

Ijaz, M., Nawaz, A., UlAllah, S., Rizwan, M. S., Ullah, A., Hussain, M., et al. (2019). "Crop diversification and food security," in Agronomic Crops, ed M. Hasanuzzamanm (Singapore: Springer), 607–621. doi: 10.1007/978-981-32-9151-5_26

INSTAT and AFRISTAT (2019). *Enquete Regionale Integree sur l' Emploi et Secteur Informe, 2017*. Bamako, Mali: INSTAT and AFRISAT.

Joseph, G., and Wodon, Q. (2008). *Assessing the Potential Impact on Poverty of Rising Cereals Prices: The Case of Mali.* World Bank Policy Research Working Paper. p. 4744. doi: 10.1596/1813-9450-4744

Kearney, J. (2010). Food consumption trends and drivers. *Philos. Trans. R. Soc. B* 365, 2793–2807. doi: 10.1098/rstb.2010.0149

Kennedy, G., Fanou, N., Segheiri, C., and Brouwer, I. D. (2009). *Performance of Eight Food Group Diversity Indicators to Predict Probability of Adequate Intake of Micronutrients in Women of Reproductive Age in Urban Mali*. Thesis Wageningen University, Wageningen, The Netherlands. With abstract – references – with summaries in English and Dutch.

Konate, F., Iknane, A. A., Diawara, F., Coulibaly, D., and Kone, A. D. (2020). Nutrition and food security in Mali from 2010 to 2020. *North Afr. J. Food Nutr. Res.* 4, S1–S6. doi: 10.51745/najfnr.4.9.S1-S6

Kouressy, M., Sultan, B., Vaksmann, M., Belires, J. F., Claessens, L., Dingkuhn, M., et al. (2019). Climate change and cereal production evolution trend in the Sahel: case study in Mali from 1951 to 2010. *Sustain. Agric. Res.* 8, 68. doi: 10.5539/sar.v8n2p68

Maiga, O., Tounkara, M., Doumbia, S., and Sangho, H. (2019). *Mali Political Economy Analysis*. Report Research Technical Assistance Center, Washington, DC.

Makombe, T., Lewin, P., and Fisher, M. (2010). *The Determinants of Food Insecurity in Rural Malawi: Implications for agricultural Policy*. Lilongwe: Malawi Strategy Support Program (MaSSP).

Mango, N., Makate, C., Mapemba, L., Mapembamm, L., and Sopo, M. (2018). The role of crop diversification in improving household food security in central Malawi. *Agric. Food Secur.* 7, 7. doi: 10.1186/s40066-018-0160-x

Republique du Mali (2019). *Enqute Nationale Nutritionnelle Anthropomtrique et de Mortalit Rtrospective Suivant la Mthodologie SMART-2019*, Mali. p. 86.

Sanga, U., Sidibé, A., and Olabisi, L. S. (2021). Dynamic pathways of barriers and opportunities for food security and climate adaptation in Southern Mali. *World Dev.* 148, 105663. doi: 10.1016/j.worlddev.2021.105663

Tefft, J., Penders, C., Kelly, V., Staatz, J. M., Yade, M., and Wise, V. (2000). *Linkages Between Agricultural Growth and Improved Child Nutrition in Mali.* MSU International Development Papers. p. 70.

Tounkara, M., Diarra, S., Maiga, O., Sangare, H., Diawara, S. I., Diawara, F., et al. (2019). *Food Security in Mali. Desk Review*. Research Technical Assistance Center, Washington, DC.

World Bank (2018). *Report Growth and Diversification, Mali (I) No: AUS00008.*

Youssouf, D. (2017). *Determinants of Food Insecurity in the Circle of Bandiagara in Mali*. Thesis, Gaston Berger University of Saint Louis. p. 99.

Author Disclaimer: The author's views expressed in this publication do not reflect the views of the European Union Delegation in Mali.

Conflict of Interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's Note: All claims expressed in this article are solely those of the authors and do not necessarily represent those of their affiliated organizations, or those of the publisher, the editors and the reviewers. Any product that may be evaluated in this article, or claim that may be made by its manufacturer, is not guaranteed or endorsed by the publisher.

frontiers in Sustainable Food Systems

ORIGINAL RESEARCH
published: 01 February 2022
doi: 10.3389/fsufs.2021.735356

Insights of Nutritional and Anti-nutritional Retention in Traditionally Processed Millets

H. V. Sheethal, Chandrama Baruah, Kalpuri Subhash, Rajendran Ananthan* and T. Longvah

Food Chemistry Division, ICMR-National Institute of Nutrition, Hyderabad, India

OPEN ACCESS

Edited by:
Ramadjita Tabo,
International Crops Research Institute for the Semi-Arid Tropics, Mali

Reviewed by:
Alexandru Rusu,
Biozoon Food Innovations GmbH, Germany
C. K. Sunil,
Indian Institute of Food Processing Technology, India

****Correspondence:***
Rajendran Ananthan
ananthan.nin@gmail.com

Specialty section:
This article was submitted to Nutrition and Sustainable Diets, a section of the journal Frontiers in Sustainable Food Systems

Received: *02 July 2021*
Accepted: *16 December 2021*
Published: *01 February 2022*

Citation:
Sheethal HV, Chandrama Baruah, Kalpuri Subhash, Rajendran Ananthan and Longvah T (2022) Insights of Nutritional and Anti-nutritional Retention in Traditionally Processed Millets.
Front. Sustain. Food Syst. 5:735356.
doi: 10.3389/fsufs.2021.735356

Millets are nutritionally superior indigenous staple crops packed with high protein, vitamins, and minerals. However, anti-nutrients such as phytic acid, tannins, and polyphenols present in the millets tend to reduce the bio-accessibility of minerals (iron and zinc), due to which the millet diets are greatly compromised. Although most of the cereals, such as wheat flour, brown rice, and barley, contain phytic acid to a level far more than that of the millets, it is important to develop feasible household methods to reduce the level of phytic acid so as to enhance nutrient absorption. The present study was carried out to investigate the effect of traditional processing on nutrient and anti-nutrient retention of three majorly consumed millets, namely, sorghum, finger millet, and pearl millet. These millets were traditionally cooked and then fermented overnight with water and curd. The results show that this type of simple, traditional household-level process significantly reduced the phytic acid content by 62.9% in sorghum, 34.1% in finger millet, and 29.35% in pearl millet. There is a considerable decrease in phytic acid–zinc molar ratio by 71.38, 61.15, and 33.47% and in phytic acid–iron molar ratio by 73.52, 48.07, and 66.39% in sorghum, finger millet, and pearl millet, respectively. Among the macronutrients, the protein and ash contents were significantly increased. A high retention of water-soluble vitamins was observed in the processed millets. Overall, the traditionally cooked millet, fermented overnight and then added with curd, enhanced many essential macro- and micronutrients and concurrently reduced phytic acid, thus forming a sustainably simple household method for improving dietary nutrients.

Keywords: millets, traditional cooking, fermentation, antinutrients, nutrition retention

INTRODUCTION

Millets are small-seeded annual grasses belonging to the Graminae (Poaceae) family. They are generally resistant to pests and diseases and have the ability to grow in less fertile, dry land, with a harvesting time of 70–80 days (Devi et al., 2014). Their long storability under normal conditions made them "famine reserves". Millets are also called "nutri-cereals" due to their high nutritive value (Bhat et al., 2018). Millets are mainly cultivated in African and Asian countries. In 2019, millet cultivation in Central Africa was at 1,120.7 kg/ha, while in India it was 1,211.4 kg/ha (FAOSTAT, 2019). Due to the adaptation of improved technologies, millet production has increased drastically in recent years. The production of millets increased from 87.7 thousand tons (2009) to 1 lakh tons (2019) (FAOSTAT, 2019) in the last decade. In particular, sorghum and finger millet average productivity has improved by 75 and 41%, respectively, during 2015–2016 (Chapke et al., 2018).

However, in the past few decades, the global millet consumption has declined at a rate of 0.9%; the *per capita* consumption of millets fell marginally from 4.6 in 1982 to 3.6 kg around the world, while in India it was 12 kg in 1982 and subsequently reduced to 8 kg during the year 2009 (Rao and Basavaraj, 2015). Millet consumption was reported to be much higher in rural (58.6%) compared to urban (27.5%) populations in India (NSSO, 2012). Some of the reasons for reduced millet consumption are the availability of rice and wheat through the public distribution system, increased *per capita* income, growing urbanization, and changing tastes and preferences (Bhagavatula et al., 2013). Hence, consumption has shifted from millets to refined cereals. Nevertheless, in recent times, millet consumption has been increased from 43.2 million metric tons (MMT) in 2018 to 45.4 MMT in 2019 (Wallace and Singh, 2019). Sorghum, pearl millet, and finger millet are the most commonly consumed millets among the different millets. In India, pearl millet appeared to be popular in the northern and eastern parts, while sorghum appeared to be popular in the west and south of India and in Eastern India. India is the largest producer of pearl millet, and it is largely concentrated in the states of Rajasthan, North and Central Maharashtra, Gujrat, and Northern Karnataka (Rao and Basavaraj, 2015), whereas finger millet appeared to be most popular in the western and southern parts of India (Muniappan et al., 2018).

Most commonly, millets are consumed in the form of a thick porridge, rotis (Indian flat bread), and dumplings and cooked with vegetables (Rao et al., 2006). Different traditional methods also used for millet processing include boiling, pounding, soaking, fermenting, malting, popping, flaking, and roasting. In recent times, there are many industries and research institutes that developed different processing methods using modern equipment to prepare ready-to-eat and ready-to-cook products like semolina, flakes, pasta, vermicelli, dehulled millets, millet-rich multigrain, and millet-rich multigrain roti.

Cereals and grains not only provide more than 50% of the caloric intake and protein intake of the world but are also a good source of other micronutrients (BNF, 2004). Whole grains are rich sources of fiber, vitamins, minerals, and phytochemicals, such as phenolics, lignans, b-glucan, inulin, resistant starch, and sterols. Millets contain high level of protein, essential fatty acids, dietary fiber, vitamin B, and minerals, such as calcium, iron, zinc, potassium, and magnesium, and help in rendering health benefits like reduction in blood sugar level (diabetes), blood cholesterol and pressure regulation, preventing thyroid disorders, reducing the risk of developing cardiovascular disease, celiac disease, and many other age-related chronic diseases (Jacobs et al., 1995, 1998; Liu et al., 1999, 2000; Anderson et al., 2000; Meyer et al., 2000; Nicodemus et al., 2001; Liu, 2002; Anitha et al., 2021). However, studies also reported that cereals contain "anti-nutrients", such as phytic acid, that interfere with nutrient absorption in the human body. Not only millet but also other monocotyledonous seeds like wheat, barley, and rice accumulate phytic acid mostly in the aleurone layer, and the level varies between 0.5 and 2.0%, with brown rice at 0.84–0.94%, milled rice at 0.20%, wheat flour at 0.96%, barley at 1.19%, and whole corn at 1.05% (Reddy and Sathe, 2001; Okazaki and Katayama, 2005; Longvah et al., 2017).

Millets have about 0.61% of tannin, 0.48% of phytic acid, 0.2–3.0% of polyphenol, and trypsin inhibitors (Thompson, 1993), out of which phytic acid is a matter of concern. Although the level of phytic acid in millet is far less than that in wheat flour, brown rice, barley, and whole corn, it is still important to reduce the phytic acid content to enhance the bio-accessibility of major nutrients. Phytic acid is the organic form of phosphorous (myoinositol 1,2,3,4,5,6-hexakis dihydrogen phosphate) occurring in plant constituents as the major portion of total phosphorus (Guttieri et al., 2004), with a highly negative charge and a reactive compound that attracts and binds positively charged mineral ions such as iron, zinc, and calcium. This binding changes nutrient digestibility and bioavailability, as monogastric animals (poultry and humans) cannot metabolize phytic acid due to the absence of phytase enzymes in their digestive tract (Lopez et al., 2002; Vats and Banerjee, 2004). Concurrently, reports also reveal that only the highly phosphorylated inositol phosphates, i.e., IP5 and IP6, interfered with mineral utilization but not the lower inositol phosphates, namely, IP1, IP2, IP3, and IP4 (Lönnerdal et al., 1989).

Traditional processing methods are considered to reduce the phytic acid level in cereals and millets. More reduction has been reported especially in traditional Indian cooking style, like pressure cooking, prolonged boiling, steaming, etc., compared to other countries (Agte et al., 1999). In addition, soaking, fermentation, and germination also reduced the phytic acid content (Liang et al., 2008) in millets. It has been reported that fermentation provides optimum pH to degrade phytic acid and increases the availability of minerals and vitamin B (Haard, 1999). The synthesis of vitamins during germination increased several times and reduced the phytic acid concentration (FAO/WHO, 2001), but roasting, puffing, flaking, and decortication showed relatively lesser outcomes on phytic acid reduction. A combination of different processing methods, such as autoclaving along with fermentation, also showed a promising role in phytic acid reduction by 63% (Binita and Khetarpaul, 1997).

Although many pieces of research have been reported on the determination of phytic acid content in different processing

Abbreviations: AOAC, Association of Official Agricultural Chemists; PA, phytic acid; MW, molecular weight; RF, retention factor; YF, yield factor; SMR, raw sorghum millet; SMC, cooked sorghum millet; SMFC, sorghum millet cooked and fermented with curd overnight; SMFW, sorghum millet cooked and fermented with water overnight; SMFWC, sorghum millet cooked and fermented with water overnight and added with curd; PMR, raw pearl millet; PMC, cooked pearl millet; PMFC, pearl millet cooked and fermented with curd overnight; PMFW, pearl millet cooked and fermented with water overnight; PMFWC, pearl millet cooked and fermented with water overnight and added with curd; FMR, raw finger millet; FMC, cooked finger millet; FMFC, finger millet cooked and fermented with curd overnight; FMFW, finger millet cooked and fermented with water overnight; FMFWC, finger millet cooked fermented with water overnight and added with curd; SDF, soluble dietary fiber; IDF, insoluble dietary fiber; TDF, total dietary fiber; FAO, Food and Agriculture Organization; IP, inositol monophosphate; IP2, inositol bisphosphate; IP3, inositol trisphosphate; IP5, inositol pentakisphosphate; IP6- inositol hexaphosphate; RTE, ready-to-eat; RTC, ready-to -cook; Fe, iron; Zn, zinc; Ca, calcium; Mg, magnesium; Na, sodium; P, phosphorous; K, potassium; DW, dry weight; IZiNCG, International Zinc Nutrition Consultative Group; PDS, public distribution system.

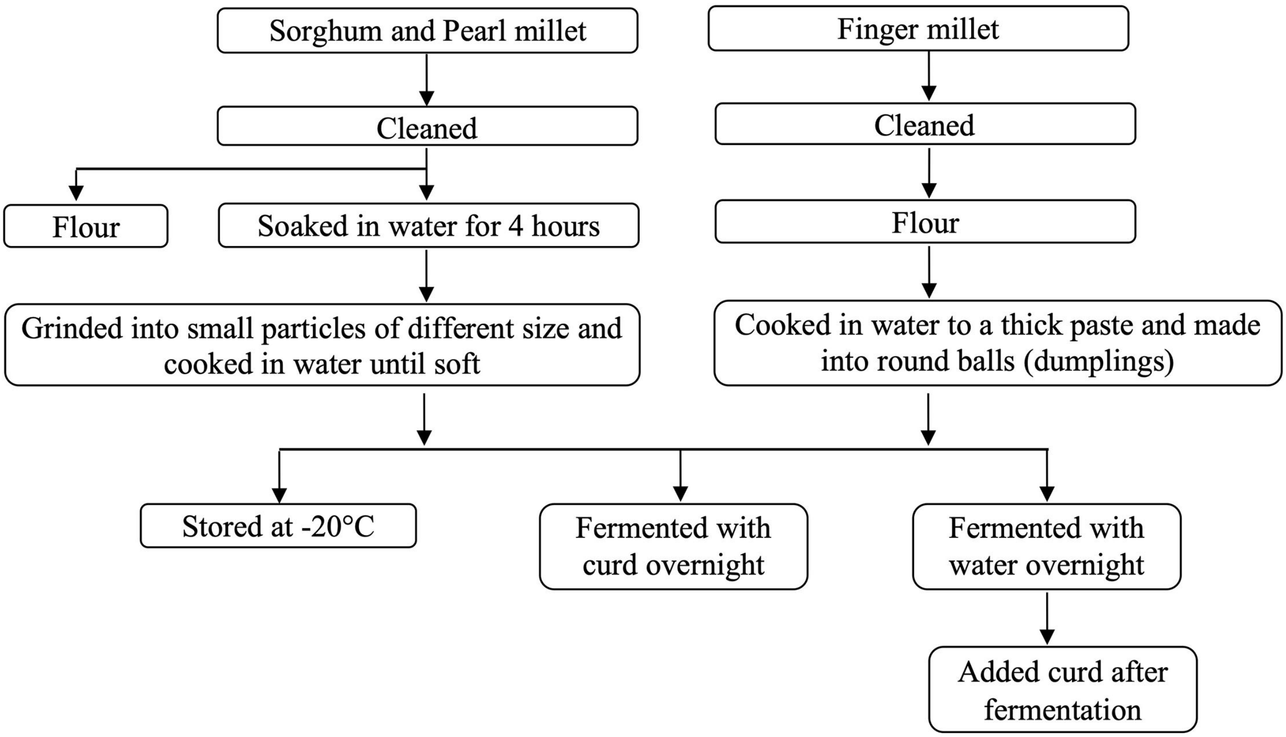

FIGURE 1 | Sample preparation flow chart.

methods to reduce the phytic acid levels and its effect on mineral and vitamin availability, there is no report found on the effect of traditionally cooked millet along with curd/yogurt which contain *Lactobacillus* sp. on improving the nutrition quality of millets. We hypothesized that traditionally cooked and processed millets along with curd attenuate the phytic acid level and enhance the water-soluble vitamin content. Thus, the present study was carried out to observe the trends of phytic acid reduction and predict mineral bioavailability in three commonly consumed millets (sorghum, pearl millet, and finger millet) after the application of various traditional processing methods.

MATERIALS AND METHODS

Sample Collection

Three millets, namely, sorghum (*Sorghum vulgare*), pearl millet (*Pennisetum typhoideum*), and finger millet (*Eleusine coracana*), were purchased at 2 kg each from three different markets of Secunderabad and Hyderabad (Telangana) and combined into a single (total of 6 kg) sample for the subsequent studies. Traditionally prepared curd was purchased from a local shop in Secunderabad, Telangana.

Sample Preparation

All the millets were cleaned by removing unwanted foreign particles if there was any (**Figure 1**). The whole quantity of sorghum and pearl millet were soaked in water for 4 h, and then the water was drained. The soaked millets were spread on a blotting paper at room temperature for 30 min. They were coarsely ground using a domestic mixer grinder. The pearl and powder were separated using a sieve (60 mesh, 0.25 mm). Initially, the pearl was added to boiling water and cooked until it turned soft, and then the powder was added and allowed to cook further. Finger millet was made into fine powder using a commercial mill. The powder was added to boiling water (in a ratio of 1:2 w/v) and stirred continuously to avoid the formation of lumps. The sample was cooked until it turns into a thick paste. Then, the paste was made into small round balls (dumplings). All the three cooked millets were allowed to cool down to room temperature and then divided into four equal parts. The first part was stored at −20°C in air-tight containers until further analysis. The second part was mixed with an equal amount of curd and kept for overnight fermentation. Water was added (up to submersible level) to the third and fourth part of the cooked millet, and this was stored at room temperature overnight for fermentation. After overnight fermentation, the fourth part was mixed with an equal quantity of curd. All the samples were homogenized using a domestic mixer grinder and stored at −20°C in air-tight containers until further analysis. All the cooking procedures and subsequent processing were carried out using stainless steel vessels.

Proximate Analysis

Proximate composition was determined using Association of Official Agricultural Chemists (AOAC) official methods. Moisture was analyzed as per AOAC 934.01 methods. Using

MRC (DFO 36-240 SERIES) hot-air oven, ash was quantified using AOAC 942.05 methods in a Thermo Fischer, Heraeus muffle furnace. The total fat content was determined by mixed solvent extraction method (chloroform and methanol, 2:1 ratio) using AOAC 922.06, and dietary fiber was done by enzymatic–gravimetric method using AOAC 991.43. Protein was estimated using AOAC 954.01 protocol by Kjeldahl method (gravimetric and titration), using an automated FOSS Kjeltec™ 8400 Kjeldahl analyzer, with a conversion factor of 5.95. The total carbohydrate was estimated by differential method using the following formula: total carbohydrate = [100 – (moisture + ash + fat + total protein + fiber)] (g/100 g).

Determination of Phytic Acid, Minerals, and Phytic Acid–Mineral Molar Ratio

Total phytic acid (IP6) was analyzed by the anion exchange method (AOAC 986.11). Phytic acid was extracted with 2.4% HCl. First, columns were prepared by adding 0.5 g AG 1-X4 resin; then, 5 ml of distilled water was added to form a resin bed. Furthermore, 15 ml of 0.1 M NaCl was added to remove any contaminating phosphate ions, and 15 ml of water was added again to wash the columns. The samples were prepared with EDTA-NaOH reagent and poured into columns. The columns were allowed to stand for 20 min and then washed with 15 ml water and 0.1 M NaCl to remove unbound foreign materials and lower inositol phosphates, respectively. The resin was eluted with 15 ml 0.7 M NaCl to release the bound inositol hexaphosphate (phytic acid) and collected into 100-ml Kjeldahl flasks. Glass beads (no. 3), 3.0 ml HNO_3, and 0.5 ml H_2SO_4 were added to the Kjeldahl flasks and digested under the hood over medium heat until a cloud of thick yellow vapor fills the neck of the flasks. The, the flasks were cooled, and the salts formed were dissolved in water and then transferred into 50-ml volumetric flasks. Ammonium molybdate solution (2.0 ml) and sulfonic acid reagent (1.0 ml) were added and mixed well and made up the final volume with distilled water. The mixture was incubated for 15 min at room temperature, and the absorbance was measured at 640 nm using a UV–visible spectrophotometer.

The phytic acid concentration in food samples was calculated (phytic acid contains 28.2% phosphorus) as follows:

$$Phytic\ acid\ (mg/g\ sample) = \frac{Content\ volume\ \times\ mean\ K\ \times\ absorbance}{Weight\ of\ the\ sample \times\ 0.282\ \times\ 1,000}$$

where mean K is the mean of the concentrations of standard divided by the absorbance of standard.

Mineral content in millet samples was determined by AOAC (2016) using flame atomic absorption spectrophotometry. The finely ground sample (∼1 g) was digested using Supra-pure nitric acid (67%) and hydrogen peroxide (30%) at the ratio of 2:1 (v/v) in a microwave Mars Xpress CEM. Then, the samples were allowed to cool to room temperature and made up to 25 ml using volumetric flasks. Elements such as Fe and Zn were determined using a flame-atomic absorption spectrophotometer (Analytikjena ContrAA 700) operated with Aspect CS 2.2.1.0 tech software. Absorbance for Fe was taken at 248.32 nm and for Zn at 213.86 nm.

The molar ratios between phytic acid and minerals were calculated by dividing the mole of phytic acid with a mole of mineral content using the following formula (Dahdouh et al., 2019):

$$Phytic\ acid - mineral\ molar\ ratio = \frac{\frac{PA}{MW(PA)}}{\frac{Min}{MW(Min)}}$$

where PA = phytic acid analyzed, MW(PA) = phytic acid molecular weight (660.06 Da), Min = mineral content (Zn/Fe), and MW(Min) = mineral molecular weight (Fe = 55.845 Da; Zn = 65.38 Da).

Determination of Water-Soluble Vitamins

Water soluble vitamins, namely, B_2, B_3, B_5, B_6, B_9, and C, were quantified by HPLC techniques using ultra-high-performance liquid chromatography (U-HPLC; Dionex Ultimate 3000 RSLC, USA, with Chromeleon software). For the determination of vitamins B_2 and B_3, a sample (1 g) was extracted with 0.1 M HCl, and the tubes were centrifuged at 4,000 rpm at 10°C for 10 min. The supernatant was collected into amber-colored HPLC auto-sampler vials after filtering through a 0.45-μm polyvinylidene fluoride (PVDF) syringe filter. The vitamins were separated on a reverse-phase chromatographic column (Thermo Scientific BDS Hypersil C18 column 250 × 4.6 mm; particle size, 5 μm). The column temperature was maintained at 40°C. Phosphate buffer (0.05 M, pH 3.2) and acetonitrile were used as mobile phase at the ratio of 70:30 (v/v) and flow rate of 1 ml/min. Fluorescence detector (FLD) was used with excitation λ at 445 nm and emission λ at 522 nm for the quantification of vitamin B_2, whereas for vitamin B3, phosphate buffer (25 mM, pH 3.02) and acetonitrile at the ratio of 95:5 (v/v) were used as mobile phase at 1-ml/min flow rate and detected at 260 nm by photodiode array detector (PDA). Vitamin B_5 was determined by the method suggested by Woollard et al. (2000). Briefly, 1 g of sample was extracted with 3% acetic acid and centrifuged at 4,000 rpm for 10 min (10°C). The supernatant was collected into HPLC auto-sampler vials after filtering through a 0.45-μm PVDF syringe filter. A Thermo Scientific BDS Hypersil C18 column 250 × 4.6 mm, a 5-μm-particle-sized column (maintained at 40°C), was used with phosphate buffer (0.1 M, pH 2.25) and acetonitrile (95:5) as mobile phase adjusted to a flow rate of 1 ml/min and detected at 205 nm using PDA. The quantification of vitamin B_6 was carried out with reference to Valls et al. (2001). Briefly, the sample was extracted with 5% metaphosphoric acid and centrifuged at 4,000 rpm for 10 min at 10°C. The supernatant was filtered using a 0.45-μm PVDF syringe filter and injected. The isocratic mobile phase consisting of 25 mM phosphate buffer (pH 3.2) and acetonitrile (70:30 v/v) was passed through a Thermo Scientific BDS Hypersil C18 column (250 × 4.6 mm; particle size, 5 μm). The column was maintained at 35°C with a mobile phase flow at 0.8 ml/min. FLD was used to determine B_6 vitamers fixing the excitation λ at 290 nm and emission λ at 395 nm (Valls et al., 2001). Vitamin B_9 was quantified by U-HPLC after extracting with the tri-enzyme technique.

Briefly, the sample was extracted with phosphate (K_2HPO_4) buffer containing ascorbic acid, sodium azide, and 2-mercapto ethanol. The sample was subsequently treated with enzymes (α-amylase, protease, and deconjugase) one by one at an appropriate temperature and finally centrifuged at 4,000 rpm (10°C). The supernatant was purified by passing through a strong anion exchange cartridge (SEP-PAK cartridge), filtered using 0.22-µm PVDF syringe filter, and injected into U-HPLC. Phosphate buffer (pH 2.2) and acetonitrile at the ratio of 95:5 (v/v) were passed through a Phenomenex Luna C18 (150 × 2 mm; 3 µm) column at a flow rate of 0.5 ml/min, and the folate vitamers were detected using FLD with excitation and emission λ at 220 and 440 nm, respectively (Rader et al., 1998; Brouwer et al., 2008). Vitamin C (total ascorbic acid) content in the raw and processed millets was analyzed by extracting the sample (1 g) with 3% metaphosphoric acid (w/v) and 8% glacial acetic acid (v/v). The samples were centrifuged, and the supernatant was filtered through a 0.45-µm PVDF syringe filter into HPLC auto-sampler vials and injected in U-HPLC equipped with PDA. Phosphate buffer (0.05 M, pH 3.2) and acetonitrile at the ratio of 90:10 was used as isocratic mobile phase with 1 ml/min flow rate. A Thermo Scientific BDS Hypersil C18 column (250 × 4.6 mm; particle size, 5 µm) was used as stationary phase, and ascorbic acid was detected at 244 nm using PDA (Ekinci and Kadakal, 2005; Hernandez et al., 2006; Phillips et al., 2010).

Nutrient Retention Factor

Nutrient retention factor is the amount of nutrients retained in foods after preparation, processing, or other treatments. It depends on several factors, such as temperature, time, pressure, and many other cooking practices (Vásquez-Caicedo et al., 2008).

The retention factor for water-soluble vitamins and phytic acid was calculated by using the given formula below and expressed as values between 0 and 1 or as a percentage of retention (0–100%). Nutrient content could be expressed in grams, milligrams, or micrograms, depending on the nutrient.

$$Nutrient\ retention\ factor = \frac{Nutrient\ content\ per\ 100\ g\ of\ dish,\ edible\ part\ \times\ yield\ factor\ (YF)}{Nutrient\ content\ per\ 100\ g\ of\ ingredients\ (ready-to-cook)}$$

$$YF = \frac{Prepared\ dish,\ including\ waste,\ in\ grams}{Total\ quantity\ of\ ingredients\ \left(ready-to-cook\right)\ in\ grams}$$

Statistical Analysis

Descriptive statistical analysis was done using the SPSS package (SPSS for Windows, version 16.0. Chicago, USA). The experiment for phytic acid was carried out in quadruplicate, while all the other experiments were carried out in triplicate analyses, and the results were expressed as mean ± standard deviation (SD). One-way ANOVA was performed to evaluate the significance of differences within the treatments at $p < 0.05$ level of significance.

RESULTS

Proximate Principles

The proximate principles (moisture, protein, ash, fat, and dietary fiber) were analyzed in the raw pearl millet (PMR), finger millet (FMR), and sorghum millet (SMR), the representative samples of which were traditionally processed, and the results are given in **Table 1**. All proximate values are expressed as grams in 100 g of the edible portion on dry weight basis. The protein content of raw millets, such as PMR, FMR, and SMR, was 9.45 ± 0.37, 7.18 ± 0.42, and 10.60 ± 0.26 g/100 g, respectively. Among the traditionally processed millet foods, the significantly highest ($p < 0.05$) amount of protein was found in SMFWC (20.57 ± 0.37 g/100 g) and PMFWC (20.27 ± 0.27 g/100 g), followed by FMFWC (16.92 ± 0.07 g/100 g). The lowest amount of protein was found in SMC (9.91 ± 0.18 g/100 g), PMC (9.30 ± 0.06 g/100 g), and FMFW (7.88 ± 0.12 g/100 g).

FMR contains the highest amount of ash (2.14 ± 0.04 g/100 g) compared to PMR (1.24 g/100 g) and SMR (1.35 g/100 g). Among the different processed foods, the millet cooked, fermented overnight, and added with curd had the highest ash content in all the three millets studied (SMFWC-2.56 ± 0.06, PMFWC-2.61 ± 0.14, and FMFWC-3.81 ± 0.05 g/100 g). The fat content of raw pearl millet was the highest (4.3 g/100 g) among all raw and processed millets. However, SMR and FMR had higher values than their cooked forms (1.40 and 1.36/100 g, respectively). Among the different processing techniques, there was a significant reduction observed in the cooked pearl millet fermented overnight and then added with curd (0.64/100 g). In contrast, the lowest fat content among the differently processed finger millet samples was observed in the cooked finger millet (0.58/100 g). Data on dietary fiber (both insoluble and soluble) analyzed in the raw and cooked millets are presented in **Table 1**. The total dietary fiber content of sorghum was between 9.33 and 9.97/100 g, while it was 10.13 and 11.4 g/100 g in pearl millet and finger millet, respectively. Among the two dietary fiber fractions, more than 80% are from insoluble dietary fiber. Among three millets, carbohydrate content was observed to be higher in both finger millet and sorghum (around 77%) than in pearl millet (75%). The cooked millets added with curd either before or after fermentation were found to have lesser carbohydrate than the other processed foods.

Total Phytic Acid Content and Its Retention

Data on the total phytic acid content and its retention in traditionally processed sorghum, pearl millet, and finger millet are summarized in **Figure 2**. The total phytic acid content in raw millets was found to be 8.6 ± 0.15 mg/g (SMR), 5.69 ± 0.19 mg/g (FMR), and 4.77 ± 0.07 mg/g (PMR). Reduction of phytic acid was observed in all the millets after cooking (minimum of 16.14% and maximum of 49.18%) and in subsequent processing where the samples were fermented with or without curd overnight (minimum of 20.96% and maximum of 54.53%). Among the different processes, the maximum reduction in total phytic acid

TABLE 1 | Proximate principles of raw and traditionally processed sorghum, pearl millet, and finger millet (g/100 g) in dry weight except for moisture.

Sample name	Protein	Ash	Fat	Dietary fiber			Carbohydrate	Moisture
				IDF	SDF	TDF		
	g/100 g							
Sorghum								
SMR	10.60 ± 0.26^{c}	1.35 ± 0.05^{c}	1.40 ± 0.04^{a}	8.33 ± 0.02^{a}	1.09 ± 0.03^{a}	9.42 ± 0.1^{a}	77.23 ± 0.21^{a}	7.87 ± 0.07^{d}
SMC	9.91 ± 0.18^{d}	1.25 ± 0.04^{c}	1.24 ± 0.05^{b}	8.05 ± 0.14^{a}	1.28 ± 0.01^{a}	9.33 ± 0.14^{a}	78.28 ± 0.43^{a}	75.38 ± 0.5^{c}
SMFC	15.60 ± 0.07^{b}	2.36 ± 0.03^{b}	0.90 ± 0.09^{c}	7.96 ± 0.01^{a}	1.46 ± 0.02^{a}	9.42 ± 0.02^{a}	71.71 ± 0.90^{c}	83.61 ± 0.56^{b}
SMFW	10.13 ± 0.09^{cd}	1.22 ± 0.00^{c}	0.62 ± 0.02^{e}	8.00 ± 0.01^{a}	1.96 ± 0.01^{a}	9.97 ± 0.01^{a}	78.05 ± 0.49^{b}	87.86 ± 1.56^{a}
SMFWC	20.57 ± 0.37^{a}	2.56 ± 0.06^{a}	0.78 ± 0.01^{d}	8.01 ± 0.04^{a}	1.58 ± 0.41^{a}	9.60 ± 0.46^{a}	66.44 ± 0.81^{d}	88.80 ± 0.34^{a}
Pearl millet								
PMR	9.45 ± 0.37^{d}	1.24 ± 0.2^{b}	4.30 ± 0.02^{a}	9.02 ± 0.01^{a}	1.09 ± 0.02^{c}	10.11 ± 0.02^{c}	74.88 ± 0.43^{a}	7.87 ± 0.09^{d}
PMC	9.30 ± 0.06^{d}	1.35 ± 0.04^{b}	2.04 ± 0.12^{b}	8.34 ± 0.01^{c}	1.79 ± 0.03^{b}	10.13 ± 0.05^{c}	77.14 ± 0.25^{a}	72.97 ± 0.93^{c}
PMFC	16.46 ± 0.18^{b}	2.56 ± 0.04^{a}	1.43 ± 0.00^{c}	8.42 ± 0.03^{c}	1.85 ± 0.04^{ba}	10.28 ± 0.02^{b}	69.26 ± 0.68^{c}	81.80 ± 0.44^{b}
PMFW	10.49 ± 0.11^{c}	1.41 ± 0.48^{b}	0.91 ± 0.03^{d}	8.70 ± 0.03^{b}	1.93 ± 0.01^{ba}	10.64 ± 0.04^{a}	76.52 ± 0.79^{b}	87.81 ± 0.20^{a}
PMFWC	20.27 ± 0.27^{a}	2.61 ± 0.14^{a}	0.64 ± 0.02^{e}	8.11 ± 0.02^{d}	2.02 ± 0.02^{a}	10.13 ± 0.04^{c}	66.32 ± 0.38^{d}	88.24 ± 0.34^{a}
Finger millet								
FMR	7.18 ± 0.42^{d}	2.14 ± 0.04^{b}	1.36 ± 0.03^{a}	9.42 ± 0.13^{a}	2.08 ± 0.04^{a}	11.41 ± 0.10^{a}	77.91 ± 1.31^{a}	10.50 ± 0.00^{e}
FMC	8.09 ± 0.08^{c}	2.27 ± 0.09^{b}	0.58 ± 0.06^{d}	9.04 ± 0.03^{ab}	2.00 ± 0.02^{a}	11.13 ± 0.07^{ab}	77.95 ± 0.48^{a}	68.03 ± 0.51^{d}
FMFC	15.68 ± 0.09^{b}	3.70 ± 0.04^{a}	0.98 ± 0.01^{b}	8.66 ± 0.04^{bc}	1.79 ± 0.14^{a}	10.45 ± 0.10^{cb}	69.10 ± 1.03^{b}	77.88 ± 0.47^{c}
FMFW	7.88 ± 0.12^{c}	2.41 ± 0.07^{b}	0.64 ± 0.02^{d}	8.42 ± 0.02^{c}	1.85 ± 0.01^{a}	10.27 ± 0.03^{c}	78.79 ± 0.72^{a}	82.55 ± 0.79^{b}
FMFWC	16.92 ± 0.07^{a}	3.81 ± 0.05^{a}	0.83 ± 0.01^{c}	9.05 ± 0.01^{ab}	1.81 ± 0.02^{a}	10.86 ± 0.03^{b}	67.68 ± 0.72^{b}	84.12 ± 0.38^{a}

Values represent mean ± standard deviation of triplicate analyses, and values with the same superscript letter within the column do not differ significantly at $p < 0.05$ by one-way ANOVA.

was found in the millets fermented overnight and then added with curd (FMFWC: 3.75 ± 0.06; PMFWC: 3.37 ± 0.17; SMFWC: 3.28 ± 0.09 mg/g).

Phytic acid retention factor in the three millets was exhibited in all the four different processing methods employed (**Figure 3**). In processed sorghum, least retention of phytic acid was observed in SMFWC (0.31), followed by SMFW and SMC (0.35). In comparison, maximum phytic acid retention was found in SMFW (0.47) because the samples treated with curd create a more appropriate environment to activate the phytase enzyme compared to samples without curd fermentation. Among the traditionally processed pearl millet, minimum retention of phytic acid was detected in PMFWC (0.48), and maximum retention was observed in PMFC (0.57), which may be due to the contribution of phytic acid from curd. The other processed samples, PMC (0.56) and PMFW (0.52), retained more phytic acid. The traditionally processed finger millet samples treated with curd retained less phytic acid, i.e., FMFWC had 0.49, and FMFC retained 0.51. The retention factor for other samples, FMFW and FMC, was 0.69 and 0.52, respectively, compared to the raw sample.

Mineral Composition

Data on the iron (Fe) and zinc (Zn) content of raw and domestically processed sorghum, pearl millet, and finger millet are presented in **Table 2**. Among the three raw millets analyzed, pearl millet had the highest zinc (3.32 ± 0.15 mg/100 g), followed by finger millet (2.09 ± 0.09 mg/100 g) and sorghum (1.95 ± 0.01 mg/100 g). Between the processed millet samples, zinc content was found to increase in the fermented sorghum (with or without curd) samples. The raw sorghum contains 1.95 mg/100 g, which was increased up to 2.72 mg/100 g in SMFW. However, no significant ($p < 0.05$) change was observed among the raw and processed pearl millet and finger millet samples (**Table 2**). Determination of iron content in the three different millets revealed that raw finger millet contained the highest level of iron (5.15 ± 0.75 mg/100 g), followed by sorghum (3.32 ± 0.12 mg/100 g) and pearl millet (3.29 ± 0.19 mg/100 g). Among traditionally processed millet foods, elevated Fe content was distinguished in the millets fermented overnight after traditional cooking (SMFW, 7.24 ± 0.52 mg/100 g; PMFW, 8.40 ± 0.334 mg/100 g; FMFW, 8.95 ± 0.63 mg/100 g). The cooked millet fermented along with curd seems to have the second-highest Fe content. However, all the differently processed millet foods appear with an increased iron concentration in all the three millets studied here.

Estimation of Phytic Acid–Mineral Molar Ratio

Phytic acid–mineral molar ratios (zinc and iron) were determined, and the data are given in **Table 2**. The raw millets were found to have higher ratio values than the processed samples. The highest molar ratio between phytic acid–Zn and phytic acid–Fe was recorded in raw sorghum (43.79 and 21.94, respectively). The molar ratio between phytic acid and Zn was 14.25 in raw pearl millet and 26.98 in finger millet. The Zn molar ratio was significantly reduced in all the processed millets after fermenting, followed by the addition of curd (SMFWC, 13.60;

FIGURE 2 | Raw, cooked, and fermented millet foods. **(A1–A5)**: Sorghum (**A1**—raw, **A2**—cooked, **A3**—cooked and fermented with curd overnight, **A4**—cooked and fermented with water overnight, **A5**—cooked and fermented with water overnight and added with curd). **(B1–B5)**: Pearl millet (**B1**—raw, **B2**—cooked, **B3**—cooked and fermented with curd overnight, **B4**—cooked and fermented with water overnight, **B5**—cooked and fermented with water overnight and added with curd). **(C1–C5)**: Finger millet (**C1**—raw, **C2**—cooked, **C3**—cooked and fermented with curd overnight, **C4**—cooked and fermented with water overnight, **C5**—cooked and fermented with water overnight and added with curd).

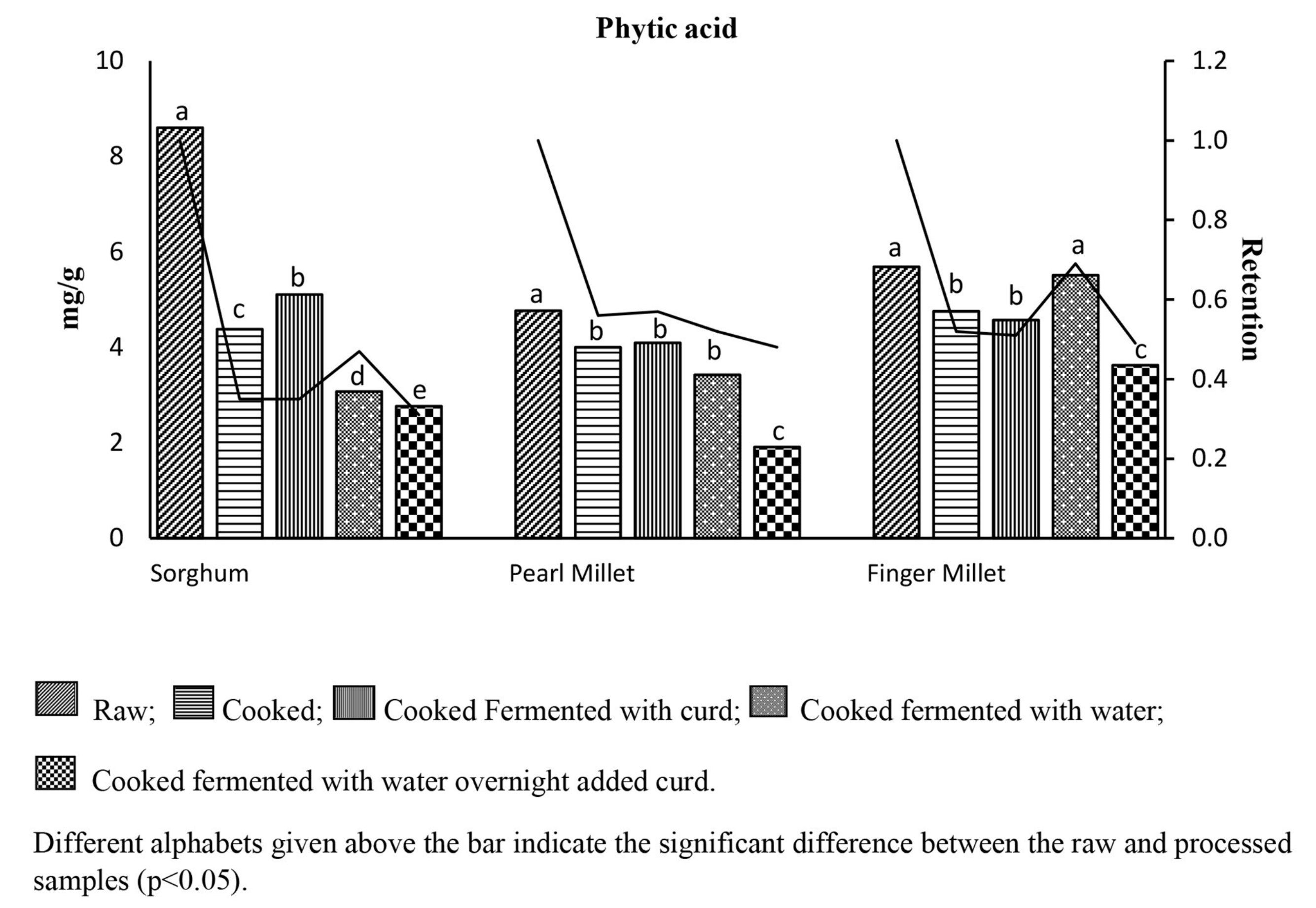

FIGURE 3 | Phytic acid content and its retention in raw and traditionally cooked millets.

PMFWC, 9.48; FMFWC, 10.48). The molar ratio between phytic acid and Fe in raw sorghum was 21.94, followed by raw pearl millet (12.26) and finger millet (9.34). Cooking and processing of millet were found to reduce the phytic acid–Fe molar ratios. The lowest Fe molar ratio was found in the cooked millets subsequently fermented overnight in the case of pearl millet and sorghum (4.12 and 5.97, respectively), whereas the cooked finger millet which was then fermented and added with curd seems to be better for the lowest phytic acid–Fe molar ratio (4.85).

Water-Soluble Vitamin Analysis

The effect of traditional processing on water-soluble vitamins in sorghum, pearl millet, and finger millet is presented in **Figures 4A–F**. Pearl millet was the chief source of vitamin B_2 (0.223 ± 0.018 mg/100 g) compared to the other millets. Among traditionally processed millets, vitamin B_2 was significantly ($p < 0.05$) higher in PMFC (0.173 ± 0.002 mg/100 g), followed by SMFW (0.136 ± 0.039 mg/100 g) and FMFWC (0.115 ± 0.012 mg/100 g). However, maximum B_2 retention was seen in SMFW (64.4%). Vitamin B_3 content was higher in sorghum (2.588 ± 0.112 mg/100 g) and lowest in finger millet (1.723 ± 0.108 mg/100 g). Vitamin B_3 content was reduced to 1.806 ± 0.132 mg/100 g in sorghum (SMFWC), with 59% retention, whereas B_3 was reduced to 1.349 ± 0.067 mg/100 g in pearl millet (PMFW), with 46% retention, and 0.629 ± 0.021 mg/100 g in finger millet (FMC), with 23% retention.

Vitamin B_5 was highest in the pearl millet (0.633 ± 0.021 mg/100 g) among the three raw millets analyzed. However, the processing reduced the B_5 content in all the millets. The cooked millet fermented overnight and then added with curd was found to have more vitamin B_5 compared to those under the other processes (SMFWC, 0.204 ± 0.008 mg/100 g; PMFWC, 0.411 ± 0.011 mg/100 g; FMFWC, 0.253 ± 0.015 mg/100 g). A maximum of 68% retention was observed in FMFWC, followed by 54% in SMFWC and 39% in PMFWC. Vitamin B_6 content in the raw and processed millets is illustrated in **Figure 4D**. Among the raw millets, highest B_6 content was seen in pearl millet (0.291 ± 0.015 mg/100 g) and the lowest in finger millet (0.071 ± 0.001 mg/100 g). The traditionally cooked millets have higher levels of B_6 (SMC, 0.041 ± 0.002 mg/100 g; PMC, 0.083 ± 0.004 mg/100 g; FMC, 0.028 ± 0.002 mg/100 g), with retention of 10.4, 19, and 29.7% in SMC, PMC, and FMS, respectively.

Quantification of vitamin B_9 by the U-HPLC technique in the raw and traditionally processed millets is shown in **Figure 4E**.

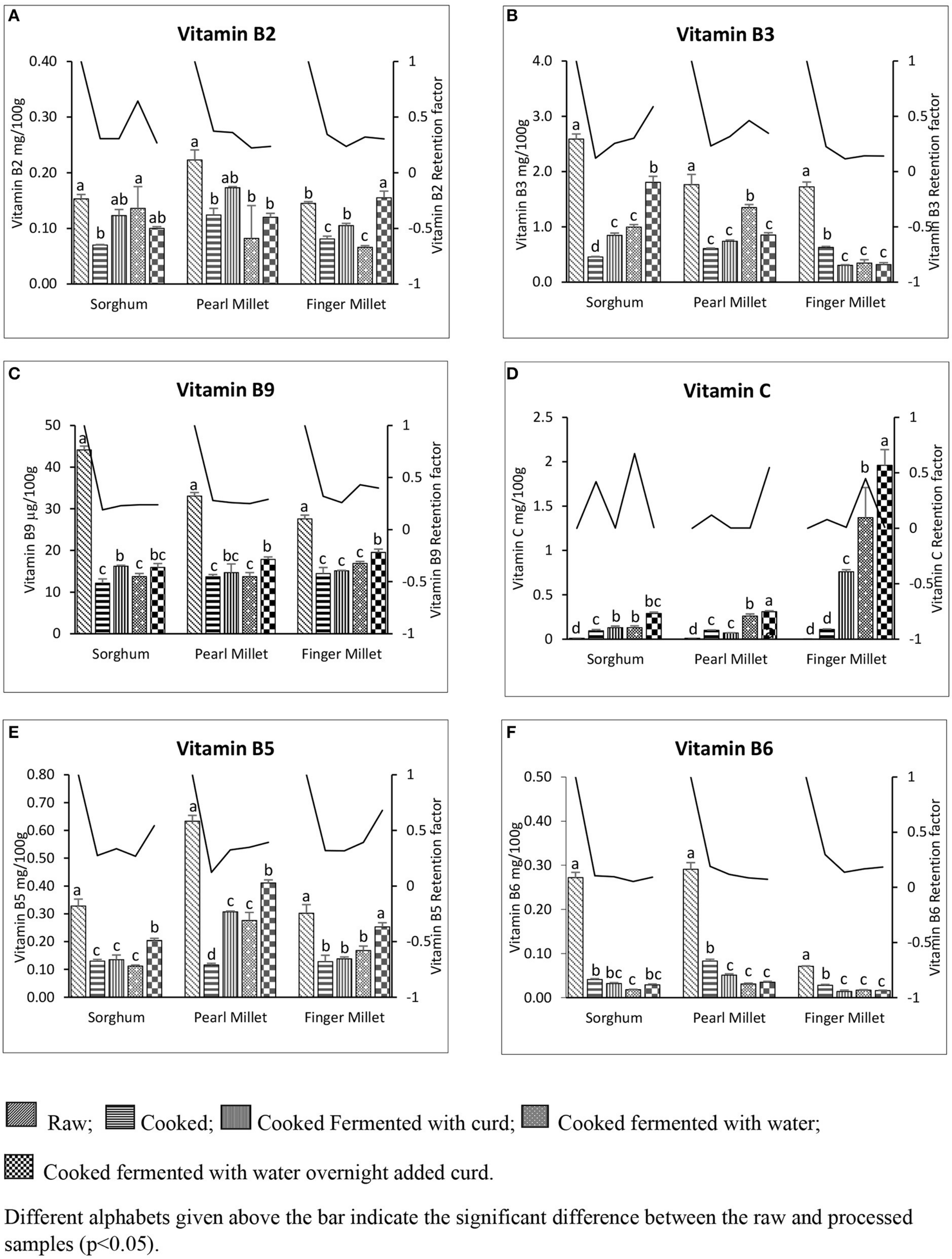

FIGURE 4 | Water-soluble vitamin concentration and its retention in the different raw and cooked millets. **(A)** Vitamin B2. **(B)** Vitamin B3. **(C)** Vitamin B5. **(D)** Vitamin B6. **(E)** Vitamin B9. **(F)** Vitamin C. Different alphabets given above the bar indicate the statistical difference between the raw and processed samples ($p < 0.005$).

TABLE 2 | Mineral content and phytic acid–mineral molar ratio of traditionally processed sorghum, pearl millet, and finger millet samples.

Sample name	Zinc	Iron	Phytic acid–mineral molar ratio	
	mg/100 g		PA/Zn	PA/Fe
Sorghum				
SMR	1.95 ± 0.01[b]	3.32 ± 0.12[b]	43.79	21.94
SMC	2.19 ± 0.11[b]	3.88 ± 0.12[b]	19.80	9.50
SMFC	2.55 ± 0.05[a]	6.09 ± 0.91[a]	15.22	5.81
SMFW	2.72 ± 0.05[a]	7.24 ± 0.52[a]	18.61	5.97
SMFWC	2.59 ± 0.02[a]	4.16 ± 0.04[b]	13.60	7.24
Pearl millet				
PMR	3.32 ± 0.15[a]	3.29 ± 0.19[c]	14.25	12.26
PMC	3.34 ± 0.13[a]	5.77 ± 0.34[b]	11.86	5.87
PMFC	3.51 ± 0.10[a]	6.75 ± 0.616[b]	10.56	4.73
PMFW	3.54 ± 0.04[a]	8.40 ± 0.334[a]	11.46	4.12
PMFWC	3.52 ± 0.02[a]	5.71 ± 0.215[b]	9.48	5.00
Finger millet				
FMR	2.09 ± 0.09[a]	5.15 ± 0.75[b]	26.98	9.34
FMC	2.21 ± 0.04[a]	6.87 ± 0.46[ba]	21.35	5.85
FMFC	3.19 ± 0.10[a]	7.81 ± 0.91[a]	14.19	4.95
FMFW	3.39 ± 0.738[a]	8.95 ± 0.63[a]	21.53	5.21
FMFWC	3.54 ± 0.231[a]	6.55 ± 0.64[ba]	10.48	4.85

Values represent mean ± standard deviation of triplicate analyses, and values with the same superscript within the column do not differ significantly at p < 0.05 by one-way ANOVA.

SMR, raw sorghum; SMC, sorghum cooked with water; SMFC, sorghum cooked and fermented with curd overnight; SMFW, sorghum cooked and fermented with water overnight; SMFWC, sorghum cooked and fermented with water overnight and added with curd; PMR, raw pearl millet; PMC, pearl millet cooked with water; PMFC, pearl millet cooked and fermented with curd overnight; PMFW, pearl millet cooked and fermented with water overnight; PMFWC, pearl millet cooked and fermented with water overnight and added with curd; FMR, raw finger millet; FMC, finger millet cooked with water; FMFC, finger millet cooked and fermented with curd overnight; FMFW, finger millet cooked and fermented with water overnight; FMFWC, finger millet cooked and fermented with water overnight and added with curd.

Vitamin B_9 (total folate) was more in sorghum (44.135 ± 1.30 μg/100 g), followed by pearl millet (33.309 μg/100 g) and finger millet (27.576 ± 1.23 μg/100 g). The processing was found to reduce the folate content significantly in all the millets studied. Maximum retention of 43% was found in FMFW (19.549 ± 1.08 μg/100 g) among the other processes in finger millets. The cooked millet samples fermented overnight and then added with curd were found to have maximum retention in pearl millet (29%) and sorghum (24%), with folate content of 17.852 ± 0.79 and 16.216 ± 0.28 μg/100 g, respectively. Analyses of vitamin C in the three raw samples and traditionally processed millets are illustrated in **Figure 4F**. The vitamin C (total ascorbic acid) content in raw millet was found to be below the detectable limit. However, a quantifiable amount of vitamin C was recorded in all the processed millets. The highest vitamin C content was found in the millets cooked and fermented overnight and then added with curd. Finger millet had the highest vitamin C content (FMFWC, 1.96 ± 0.18 mg/100 g), followed by pearl millet (PMFWC, 0.311 mg/100 g) and sorghum (SMFWC, 0.288 mg/100 g).

DISCUSSION

Millets are nutritionally enriched crops which require less maintenance in terms of water, fertilizers, pesticides, etc., compared to other grains and provide reliable harvest. Due to these characteristics, millets are termed next-generation crops (Saleh et al., 2013; Devi et al., 2014). However, it was hypothesized that millets contain phytic acid, which makes them nutritionally inferior in terms of mineral availability. All cereal grains contain phytic acid, which is mainly concentrated in the bran layer, except for maize where 80% was found in the germ. It was greatly emphasized that phytic acid can be reduced by milling, cooking, germination, fermentation, etc.—for example, average phytic acid content in brown rice was estimated to be between 541 and 742 mg/100 g, which can be reduced to 37–64% by milling and a further 20% by cooking (Okazaki and Katayama, 2005; Gupta et al., 2015; Longvah et al., 2017). As high as 742 mg/100 g of phytic acid has been reported in brown rice collected all over India. Reduction of phytic acid content in millets is a great challenge of this hour. Traditional processing/cooking may prove to be a better and simple way in reducing phytic acid content, hence increasing the mineral bioavailability. The present study aimed to investigate the effect of traditional processing on the nutrient and anti-nutrient (phytic acid) components of three major millets, namely, sorghum, pearl millet, and finger millet. The results showed that cooking reduced the protein content in SMC (9.91 ± 0.18) and PMC (9.30 ± 0.06 g/100 g), which may be due to the leaching of soluble nitrogen into the desired solution (water) (Njoki et al., 2014). Protein content was found to be significantly ($p < 0.05$) higher in cooked millet samples fermented overnight in water and then added with curd (SMFWC, PMFWC, and FMFWC), followed by the samples fermented directly with curd (SMFC, PMFC, and FMFC), as the fermentation process tend to increase the digestibility of protein. Similar findings were reported by Mallasy et al. (2011), where the protein digestibility increased significantly ($p < 0.05$) from 56.03 to 83.65% in pearl millet supplemented with whey protein and fermented for a period of 14 h. Mariod et al. (2016) and Osman (2011) also demonstrated increased protein content in sorghum and pearl millet due to microbial fermentation.

The moisture content of raw millet was comparable to the reported values of Afify et al. (2012) and Longvah et al. (2017). However, a study conducted by Kulthe et al. (2016) reported a slightly higher moisture content (11.78 g/100 g) in pearl millet. It was observed that the moisture content in all the cooked samples (SMC, FMC, and PMC) increased significantly ($p < 0.05$) due to the addition of water during cooking (**Table 1**). The addition of water/curd for overnight fermentation further increases the moisture content of all the samples. Ogodo et al. (2019) described that, with increases in fermentation time, moisture content increases in sorghum, which is attributed to the addition of water to the substrate prior to fermentation. Ojokoh et al. (2015) also revealed a higher moisture content of fermented pearl millet blends compared to unfermented samples. In our study, the highest moisture was found in SMFWC (88.80 ± 0.34 g/100 g), PMFWC (88.24 ± 0.34 g/100 g), and FMFWC (84.12 ± 0.38 g/100 g) because of added curd after overnight fermentation.

Ash content was found to be significantly ($p < 0.05$) highest in FMFWC (3.81 ± 0.05 g/100 g), PMFWC (2.61 ± 0.14 g/100 g), and SMFWC (2.56 ± 0.06 g/100 g), followed by FMFC (3.70 ± 0.04 g/100 g), PMFC (2.56 ± 0.04 g/100 g), and SMFC (2.36 ± 0.03 g/100 g). This can be attributed to the addition of curd in both treatments, whereas SMFW, PMFW, and FMFW showed no significant ($p < 0.05$) increase in ash content compared to the cooked and raw samples. Similarly, Pelig-Ba (2009) and Osman (2011) also observed no increase in ash content in fermented millet.

Among all the traditional processing methods, SMFC (0.90 ± 0.09), PMFC (1.43 ± 0.00), and FMFC (0.98 ± 0.01) showed a significantly ($p < 0.05$) high fat content due to the addition of curd. On the other hand, fat content was reduced in the processed millets since these were fermented only in water (SMFW, 0.62 ± 0.02 g/100 g; PMFW, 0.91 ± 0.03 g/100 g; FMFW, 0.64 ± 0.02 g/100 g). Similar results were reported by Sade (2009) in fermented pearl millet, where fat content was reduced after fermentation from 5.7 to 2.4 g/100 g. Mariod et al. (2016) also reported a reduction in fat content from 3.10 to 2.06 g/100 g in fermented sorghum. Fermentation also reduces the total carbohydrate content of the traditionally processed samples. Cooking did not reduce the carbohydrate content significantly ($p > 0.05$), whereas samples fermented and added with curd had significantly reduced carbohydrate ($p < 0.05$). Mariod et al. (2016) stated that microbial fermentation and baking of sorghum decreased the carbohydrate content, a finding which is parallel to that of the present study, where carbohydrate content decreased from 77.23 ± 0.21 (SM) to 66.44 ± 0.81 g/100 g (SMFWC). Nevertheless, an increased amount of IDF, SDF, and TDF was observed among all the fermented samples.

Phytic acid is the principal storage form of phosphorus in seeds which forms insoluble complexes with minerals, such as zinc, calcium, magnesium, and iron, thereby decreasing their bioavailability. The phytic acid content of raw sorghum, pearl millet, and finger millet was 8.6 ± 0.15, 4.77 ± 0.07, and 5.69 ± 0.19 mg/g, respectively. Makokha et al. (2002) and Netravati et al. (2017) also reported similar values in millets. It was reported that cooking the millets reduced the phytic acid content between 11.71 and 16.14%. The major reason for this reduction is the leaching of phytic acid during cooking and thermal degradation (Kataria et al., 1989; Sihag et al., 2015). Our study showed a significant reduction in phytic acid content at each level of treatment, which ranged from 3.16 to 62.9%. Among the traditionally processed sorghum, SMFWC showed the highest reduction of phytic acid at up to 62.9% (3.91 ± 0.05 mg/g). A similar trend in reduction of phytic acid was seen among traditionally processed pearl millets and finger millets, where PMFWC and FMFWC showed the highest values, i.e., 29.35% (3.37 mg/g) and 34.1% (3.75 ± 0.06 mg/g) reduction, respectively. As reported by Haard (1999) and Reale et al. (2007), fermentation provides optimum pH (created by the lactic acid bacteria due to lactic acid production) that activates the phytase enzyme, eventually leading to the degradation of phytic acid and increasing the solubility of minerals. The results obtained were similar to those reported in the literature in such a way that fermentation reduced phytic acid content by 50% (Towo et al., 2006; Kayode et al., 2007; Wedad et al., 2008; Osman, 2011). It was also reported that the reduction of phytic acid increases with an increase in fermentation time (Makokha et al., 2002).

The quantification of minerals revealed a significant increase in zinc and iron content in cooked samples, which are in contrast to the results of Borade et al. (1984) and Avola et al. (2012), who explained that pressure cooking leaches out minerals and the anti-nutritional factors from pearl millet grains resulted in a reduction of mineral content. Afify et al. (2012) demonstrated that fermentation decreased the zinc content from 4.43 to 3.29 mg/100 g in sorghum. The present study reveals a slight increase in zinc content after fermentation with curd, but which is not significant at $p < 0.05$. The iron content of millets also increases after fermentation, which is similar to the finding of Kindiki et al. (2015) that 24 h of fermentation considerably increases iron in pearl millet. Zinc and iron play a significant role in growth and development. Zinc is involved in cellular growth and differentiation. Its deficiency causes impaired growth, immune dysfunction, increased morbidity and mortality, adverse pregnancy outcomes, and abnormal neurobehavioral development. It was estimated that one-third of the population of the world are at a high risk of zinc deficiency and are living in low-income countries (Bagherani and Smoller, 2016). Similarly, iron deficiency also affects cognitive development, pregnancy, resistance to infection, work capacity, productivity, formation of heme proteins, and flavoproteins (Fairweather-Tait and Hurrell, 1996; Swaminathan et al., 2019). Minerals from plant sources have very low bioavailability because they form complexes with non-digestible materials, such as phytic acid (Torre et al., 1991). Fermentation is one of the traditional methods applied to free up these mineral complexes in order to make the minerals readily available (Pranoto et al., 2013).

The phytic acid–mineral molar ratios are used to estimate the negative effect of phytic acid on mineral bioavailability (Dahdouh et al., 2019). These are associated with mineral absorption capacity; the higher the molar ratio, the lower the mineral absorption. Phytic acid–zinc molar ratio <14 and phytic acid–iron molar ratio <1 indicate a positive effect on mineral bioavailability (**Table 2**). In the present study, the phytic acid–zinc and phytic acid–iron molar ratios for SMR, PMR, and FMR were initially higher than the stated values of 14 and 1. Similar findings were also reported by Netravati et al. (2017) in sorghum and finger millet. This ratio decreased progressively after the application of different processing methods, especially those involving fermentation (SMFWC, PMFC, PMFW, PMFWC, and FMFWC showed a PA–Zn molar ratio that was <14). This reduction in molar ratio was mainly due to the reduction in phytic acid content. A study conducted by Murali and Kapoor (2003) revealed that fermentation of finger millet with individual cultures of *Lactobacillus brevis*, *Lactobacillus fermentum*, and *Saccharomyces cerevisiae* for 24 and 48 h at 37°C resulted in significant reductions in phytic acid content, subsequently resulting in a lower phytic acid–zinc molar ratio. Nair and Iyengar (2009) reported that the low bioavailability of minerals is mainly attributed to the low mineral levels and the presence of high phytic acid content and other anti-nutritional factors. Thus, the bioavailability of minerals (iron and zinc) can be improved significantly by the application of different processing methods like soaking and fermentation (Norhaizan and Nor Faizadatul Ain, 2009; Afify et al., 2011).

Quantification of water-soluble vitamins revealed a significant ($p < 0.05$) increase in vitamin B_2, B_3, B_5, and B_9 among the fermented samples (PMFC, FMFC, SMFC, SMFWC, PMFWC, and FMFWC) compared to the cooked millets. Cooking (SMC, PMC, and FMC) reduced the vitamin B_2, B_3, B_5, and B_9 levels significantly ($p < 0.05$) compared to the raw and fermented samples due to thermal degradation, as vitamins are heat sensitive. Ekinci and Kadakal (2005) and Ochanda et al. (2010) also revealed a significant increase in B-complex vitamins after fermentation. It was reported that the fermentation of cereals with *Lactobacillus* or yeast strains could increase their vitamin content to a greater extent. These microorganisms could be used as a starter to improve the nutritional quality of food (Nyanzi and Jooste, 2012).

The vitamin C content was below the detectable limit in all the raw millet samples analyzed. These findings were similar to the results reported by Shobana et al. (2013), which showed that the vitamin C content ranged from 0.0 to 0.1 mg/100 g. This content increases significantly at each level of treatment. Nutrient retention factor was calculated for the analyzed water-soluble vitamins to estimate the amount of nutrients retained in foods after traditional processing, which ranged from 0 (no retention) to 1 (complete retention). Calculation of nutrient retention for water-soluble vitamins showed 63% (vitamin C), 64% (vitamin B_2), 58% (vitamin B_3), and 68% (vitamin B_5) retention in the processed millets. Nutrient retention factor depends on several factors, such as temperature, time, pressure, and many other cooking methods (Vásquez-Caicedo et al., 2008). The USDA (2007) reported that the cooking method of white rice in the US retained 90% of riboflavin, 95% of niacin, 90% pyridoxine, 60% of folic acid, and 75% of ascorbic acid. The retention was very high compared to the values for millets in the present study, and this may be due to the long cooking time and method of cooking. Our literature survey found no previous data on nutrient retention in millets during various processing methods.

CONCLUSION

In the present study, nutrient and anti-nutrient retention of raw and traditionally processed millets was investigated. The study revealed that phytic acid content was significantly reduced in traditionally processed sorghum. The sample that was traditionally cooked, fermented overnight, and then added with curd was found to have a reduced phytic acid content to a greater extent (62.9%), which means that the process may improve the bioavailability of minerals, especially iron and zinc. High retention of water-soluble vitamins, such as B_2 (64%), B_3 (58%), and B_5 (68%), was found in the processed millets. Further investigation on the bioavailability of iron and zinc in these traditionally processed millets is desirable in order to confirm the intake of iron and zinc in millet diets.

DATA AVAILABILITY STATEMENT

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

AUTHOR CONTRIBUTIONS

RA, TL, and CB contributed to the conception and design of the study and guided the study. HS and KS executed the experiments. HS and CB wrote the first draft of the manuscript and carried out the statistical analysis. All authors contributed to the preparation and revision of the manuscript.

FUNDING

This project was supported with institutional fund from ICMR-National Institute of Nutrition (17-FC02).

ACKNOWLEDGMENTS

The authors would like to express their gratitude to the Director, ICMR-Natational Institute of Nutrition, for the financial support and scientific encouragement to conduct this experiment at the said institution.

REFERENCES

Afify, A. E. M. M., El-Beltagi, H. S., Abd El-Salam, S. M., and Omran, A. A. (2011). Bioavailability of iron, zinc, phytate and phytase activity during soaking and germination of white sorghum varieties. *PLoS ONE* 6, e25512. doi: 10.1371/journal.pone.0025512

Afify, A. E. M. M., El-beltagi, H. S., Abd El-Salam, S. M., and Omran, A. A. (2012). Effect of soaking, cooking, germination and fermentation processing on proximate analysis and mineral content of three white sorghum varieties (*Sorghum bicolor* L. Moench). *Notulae Botanicae Horti Agrobotanici Cluj-Napoca*. 40, 92–98. doi: 10.15835/nbha4027930

Agte, V. V., Tarwadi, K. V., and Chiplonkar, S. A. (1999). Phytate degradation during traditional cooking: significance of the phytic acid profile in cereal-based vegetarian meals. *J. Food Composition Anal.* 12, 161–167. doi: 10.1006/jfca.1999.0826

Anderson, J. W., Hanna, T. J., Peng, X., and Kryscio, R. J. (2000). Whole grain foods and heart disease risk. *J. Am. Coll. Nutr.* 19, 291–299. doi: 10.1080/07315724.2000.10718963

Anitha, S., Kane-Potaka, J., Tsusaka, T.W., Botha, R., Rajendran, A., Givens, D.I., et al. (2021). A systematic review and meta-analysis of the potential of millets and sorghum for managing and preventing diabetes mellitus. *Front. Nutr.* 8, 687428. doi: 10.3389/fnut.2021.687428

AOAC (2016). *Official Methods of Analysis*. 20th Edn. Gaithersburg, MD: The Association of Official Analytical Chemists.

Avola, G., Patanè, C., and Barbagallo, R. N. (2012). Effect of water cooking on proximate composition of grain in three Sicilian chickpeas (*Cicer arietinum* L.). *LWT Food Sci. Technol.* 49, 217–220. doi: 10.1016/j.lwt.2012.07.004

Bagherani, N., and Smoller, B. R. (2016). An overview of zinc and its importance in dermatology-Part I: importance and function of zinc in human beings. *Glob. Dermatol.* 3, 330–336. doi: 10.15761/GOD.1000185

Bhagavatula, S., Rao, P. P., Basavaraj, G., and Nagaraj, N. (2013). *Sorghum and Millet Economies in Asia – Facts, Trends and Outlook*. Patancheruvu, Andhra Pradesh: International Crops Research Institute for the Semi-Arid Tropics.

Bhat, S., Nandini, C., and Tippeswamy, V. (2018). Significance of small millets in nutrition and health -A review. *Asian J. Diary Food Res.* 37, 35–40. doi: 10.18805/ajdfr.DR-1306

Binita, R., and Khetarpaul, N. (1997). Probiotic fermentation: effect on antinutrients and digestibility of starch and protein of indigenously developed food mixture. *Nutr. Health*. 11, 139–147. doi: 10.1177/026010609701100301

BNF (2004). *Nutritional Aspects of Cereals*. London: BNF.

Borade, V. P., Kadam, S. S., and Salunkhe, D. K. (1984). Changes in phytate phosphorus and minerals during germination and cooking of horse gram and moth bean. *Plant Foods Hum Nutr*. 34, 151–157. doi: 10.1007/BF01094843

Brouwer, V. D., Storozhenko, S., Steene, J. C. V. D., Wille, S. M. R., Stove, C. P., Straeten, D. V. D., et al. (2008). Optimisation and validation of a liquid chromatography – tandem mass spectrometry method for folates in rice. *J Chromatogr A*. 1215, 125–132. doi: 10.1016/j.chroma.2008.11.004

Chapke, R. R., Shyamprasad, G., and Das, I. K. (2018). *Improved Millets Production Technologies and Their Impact*. Technology Bulletin. Hyderabad: ICAR-Indian Institute of Millets Research.

Dahdouh, S., Grande, F., Espinosa, S. N., Vincent, A., Gibson, R., Bailey, K., et al. (2019). Development of the FAO/INFOODS/IZINCG global food composition database for phytate. *J Food Composition Anal.* 78, 42–48. doi: 10.1016/j.jfca,.2019.01.023

Devi, P. B., Vijayabharathi, R., Sathyabama, S., Malleshi, N. G., and Priyadarisini, V. B. (2014). Health benefits of finger millet (*Eleusine coracana* L.) polyphenols and dietary fiber: a review. *J. Food Sci. Technol*. 51, 1021–1040. doi: 10.1007/s13197-011-0584-9

Ekinci, R., and Kadakal, C. (2005). Determination of seven water-soluble vitamins in tarhana, a traditional Turkish cereal food, by high-performance liquid chromatography. *Acta Chromatogr*. 15, 289.

Fairweather-Tait, S., and Hurrell, R. F. (1996). Bioavailability of minerals and trace elements: members of EC flair concerted action no. 10: Measurements of micronutrient absorption and status. *Nutr. Res. Rev.* 9, 295–324. doi: 10.1079/NRR19960016

FAO/WHO (2001). *Human Vitamin and Mineral Requirements*. Report of a joint FAO/WHO expert consultation Bangkok. Thailand: FAO/WHO.

FAOSTAT (2019). *Millet Production*. Available online at: http://www.fao.org/faostat/en/#data/QC (accessed November 5, 2021).

Gupta, R. K., Gangoliya, S. S., and Singh, N. K. (2015). Reduction of phytic acid and enhancement of bioavailable micronutrients in food grains. *J. Food Sci. Technol.* 52, 676–684. doi: 10.1007/s13197-013-0978-y

Guttieri, M., Bowen, D., Dorsch, J. A., Raboy, V., and Souza, E. (2004). Identification and characterization of a low phytic acid wheat. *Crop Sci*. 44, 418–424. doi: 10.2135/cropsci2004.4180

Haard, N. F. (1999). *Fermented Cereals: A Global Perspective. FAO Agricultural Services Bulletin 138*. Rome: Food and Agriculture Organisation.

Hernandez, Y., Lobo, G. M., and Gonzalez, M. (2006). Determination of vitamin C in tropical fruits: a comparative evaluation of methods. *Food Chem*. 96, 654–664. doi: 10.1016/j.foodchem.2005.04.012

Jacobs, D. R., Meyer, K. A., Kushi, L. H., and Folsom, A. R. (1998). Whole grain intake may reduce risk of coronary heart disease death in postmenopausal women: the Iowa Women's Health Study. *Am. J. Clin. Nutr*. 68, 248–257. doi: 10.1093/ajcn/68.2.248

Jacobs, D. R., Slavin, J., and Marquart, L. (1995).Whole grain intake and cancer: a review of literature. *Nutr. Cancer* 22, 221–229. doi: 10.1080/01635589509514411

Kataria, A., Chauhan, B. M., and Punia, D. (1989). Antinutrients and protein digestibility (*in vitro*) of mung bean as affected by domestic processing and cooking. *Food Chem*. 32, 9–17. doi: 10.1016/0308-8146(89)90003-4

Kayode, A. P. P., Hounhouigan, J. D., and Nout, M. J. R. (2007). Impact of brewing process operations on phytate, phenolic compounds and *in vitro* solubility of iron and zinc in opaque sorghum beer. *LWT Food Sci Technol*. 40, 834–841. doi: 10.1016/j.lwt.2006.04.001

Kindiki, M. M., Onyango, A., and Kyalo, F. (2015). Effects of processing on nutritional and sensory quality of pearl millet flour. *Food Sci. Qual. Manage.* 42, 13–19.

Kulthe, A. A., Thorat, S. S., and Lande, S. B. (2016). Characterization of pearl millet cultivars for proximate composition, minerals and anti-nutritional contents. *Adv. Life Sci*. 5, 4672–4675.

Liang, J., Han, B. Z., Nout, M. R., and Hamer, R. J. (2008). Effects of soaking, germination and fermentation on phytic acid, total and *in vitro* soluble zinc in brown rice. *Food Chem*. 110, 821–828. doi: 10.1016/j.foodchem.2008.02.064

Liu, S. (2002). A prospective study of dietary fiber intake and risk of cardiovascular disease among women. *J. Am. Coll. Cardiol.* 39, 49–56. doi: 10.1016/S0735-1097(01)01695-3

Liu, S., Manson, J. E., Stampfer, M. J., Hu, F. B., Gio vanucci, E., Colditz, G.A., et al. (2000). A prospective study of whole grain intake and risk of type 2 diabetes mellitus in US women. *Am. J. Public Health*. 90, 1409–1415. doi: 10.2105/AJPH.90.9.1409

Liu, S., Stampfer, M. J., Hu, F. B., Giovanucci, E., Rimm, E., Manson, J. E., et al. (1999). Whole grain consumption and risk of coronary heart disease: results from the nurses' health study. *Am. J. Clin. Nutr*. 70, 412–419. doi: 10.1093/ajcn/70.3.412

Longvah, T., Ananthan, R., Bhaskarachary, K., and Venkaiah, K. (2017). *Indian Food Composition Tables*. Hyderabad: National Institute of Nutrition, Indian Council of Medical Research, 2–58.

Lönnerdal, B. O., Sandberg, A. S., Sandström, B., and Kunz, C. (1989). Inhibitory effects of phytic acid and other inositol phosphates on zinc and calcium absorption in suckling rats. *J. Nutr*. 119, 211–214. doi: 10.1093/jn/119.2.211

Lopez, H. W., Leenhardt, F., Coudray, C., and Remesy, C. (2002). Minerals and phytic acid interactions: is it a real problem for human nutrition? *Int. J. Food Sci. Technol*. 37, 727–739. doi: 10.1046/j.1365-2621.2002.00618.x

Makokha, A. O., Oniang'o, R. K., Njoroge, S. M., and Kamar, O. K. (2002). Effect of traditional fermentation and malting on phytic acid and mineral availability from sorghum (*Sorghum bicolor*) and finger millet (*Eleusine coracana*) grain varieties grown in Kenya. *Food Nutr. Bull*. 23, 241–245. doi: 10.1177/15648265020233S147

Mallasy, L. O., El Tinay, A. H., Elkhalifa, A. O., Mohiedeen, I. E., Ali, M. A. M., and Yousif, N. E. (2011). Effect of fermentation and cooking on protein characteristics of pearl millet flour supplemented with whey protein. *Electron. J. Environ. Agric. Food Chem*. 10, 2057–2064.

Mariod, A. A., Idris, Y. M., Osman, N. M., Mohamed, M. A., Sukrab, A. M., Farag, M. Y., et al. (2016). Three Sudanese sorghum-based fermented foods (kisra, hulu-mur and abreh): comparison of proximate, nutritional value, microbiological load and acrylamide content. *Ukrainian J. Food Sci*. 4, 216–228. doi: 10.24263/2310-1008-2016-4-2-4

Meyer, K. A., Kushi, L. H., Jacobs, D. R. J., Slavin, J., Sellers, T. A., and Folsom, A. R. (2000). Carbohydrates, dietary fiber, incident type 2 diabetes mellitus in older women. *Am. J. Clin. Nutr*. 71, 921–930. doi: 10.1093/ajcn/71.4.921

Muniappan, K., Raghavan, V., Nachimuthu, V., Raveendran, M., Panaiyuran, S., Vediyappan, V., et al. (2018). *Scaling Up Small Millet Post-harvest and Nutritious Food Products Project*. Technical report. CIFSRF, Canada. Available online at: http://hdl.handle.net/10625/57029 (accessed February 15, 2021).

Murali, A., and Kapoor, R. (2003). Effect of natural and pure culture fermentation of finger millet on zinc availability as predicted from HCl extractability and molar ratios. *J. Food Sci. Technol.* 40, 112–114.

Nair, K. M., and Iyengar, V. (2009). Iron content, bioavailability & factors affecting iron status of Indians. *Indian J. Med. Res.* 130, 634–645.

Netravati, H., Geetha, K., Vikram, S.R., and Reddy, Y.A.N. (2017), Effect of household germination on phytic acid and iron in finger millet [*Eleusine coracana* (L.) Gaertn]. *Indian J. Pure Appl. Biosci.* 5, 958–963. doi: 10.18782/2320-7051.5740

Nicodemus, K. K., Jacobs, D. R. J., and Folsom, A. R. (2001).Whole and refined grain intake and risk of incident postmenopausal breast cancer. *Cancer Causes Control* 12, 917–925. doi: 10.1023/A:1013746719385

Njoki, J. W., Sila, D. N., and Onyango, A. N. (2014). Impact of processing techniques on nutrient and anti-nutrient content of grain amaranth (*Amaranth albus*). *Food Sci. Qual. Manage*. 25, 10–17.

Norhaizan, M. E., and Nor Faizadatul Ain, A. W. (2009). Determination of phytate, iron, zinc, calcium contents and their molar ratios in commonly consumed raw and prepared food in Malaysia. *Malays. J. Nutr*. 15, 213–222.

NSSO (2012). *Consumption expenditure survey (68th round), International Household Survey Network Schedule 1.0 (Type 1)*. India.

Nyanzi, R., and Jooste, P. J. (2012). "Cereal-based functional foods", in *Probiotics*, ed E. Rigobelo (Rijeka: InTech Open).

Ochanda, S. O., Akoth, O. C., Mwasaru, A. M., Kagwiria, O. J., and Mathooko, F. M. (2010). Effects of malting and fermentation treatments on group B-vitamins of red sorghum, white sorghum and pearl millets in Kenya. *J. Appl. Biosci.* 34, 2128–2134.

Ogodo, A. C., Ugbogu, O. C., Onyeagba, R. A., and Okereke, H. C. (2019). Microbiological quality, proximate composition and *in vitro* starch/protein digestibility of *Sorghum bicolor* flour fermented with lactic acid bacteria consortia. *Chem. Biol. Technol. Agric*. 6, 1–9. doi: 10.1186/s40538-019-0145-4

Ojokoh, A. O., Fayemi, O. E., Ocloo, F. C. K., and Nwokolo, F. I. (2015). Effect of fermentation on proximate composition, physicochemical and microbial characteristics of pearl millet (*Pennisetum glaucum* (L.) R. Br.) and Acha (Digitaria exilis (Kippist) Stapf) flour blends. *J. Agric. Biotechnol. Sustain. Dev*. 7, 1–8. doi: 10.5897/JABSD2014.0236

Okazaki, Y., and Katayama, T. (2005). Reassessment of the nutritional function of phytic acid, with special reference to myo-inositol function. *J. Jpn. Soc. Nutr. Food Sci.* 58, 151–156. doi: 10.4327/jsnfs.58.151

Osman, M. A. (2011). Effect of traditional fermentation process on the nutrient and antinutrient contents of pearl millet during preparation of Lohoh. *J. Saudi Soc. Agric. Sci*. 10, 1–6. doi: 10.1016/j.jssas.2010.06.001

Pelig-Ba, K. B. (2009). Effect of ash, KOH and millet on the fermentation of *Parkia biglobosa* seeds to form a condiment. *Pakistan J. Nutr.* 8, 1548–1554. doi: 10.3923/pjn.2009.1548.1554

Phillips, K. M., Tarrag'o-Trani, M. T., Gebhardt, S. E., Exler, J., Patterson, K. Y., Haytowitz, D. B., et al. (2010). Stability of vitamin C in frozen raw fruit and vegetable homogenates. *J. Food Composition Anal*. 23, 253–259. doi: 10.1016/j.jfca.2009.08.018

Pranoto, Y., Anggrahini, S., and Efendi, Z. (2013). Effect of natural and *Lactobacillus plantarum* fermentation on *in-vitro* protein and starch digestibilities of sorghum flour. *Food Biosci*. 2, 46–52. doi: 10.1016/j.fbio.2013.04.001

Rader, J. I., Weaver, C. M., and Angyal, G. (1998). Use of a microbial assay with tri-enzyme extraction for measurement of pre-fortification levels of folates in enriched cereal grain products. *Food Chem*. 62, 451–465. doi: 10.1016/S0308-8146(98)00089-2

Rao, P. P., and Basavaraj, G. (2015). *Status and Prospects of Millet Utilization in India and Global Scenario (pp-197-209)*. Hyderabad: ICAR-Indian Institute of Millets Research.

Rao, P. P., Birthal, P. S., Reddy, B. V., Rai, K. N., and Ramesh, S. (2006). Diagnostics of sorghum and pearl millet grains-based nutrition in India. *Int. Sorghum Millets Newslett*. 47, 93–96.

Reale, A., Konietzny, U., Coppola, R., Sorrentino, E., and Greiner, R. (2007). The importance of lactic acid bacteria for phytate degradation during cereal dough fermentation. *J. Agric. Food Chem*. 55, 2993–2997. doi: 10.1021/jf063507n

Reddy, N. R., and Sathe, S. K. (2001). *Food Phytates*. New York, NY: CRC Press.

Sade, F. O. (2009). Proximate, antinutritional factors and functional properties of processed pearl millet (*Pennisetum glaucum*). *J. Food Technol*. 7, 92–97.

Saleh, A. S. M., Zhang, Q., Chen, J., and Shen,. Q. (2013). Millet grains: nutritional quality, processing, and potential health benefits. *Compreh. Rev. Food Sci. Food Saf*. 12, 281–295. doi: 10.1111/1541-4337.12012

Shobana, S., Krishnaswamy, K., Sudha, V., Malleshi, N. G., Anjana, R. M., Palaniappan, L., et al. (2013). Finger millet (Ragi, *Eleusine coracana* L.): a review of its nutritional properties, processing, and plausible health benefits. *Adv. Food Nutr. Res*. 69, 1–39. doi: 10.1016/B978-0-12-410540-9.00001-6

Sihag, M. K., Sharma, V., Goyal, A., Arora, S., and Singh, A. K. (2015). Effect of domestic processing treatments on iron, β-carotene, phytic acid and polyphenols of pearl millet. *Cogent Food Agric*. 1, 1–12. doi: 10.1080/23311932.2015.1109171

Swaminathan, S., Hemalatha, R., Pandey, A., Kassebaum, N. J., Laxmaiah, A., Longvah, T., et al. (2019). The burden of child and maternal malnutrition and trends in its indicators in the states of India: the Global Burden of Disease Study 1990-2017. *Lancet Child Adolesc. Health* 3, 855–870. doi: 10.1016/S2352-4642(19)30273-1

Thompson, L. U. (1993). Potential health benefits and problems associated with antinutrients in foods. *Food Res. Int*. 26, 131–149. doi: 10.1016/0963-9969(93)90069-U

Torre, M., Rodriguez, A. R., and Saura-Calixto, F. (1991). Effects of dietary fiber and phytic acid on mineral availability. *Crit. Rev. Food Sci. Nutr*. 30, 1–22. doi: 10.1080/10408399109527539

Towo, E., Matuschek, E., and Svanberg, U. (2006). Fermentation and enzyme treatment of tannin sorghum gruels: effects on phenolic compounds, phytate and *in vitro* accessible iron. *Food Chem.* 94, 369–376. doi: 10.1016/j.foodchem.2004.11.027

USDA (2007). *Table of Nutrient Retention Factors, Release 6, Beltsville Human Nutrition Research Center (BHNRC), Agricultural Research Service* (ARS), U.S. Department of Agriculture.

Valls, F., Sancho, M. T., Fernandez-Muino, M. A., and Checa, M. A. (2001). Determination of vitamin B6 in cooked sausages. *J. Agric. Food Chem*. 49, 38–41. doi: 10.1021/jf0003202

Vásquez-Caicedo, A. L., Bell, S., and Hartmann, B. (2008). *Report on Collection of Rules on Use of Recipe Calculation Procedures Including the Use of Yield and Retention Factors for Imputing Nutrient Values for Composite Foods*. Brussels: European Food Information Resource Network.

Vats, P., and Banerjee, U. C. (2004). Production studies and catalytic properties of phytases (myo-inositolhexakis phosphate phosphohydrolases): an overview. *Enzyme Microb. Technol*. 35, 3–14. doi: 10.1016/j.enzmictec.2004.03.010

Wallace, M., and Singh, S. K. (2019). *Assessments of Commodity and Trade Issues by USDA*. Washington, DC: USDA Foreign Agricultural Service. 11p.

Wedad, H. A., El Tinay, A. H., Mustafa, A. I., and Babiker, E. E. (2008). Effect of fermentation, malt-pretreatment and cooking on antinutritional factors and protein digestibility of sorghum cultivars. *Pakistan J. Nutr.* 7, 335–341. doi: 10.3923/pjn.2008.335.341

Woollard, D. C., Indyk, H. E., and Christiansen, S. K. (2000). The analysis of pantothenic acid in milk and infant formulas by HPLC. *Food Chem*. 69, 201–208. doi: 10.1016/S0308-8146(99)00255-1

Conflict of Interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

frontiers in Nutrition

ORIGINAL RESEARCH
published: 02 February 2022
doi: 10.3389/fnut.2021.746625

Association of Grain Iron and Zinc Content With Other Nutrients in Pearl Millet Germplasm, Breeding Lines, and Hybrids

Mahalingam Govindaraj[1,2]*, *Anand Kanatti*[1], *Kedar Nath Rai*[1], *Wolfgang H. Pfeiffer*[3] and *Harshad Shivade*[1]

[1] International Crops Research Institute for the Semi-arid Tropics (ICRISAT), Patancheru, India, [2] Alliance of Bioversity International and the International Center for Tropical Agriculture (CIAT), Cali, Colombia, [3] HarvestPlus Program, International Food Policy Research Institute (IFPRI), Washington, DC, United States

Micronutrient deficiency is most prevalent in developing regions of the world, including Africa and Southeast Asia where pearl millet (*Pennisetum glaucum* L.) is a major crop. Increasing essential minerals in pearl millet through biofortification could reduce malnutrition caused by deficiency. This study evaluated the extent of variability of micronutrients (Fe, Zn, Mn, and Na) and macronutrients (P, K, Ca, and Mg) and their relationship with Fe and Zn content in 14 trials involving pearl millet hybrids, inbreds, and germplasm. Significant genetic variability of macronutrients and micronutrients was found within and across the trials (Ca: 4.2–40.0 mg 100 g^{-1}, Fe: 24–145 mg kg^{-1}, Zn: 22–96 mg kg^{-1}, and Na: 3.0–63 mg kg^{-1}). Parental lines showed significantly larger variation for nutrients than hybrids, indicating their potential for use in hybrid parent improvement through recurrent selection. Fe and Zn contents were positively correlated and highly significant ($r = 0.58$–0.81; $p < 0.01$). Fe and Zn were positively and significantly correlated with Ca ($r = 0.26$–0.61; $p < 0.05$) and Mn ($r = 0.24$–0.50; $p < 0.05$). The findings indicate that joint selection for Fe, Zn, and Ca will be effective. Substantial genetic variation and high heritability (>0.60) for multiple grain minerals provide good selection accuracy prospects for genetic enhancement. A highly positive significant correlation between Fe and Zn and the nonsignificant correlation of grain macronutrients and micronutrients with Fe and Zn suggest that there is scope to achieve higher levels of Fe/Zn simultaneously in current pearl millet biofortification efforts without affecting other grain nutrients. Results suggest major prospects for improving multiple nutrients in pearl millet.

Keywords: biofortification, iron, macronutrients, micronutrients, pearl millet, zinc

OPEN ACCESS

Edited by:
Stefania Grando, Consultant, Ascoli Piceno, Italy

Reviewed by:
Sapna Langyan, National Bureau of Plant Genetic Resources (ICAR), India
Henry Fred Ojulong, ICRISAT, Kenya

***Correspondence:**
Mahalingam Govindaraj
m.govindaraj@cgiar.org

Specialty section:
This article was submitted to Nutrition and Sustainable Diets, a section of the journal Frontiers in Nutrition

Received: 24 July 2021
Accepted: 31 December 2021
Published: 02 February 2022

Citation:
Govindaraj M, Kanatti A, Rai KN, Pfeiffer WH and Shivade H (2022) Association of Grain Iron and Zinc Content With Other Nutrients in Pearl Millet Germplasm, Breeding Lines, and Hybrids. Front. Nutr. 8:746625. doi: 10.3389/fnut.2021.746625

INTRODUCTION

Micronutrient malnutrition affects more than two billion people worldwide (1–4). The most prevalent forms of malnutrition are those arising from deficiencies of iron (Fe), zinc (Zn), vitamin A, and iodine (I), which occur particularly among women and children in developing countries. In these countries, more than 40% of preschool children are stunted because of Zn deficiency, whereas 30% of preschool children are anemic because of Fe deficiency (5, 6). For instance, India loses about 4 million children every year to disability-adjusted life years (DALYs) caused by Fe deficiency or anemia (7), with another 2.8 million children lost to DALYs because of stunted growth caused by Zn deficiency (8, 9). Humans require more than 40 nutrients that are essential to meet the metabolic needs of the body including proteins, lipids, macronutrients, micronutrients, and vitamins. Inadequate consumption of any of these will result in adverse metabolic disturbances, leading to sickness, poor health, impaired development in children, and a large economic cost to society (1). Men and women aged between 25 and 50 years require a daily intake of 800 mg of calcium (Ca) and phosphorus (P), 280–350 mg of magnesium (Mg), 2,000 mg of potassium (K), 10–15 mg of Fe and Zn, 2–5 μg of manganese (Mn), and 500 mg of sodium (Na) to meet the Recommended Dietary Allowance (RDA) (10–12). This is reason enough for developing public health policies that encourage the consumption of micronutrients at the RDA levels. Evidence suggests that the main cause of hidden hunger in developing countries is the unavailability of essential minerals in staple diets, particularly those comprising cereal-based foods that are inherently low in micronutrients such as Fe, Zn, and vitamin A (13–16). Efforts are underway to breed for increased Fe, Zn, and vitamin A content (17, 18). The agronomic or genetic enhancement of essential micronutrients and vitamins in edible parts of staple food crops is called biofortification. Genetic biofortification is a one-time investment and has no genetic erosion such as the dwarfing genes that catalyzed the Green Revolution in wheat and rice. Biofortification breeding is currently limited to a few crops, including iron-fortified pearl millet and beans, zinc-fortified wheat, rice, and maize, and vitamin A-fortified orange sweet potato, cassava, and maize (18) (www.harvestplus.org). There are biofortification initiatives in other crops such as lentils (19).

Pearl millet is grown on 26 million ha globally, of which 7.4 m ha are in the most marginal arid and semiarid tropical regions of India, particularly in Maharashtra, Rajasthan, Gujarat, and Uttar Pradesh states (20). It is an important staple food for millions of people and a major source of dietary energy and nutritional security for the vast rural communities in these regions (21). Pearl millet is also the cheapest source of not only energy and protein but also of Fe and Zn (22). Given its high nutritional value, pearl millet can contribute significantly to improve the nutritional status of millions. However, all the released and commercially grown pearl millet cultivars have low levels of micronutrients, especially low Fe (42 mg kg^{-1}) and Zn (32 mg kg^{-1}) (23). A few studies (24, 25) have reported crop breeding efforts that have significantly contributed to improving grain yield in commercial cultivars, but with reduced grain nutrient concentrations compared to landraces. The International Crops Research Institute for the Semi-Arid Tropics (ICRISAT) initiated biofortification research under the umbrella of the HarvestPlus Challenge Program of the CGIAR, to develop high-Fe open-pollinated varieties (OPVs), improved breeding lines, and hybrid parents for high Fe and Zn contents. Wide variability for Fe and Zn contents and their genetic inheritance are well documented (26, 27). These two micronutrients are governed by additive-effect genes (23, 26, 28). Among the micronutrients, Fe and Zn can be significantly improved through biofortification breeding. While the pattern of association between Fe and Zn is being studied, the association of these two traits with other important macro- and micronutrients has not been studied extensively in sizeable pearl millet breeding materials. As part of the HarvestPlus-supported biofortification program, this study assessed the available variability for grain micronutrients (Mn, Na) and macronutrients (P, K, Ca, and Mg) and their relationship with Fe and Zn content (current biofortification target nutrients) in different pearl millet breeding trials, including germplasm accessions, hybrid parents, and commercial cultivars to develop cultivars with improved iron and zinc content.

MATERIALS AND METHODS

Field Trial

This study consisted of 928 entries in 14 replicated trials during the 2012–2013 crop season in India. The details of the experimental materials and trials are given in **Table 1**. All these field trials were evaluated during the rainy season using a randomized complete block design with two replications (trials 7 and 8 were replicated thrice) in an Alfisol precision field at ICRISAT, Hyderabad, India (latitude: 17.51° N, longitude: 78.27° E, altitude: 545 m) (**Table 1**). Entries in trials 1–4 were planted in two rows of 4 m-long plots. Entries in the remaining trials were planted in one-row 2 m-long plots, with an interrow spacing of 75 cm and intrarow spacing of 15 cm. In all the field trials, fertilizer was applied as per standard recommendations for the site to maintain good soil fertility of the experimental fields to ensure trial precision. Open-pollinated main panicles of five random plants with good seed sets were harvested from each plot at or after physiological maturity in all the trials. The harvested panicles were sun-dried on a tarpaulin sheet for 12–15 days, stored in cloth bags, hand threshed, and the grains were divested of glumes and foreign matter, if any, to produce grain samples for laboratory analyses.

Mineral Estimation

Grain macronutrients such as P, K, Ca, and Mg and micronutrients such as Fe, Zn, Mn, and Na were analyzed following the methods described by Wheal et al. (29) at Waite Analytical Laboratory, Adelaide University, Australia. Grain samples were finely ground and oven-dried at 60°C for 48 h before analyzing their nutrient content. This help to reduce the uniform moisture of the grain samples at ∼12%. The ground samples (0.2 g) were transferred to 25 ml polypropylene Plasma Preparation Tube (PPT) tubes and digestion was initiated by adding 2.0 ml of concentrated nitric acid (HNO_3) and 0.5 ml of 30% hydrogen peroxide (H_2O_2). Tubes were vortexed to ensure the entire sample was wetted and then predigested overnight at room temperature. Tubes were vortexed again before being placed in the digestion block. They were initially heated at 80°C for 1 h followed by digestion at 120°C for 2 h. After digestion, the volume of the digest was brought to 25 ml using distilled water and the content was agitated for a minute in the

TABLE 1 | Study materials evaluated in 14 trials and remarks.

Trial No.	Name of the trial	No. of entries	No. of replications	Remarks
Trial-1	Commercial hybrid trial	40	2	Released and commercially grown hybrids
Trial-2	Hybrid trial-1	39	2	Pipeline hybrids
Trial-3	Hybrid trial-2	36	2	Pipeline hybrids
Trial-4	Hybrid trial-3	28	2	Pipeline hybrids
Trial-5	Hybrid trial-4	30	2	Pipeline hybrids
Trial-6	Released cultivar trial	130	2	Released cultivars at the national level since 1970s
Trial-7	Hybrid parental trial-1	45	3	Inbred/hybrid parents
Trial-8	Hybrid parental trial-2	40	3	Inbred/hybrid parents
Trial-9	Testcross parental trial-1	72	2	Inbred/hybrid parents
Trial-10	Testcross parental trial-2	76	2	Inbred/hybrid parents
Trial-11	Testcross parental trial-3	66	2	Inbred/hybrid parents
Trial-12	Testcross parental trial-4	56	2	Inbred/hybrid parents
Trial-13	*Iniadi* accessions	200	2	Germplasm accessions
Trial-14	Designated B-lines	70	2	Mainstream seed parent

TABLE 2 | Analysis of variance for macronutrient and micronutrient contents in pearl millet breeding trials at ICRISAT, Hyderabad, India.

Trial no.	Trial name		P	K	Ca	Mg	Fe	Zn	Mn	Na
Hybrid trials										
Trial-1	Commercial hybrid trial	F-test	**	**	**	**	*	**	**	NS
		CV %	4.0	3.7	14.0	3.9	11.6	7.9	7.8	13.8
Trial-2	Hybrid trial-1	F-test	*	**	**	**	*	**	**	**
		CV %	4.2	4.8	13.6	6.0	10.8	7.6	9.0	13.3
Trial-3	Hybrid trial-2	F-test	*	*	**	**	**	**	**	**
		CV %	5.5	10.7	12.5	6.1	11.8	7.6	10.6	17.0
Trial-4	Hybrid trial-3	F-test	**	**	**	**	**	**	**	**
		CV %	3.3	4.2	11.4	3.1	7.1	7.2	5.8	9.8
Trial-5	Hybrid trial-4	F-test	**	**	**	**	**	**	**	**
		CV %	4.7	4.2	11.9	2.8	6.8	7.4	6.1	9.6
Trial-6	Released cultivar trial	F-test	**	**	**	**	**	**	**	*
		CV %	5.5	5.5	10.5	4.5	7.9	5.9	7.7	20.0
Breeding/parental lines										
Trial-7	Hybrid parental trial-1	F-test	**	**	**	**	**	**	**	**
		CV %	5.9	6.0	13.8	6.1	9.7	9.2	10.4	15.6
Trial-8	Hybrid parental trial-2	F-test	**	NS	**	NS	**	**	**	**
		CV %	4.7	5.3	14.6	5.3	10.9	10.1	10.7	17.1
Trial-9	Testcross parental trial-1	F-test	**	**	**	**	**	**	**	**
		CV %	4.7	4.0	15.1	4.9	10.0	8.7	7.1	18.7
Trial-10	Testcross parental trial-2	F-test	NS	**	NS	*	*	*	NS	**
		CV %	5.0	5.2	15.4	4.1	11.9	10.6	9.3	35.0
Trial-11	Testcross parental trial-3	F-test	**	**	**	**	**	**	**	**
		CV %	5.7	5.4	14.3	5.6	9.6	10.1	9.5	16.5
Trial-12	Testcross parental trial-4	F-test	**	**	**	**	**	**	**	**
		CV %	5.6	6.5	14.8	4.0	10.1	9.9	8.8	14.6
Trial-13	*Iniadi* Accessions	F-test	NS	NS	**	**	**	**	**	*
		CV %	6.3	7.9	18.5	6.1	16.7	12.8	10.9	22.0
Trial-14	Designated B-lines	F-test	**	**	**	**	**	**	**	**
		CV %	5.7	6.0	11.6	5.4	9.5	9.8	7.9	16.5

*, ***Significant at $P < 0.05$ and $P < 0.01$ probability, respectively. NS, Non-significant.*

vortex mixer. The digests were filtered and the nutrient content was determined using Inductively Coupled Plasma Optical Emission Spectroscopy (ICP-OES). Estimation of aluminum (Al) as an index of soil or dust contamination was done in the grain samples of all the trials using the procedure followed in wheat (30).

Data Analyses

Data analysis was done using SAS University Edition (SAS/STAT®, SAS Institute Incorporation, Cary, North Carolina, USA). The analyses of variance of all the trials were done following Gomez and Gomez (31). This study applied the Generalized linear model (GLM) statistical analysis since most of the trials consisted of fixed-line materials (no early stages of a selection). Broad-sense heritability (H^2) was calculated following Hallauer et al. (32). Correlation analysis among grain minerals in all the trials was done as per Al-Jibouri et al. (33) and the significance of the correlation coefficients was tested using the standard table in Snedecor and Cochran (34). Genotype (G) and traits (T) analysis were performed using the "Genotype-by-Trait" module of the genotypes, and genotype × environment interaction (GGE) biplot software (35) (http://ggebiplot.com/biplot-breeder's_kit.htm).

RESULTS AND DISCUSSION

This study emphasized total variability for multiple grain nutrients to establish a baseline for most nutrients in pearl millet. Therefore, the results and their interpretation mostly focused on the magnitude of variability of each trial (hybrid parents, commercial/released hybrids, and germplasm accession) and Fe/Zn association with other nutrients under highly managed precision fields. All the 14 trials had quality data, as indicated by

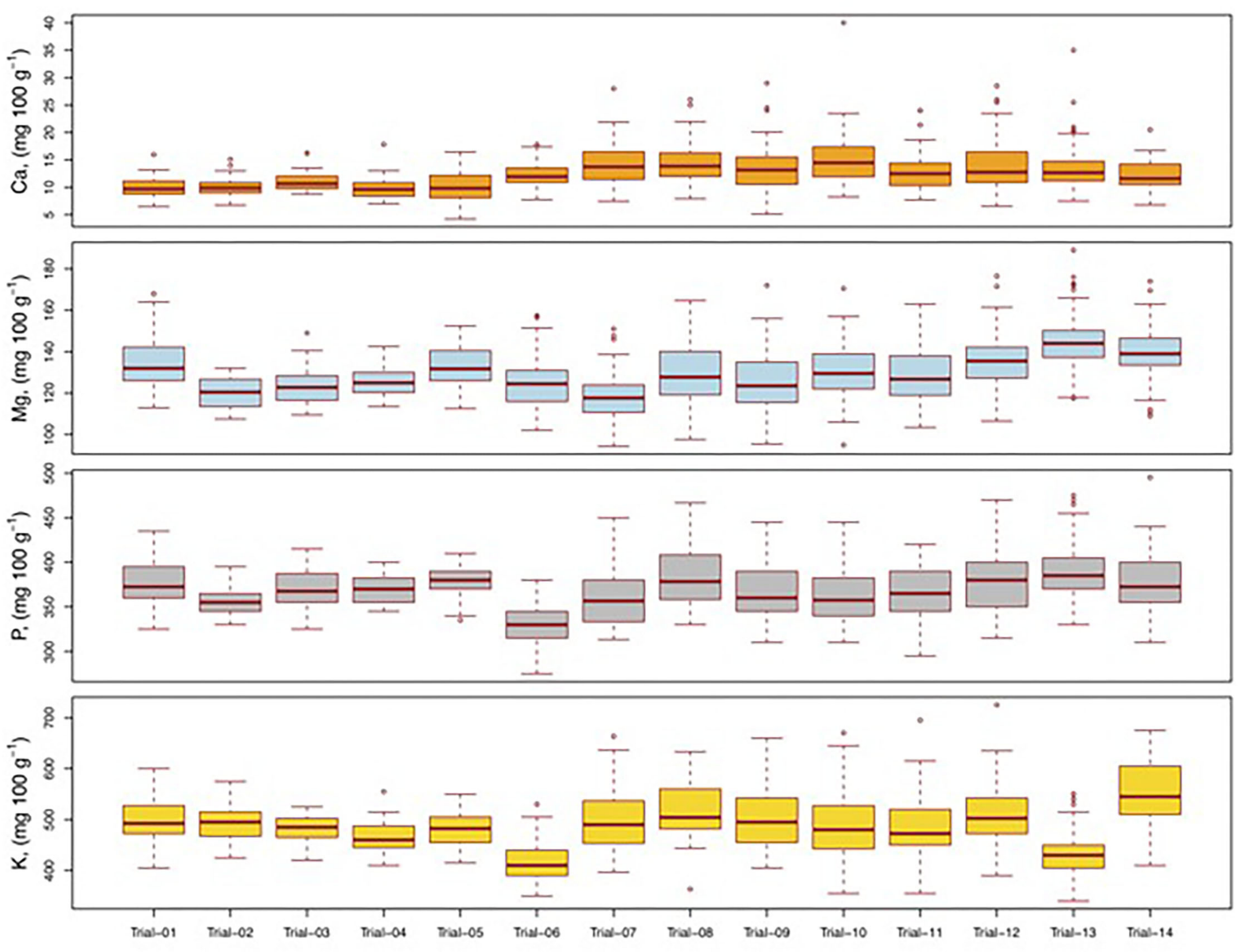

FIGURE 1 | Variability for potassium (K), phosphorus (P), magnesium (Mg), and calcium (Ca) in pearl hybrid trials (trial 1 to trial 6), inbred trials (trial 7 to trial 12 and trial 14), and germplasm accessions (trial 13) at the ICRISAT, Hyderabad, India.

the Coefficient of variation (CV)% of each trial. The magnitude and significance of genetic variability are prerequisites for an effective pre-breeding program enabled through the efficient selection of these minerals for genetic improvement.

Genetic Variability and Heritability for Grain Mineral Contents

The analysis of variance showed that the differences among the genotypes were highly significant for Fe and Zn in all the trials. Variation attributable to genotypes was not significant for Mn, Ca, Na, and Mg in one trial and P and K in two trials. Significant genotypic differences were also observed for other grain mineral content (**Table 2**). The nonsignificant values observed for very few macronutrients in three trials were not expected. This could possibly be because the trial consisted of genotypes that had been selected either for grain yield traits or partially for grain micronutrient (Fe/Zn) content during line development. A further investigation of these specific pedigrees and genetic backgrounds is warranted for a better understanding of variability. The results also showed that compared to other minerals, there was substantial genetic variability for Fe and Zn in the elite materials. For instance, the genotypes in the commercial hybrid trial, released cultivar trial, and designated parents of the seed (designated B-lines) trial were directly selected for grain yield and its components, whereas those in the other trials were mainly selected for Fe and Zn contents. Across the 14 trials, the means of P, K, Ca, and Mg content were 369, 489, 12, and 130 mg 100 g^{-1}, respectively (**Figure 1**; **Supplementary Table 1**). The variability for these macronutrients across the 14 trials ranged from 275 to 495 mg 100 g^{-1} for P, 340–725 mg 100 g^{-1} for K, 4–40 mg 100 g^{-1} for Ca, and 94–189 mg 100 g^{-1} for Mg. Similarly, the mean micronutrient content across the 14 trials was 53 mg kg^{-1} for Fe, 41 mg kg^{-1} for Zn, and 13 mg kg^{-1} for both Mn and Na (**Figure 2**; **Supplementary Table 2**). The magnitude of variability was higher for macronutrients than for micronutrients. Mean and variability range of eight minerals were in the order K > P > Mg > Ca > Fe > Zn

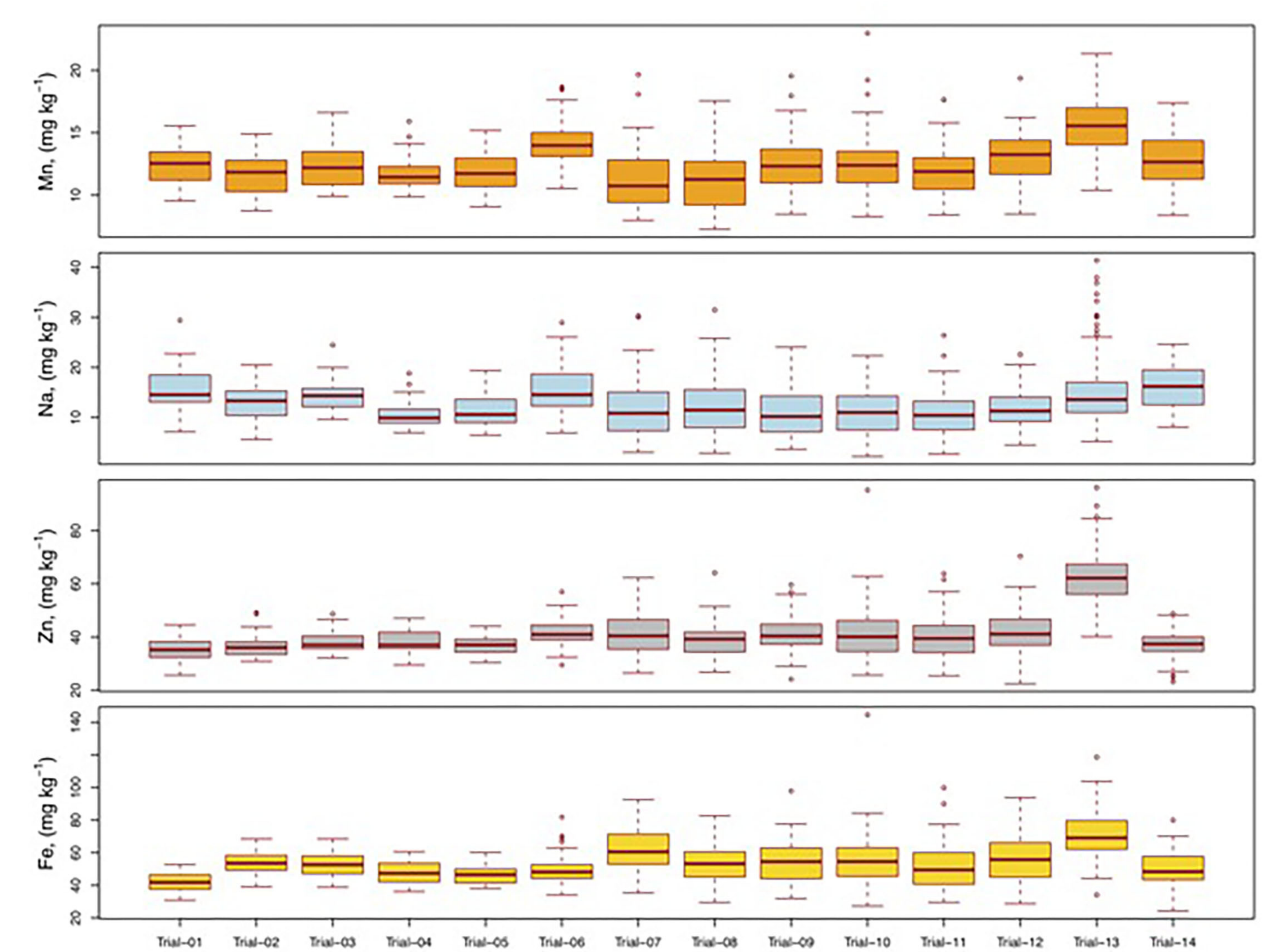

FIGURE 2 | Variability for iron (Fe), zinc (Zn), sodium (Na), and manganese (Mn) in pearl millet hybrid trials (trial 1 to trial 6), inbred trials (trial 7 to trial 12 and trial 14), and germplasm accessions (trial 13) at the ICRISAT, Hyderabad, India.

TABLE 3 | Heritability estimates (broad sense) for grain macronutrient and micronutrient contents in pearl millet breeding trials at ICRISAT, Hyderabad, India.

Trial Name	Macronutrients				Micronutrients			
	P	K	Ca	Mg	Fe	Zn	Mn	Na
Hybrid trials								
Commercial hybrid trial	0.70	0.82	0.54	0.84	0.42	0.69	0.64	–
Hybrid trial-1	0.37	0.59	0.55	0.39	0.45	0.65	0.67	0.77
Hybrid trial-2	0.37	0.51	0.54	0.47	0.44	0.56	0.55	0.51
Hybrid trial-3	0.57	0.66	0.77	0.73	0.80	0.64	0.79	0.87
Hybrid trial-4	0.43	0.67	0.84	0.88	0.79	0.54	0.81	0.88
Released cultivar trial	0.48	0.63	0.66	0.78	0.77	0.74	0.62	0.74
Breeding/parental lines								
Hybrid parental trial-1	0.69	0.82	0.79	0.77	0.82	0.83	0.81	0.92
Hybrid parental trial-2	0.72	–	0.77	–	0.82	0.75	0.77	0.87
Testcross parental trial-1	0.74	0.88	0.78	0.82	0.81	0.78	0.84	0.92
Testcross parental trial-2	–	0.86	–	0.85	0.83	0.82	–	0.90
Testcross parental trial-3	0.57	0.85	0.77	0.75	0.88	0.78	0.73	0.86
Testcross parental trial-4	0.66	0.75	0.82	0.86	0.86	0.80	0.75	0.83
Iniadi accessions	–	–	0.57	0.54	0.66	0.46	0.51	0.85
Designated B-lines	0.67	0.74	0.72	0.70	0.84	0.69	0.80	0.67
Across trials	0.58	0.72	0.71	0.73	0.73	0.70	0.72	0.67

Heritability is not estimated where non-significant variances found for few traits.

> Na > Mn. The results also revealed larger variability for P, K, Ca, Mg, Fe, Zn, Mn, and Na in parents/inbred trials compared to the hybrid trials. The variation for macronutrients (P, K, Ca, and Mg) in parent/inbred trials ranged from 25 to 157% and the variation for micronutrients (Fe, Zn, Mn, and Na) in parent/inbred trials ranged from 16 to 139% compared to those observed in the hybrid trials. These significant differences in grain mineral content among diverse sets of genetic materials suggested the promising prospect of enhancing these mineral nutrients in pearl millet, in addition to Fe and Zn. Previous studies in pearl millet have revealed wide genetic variability in grain micronutrient contents of Fe and Zn to be highly heritable (26, 36–39). A couple of studies have reported breeding approaches that have significantly improved grain yield in commercial cultivars, but reduced grain nutrient concentrations compared to old cultivars (24, 25). This study revealed the presence of adequate variation for Fe and Zn in elite genetic backgrounds for further breeding. All these lines were initially bred for yield-related traits as a part of mainstream breeding and subsequently screened for micronutrients. This showed the prospects for genetic enhancement of pearl millet with respect to these grain minerals, along with productivity traits, which would further make pearl millet a cheap source of Fe/Zn.

Heritability estimates provide information about the proportion of phenotypic variation that is genetic and allow for the prediction of genetic gains following selection. In this study, broad-sense heritability (H^2) estimates, averaged across 14 trials, ranged from 0.58 to 0.73 for macronutrients and from 0.67 to 0.70 for micronutrients (**Table 3**). This implied that, in general, slightly greater selection progress would be possible for grain micronutrients than for grain macronutrients in pearl millet. The heritability observed in inbred/parental trials was higher than that in hybrid trials for both macronutrients and micronutrients. These high heritability values suggest high genetic gains in phenotypic selection since these micronutrients are largely controlled by additive gene action (26, 28). These results are consistent with earlier studies on progeny phenotypic selection, which was highly effective in improving Fe and Zn in pearl millet (26, 40). Previous studies have reported high H^2 for grain Fe and Zn contents in pearl millet (38, 41, 42). Therefore, the availability of highly heritable variation for these macronutrients and micronutrients suggests that genetic improvement in pearl millet is highly feasible through progeny selection.

Association of Iron and Zinc With Other Mineral Content

Correlation among different traits is very important to ensure success in indirect selection in a crop breeding program. Therefore, associations between Fe and Zn and their relationship with other grain macronutrients and micronutrients are critical for the success of the genetic biofortification of pearl millet with respect to Fe and Zn. The correlation coefficient between Fe and Zn ranged from 0.58 to 0.79 in hybrids and from 0.64 to 0.81 in inbred and iniadi germplasm (early maturing, large-seeded, originated from Togo regions of West Africa), with an overall correlation coefficient of 0.79 (**Table 4**). Three hybrid trials, five inbred trials, and a germplasm trial showed a high magnitude of correlation ($r \geq 0.70$; $p < 0.01$). This implied that Fe and Zn content were closely linked within the common genomic region or via interconnected physiological mechanisms for their uptake and translocation into grains. A similar positive and significant correlation between Fe and Zn has been reported in earlier studies in pearl millet (28, 43–46), which can be attributed to

TABLE 4 | Correlation coefficient (r) of grain Fe and Zn density with other nutrients' content in pearl millet breeding trials at ICRISAT, Hyderabad, India.

Trial Name	Grain Fe vs. other nutrients							Grain Zn vs. other nutrients					
	Zn	P	K	Ca	Mg	Mn	Na	P	K	Ca	Mg	Mn	Na
Hybrid trials													
Commercial hybrid trial	0.70**	0.13	−0.08	0.18	−0.12	0.17	0.11	0.10	−0.02	0.16	−0.02	0.02	0.05
Hybrid trial-1	0.58**	−0.11	−0.16	0.27	0.19	0.46**	−0.35*	0.18	−0.33*	0.49**	0.35*	0.41**	−0.51**
Hybrid trial-2	0.59**	0.12	−0.40*	0.12	0.16	0.49**	−0.30	0.27	−0.22	0.24	0.22	0.32	−0.30
Hybrid trial-3	0.78**	−0.01	−0.13	0.00	−0.21	0.38*	−0.06	0.01	−0.10	−0.01	−0.23	0.13	−0.27
Hybrid trial-4	0.69**	0.00	−0.18	0.49**	0.00	0.37*	−0.19	0.10	−0.05	0.22	−0.07	0.15	−0.38*
Released cultivar trial	0.79**	0.40**	−0.04	0.30**	0.00	0.15	−0.13	0.47**	−0.02	0.21*	0.12	0.20*	−0.02
Breeding/parental lines													
Hybrid parental trial-1	0.74**	0.26	−0.26	0.21	0.42**	0.23	−0.44**	0.36*	−0.10	0.26	0.52**	0.36*	−0.37*
Hybrid parental trial-2	0.66**	−0.01	0.23	−0.17	−0.16	0.41**	−0.06	0.19	0.48**	−0.11	0.03	0.52**	0.03
Testcross parental trial-1	0.64**	0.05	−0.20	0.12	0.05	0.10	−0.43**	0.13	0.01	0.09	0.19	0.11	−0.37**
Testcross parental trial-2	0.81**	0.01	−0.23*	0.61**	0.13	0.41**	−0.53**	0.10	−0.20	0.53**	0.16	0.35**	−0.43**
Testcross parental trial-3	0.73**	−0.04	−0.13	−0.03	−0.29*	0.35**	−0.06	−0.01	−0.15	0.22	−0.25*	0.27*	−0.10
Testcross parental trial-4	0.81**	0.11	−0.14	−0.11	−0.16	0.27*	−0.17	0.17	−0.14	0.04	−0.03	0.09	−0.21
Iniadi accessions	0.74**	0.03	−0.12	0.34**	0.06	0.24**	−0.14	0.09	−0.16*	0.19**	0.05	0.14*	−0.11
Designated B-lines	0.75**	0.35**	0.00	0.26*	0.27*	0.50**	−0.01	0.44**	0.04	0.29*	0.31**	0.41**	0.00
Across trials	0.79**	0.14**	−0.06*	0.24**	0.23**	0.44**	−0.11**	0.08	−0.16**	0.22**	0.37**	0.53**	0.01

, Significant at $P < 0.05$ and $P < 0.01$ probability, respectively.*

the co-segregation of alleles for these micronutrients and the co-localization of quantitative trait loci (19). The results of this study suggested breeding for Zn concentration to be the secondary target while maintaining the focus on Fe content in pearl millet.

Fe and Zn showed mostly positive association with P, and the association was significant only in two trials for Fe ($r = \leq 0.40$) and three trials for Zn ($r = 0.36–0.47$). Such significant association was observed in released cultivars and designated hybrid parents trials and not in other trials. The hypothesis is, such significant association exist due to homeostasis cross-talk between P, Zn, and Fe for better crop survival and fitness (47) as the designated parents and released cultivars consisted of well-adapted materials across regions [nitrogen (N), phosphorus (P) and potassium (K) (NPK) applications for better yield], while others are in pipeline testing and yet to be tested for wider adaption. Therefore, breeding for P improvement in hybrid parents and cultivar breeding is highly possible together with Fe and Zn in pearl millet. The association of Fe and Zn with Ca was mostly in the positive direction and significant in five trials with Fe ($r = 0.26–0.61$) and Zn ($r = 0.21–0.53$). While Mn showed a positive association with Fe and Zn in all the trials, a significant association was seen in 10 trials with Fe ($r = 0.24–0.50$) and eight trials with Zn ($r = 0.14–0.52$). This positive association of Fe and Zn with Mn, and to some extent with Ca, suggested the possibility of improving Fe and Zn along with these micronutrients. Positive or negative correlations of other minerals with Fe and Zn content were not always significant. For instance, Mg had a positive and significant association with Fe in two trials ($r = 0.27–0.42$) and with Zn in three trials (0.31–0.52), while the association was significant and negative in one trial (trial 11) with both Fe ($r = -0.29$) and Zn ($r = -0.25$). Na had a negative and significant association with Fe ($r = -0.35$ to -0.53) in four trials and with Zn ($r = -0.37$ to -0.51) in five trials. Fe and Zn had a significant negative association with K only in two trials ($r = -0.16$ to -0.40). On the contrary, Zn had a positive and significant association ($r = 0.48$) with K in one trial (trial 8). Very few germplasm-based studies in pearl millet revealed that Fe and Zn were significantly negatively correlated with P and no correlation was detected between Ca and Fe, Zn, and P (42, 48). The order and magnitude of the interrelationship of Fe and Zn with these grain minerals suggest that similar genetic and physiology/molecular mechanisms control Fe and Zn mobilization, uptake, distribution, and accumulation in pearl millet without much interference or adversely affecting the accumulation of other nutrients in the grain. These two micronutrients were weakly correlated with K and Na. Such weak and negative trait linkages can be broken using a directional selection in a larger segregating population (early generations). It can also be executed using genomic marker technology, identifying single-nucleotide polymorphism (SNP) markers associated with the target nutrient traits (so-called diagnostic markers) in early generation breeding pipelines. Interestingly, positive and significant associations of Fe and Zn with P, Ca, and Mn were observed in released cultivars (trial 6) and with P, Ca, Mg, and Mn across 14 trials that consisted of hybrids, inbreds, and germplasm. This implies that genetic improvement of productivity traits in advanced breeding lines, hybrid parents, and hybrids with micronutrients and macronutrients is highly feasible in pearl millet. Correlations indicate that Fe and Zn can be improved simultaneously with a few nutrients such as Ca and Mn. Capitalizing on the available latent genetic variation for grain nutrients and the absence of significant negative associations

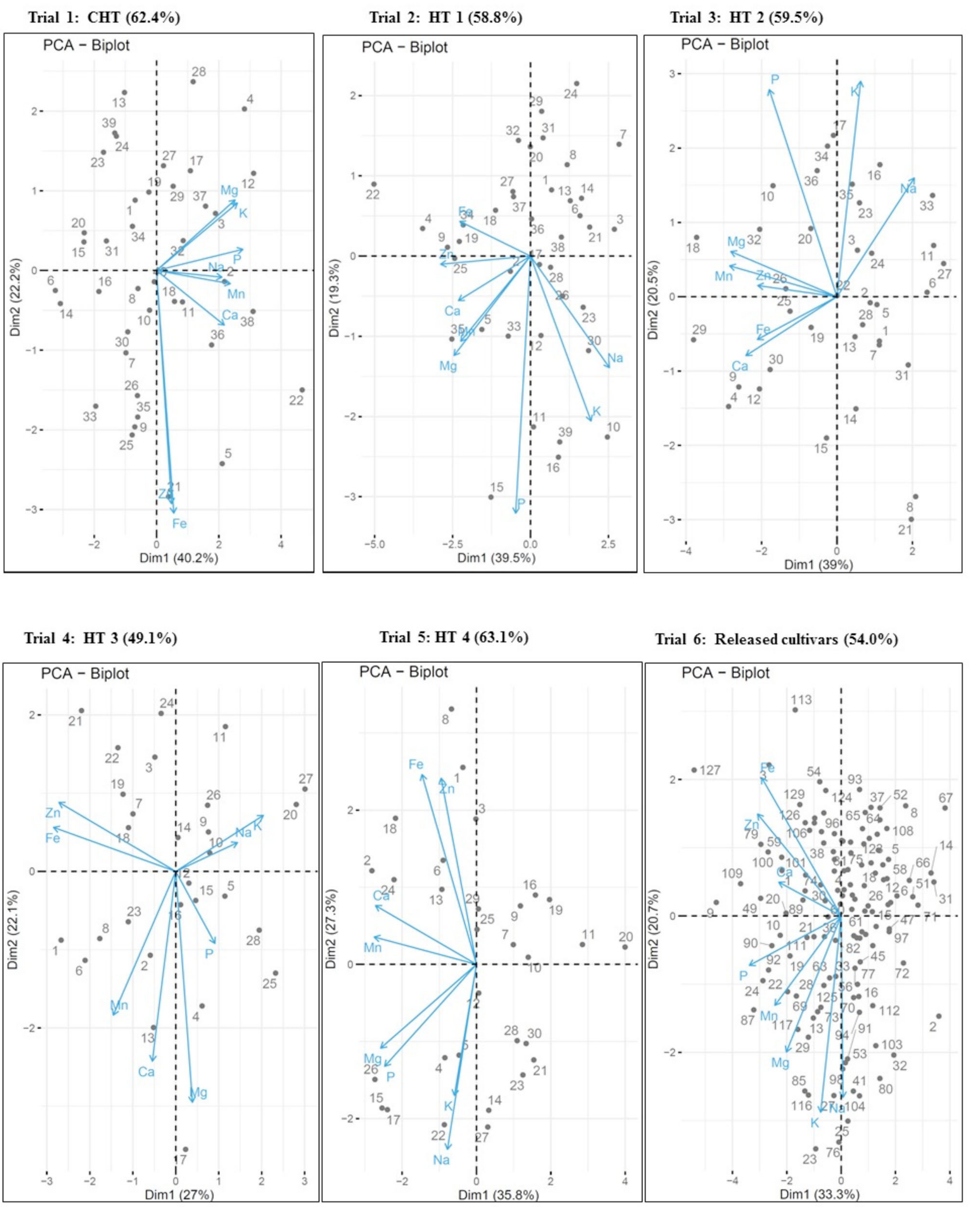

FIGURE 3 | Continued

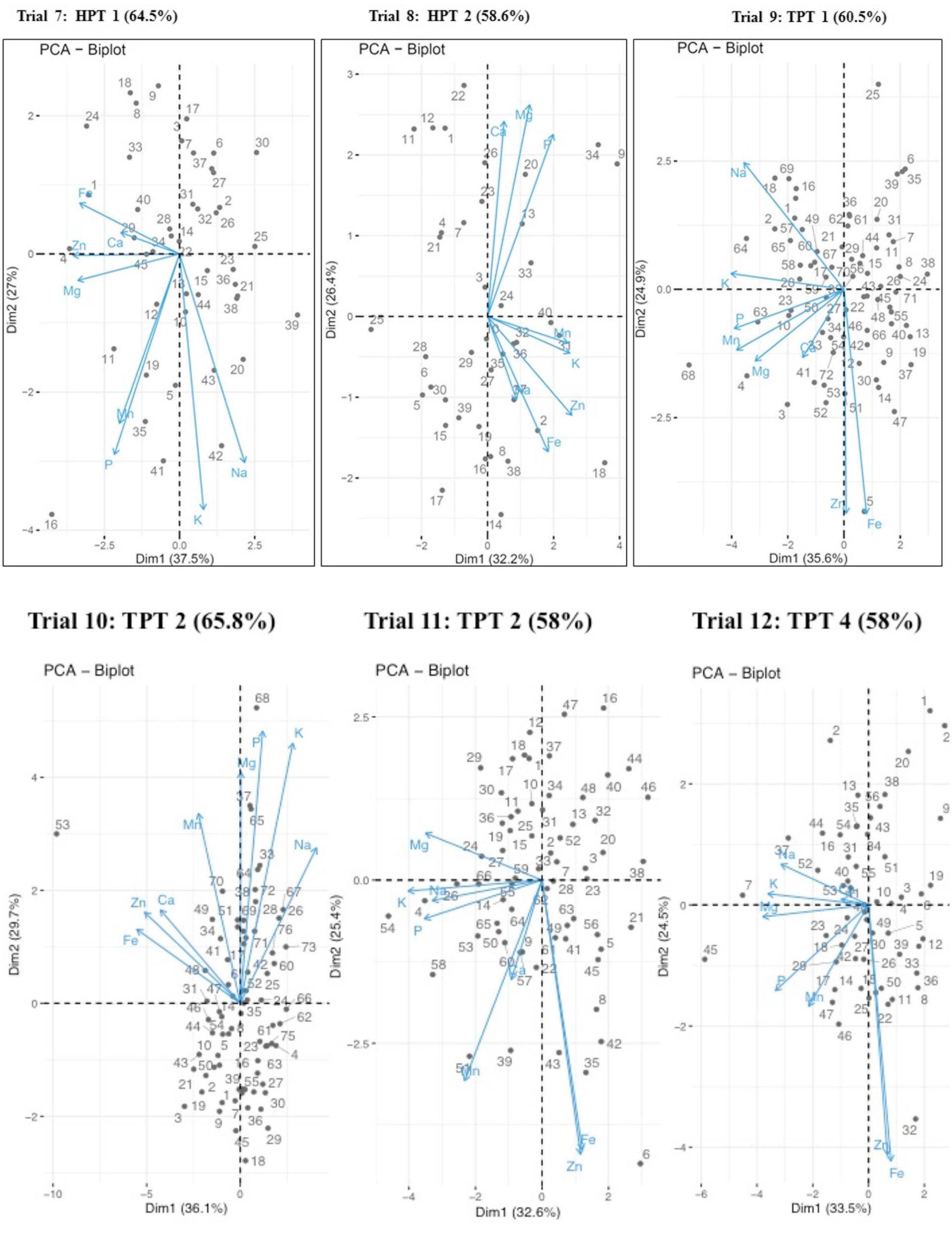

FIGURE 3 | Continued

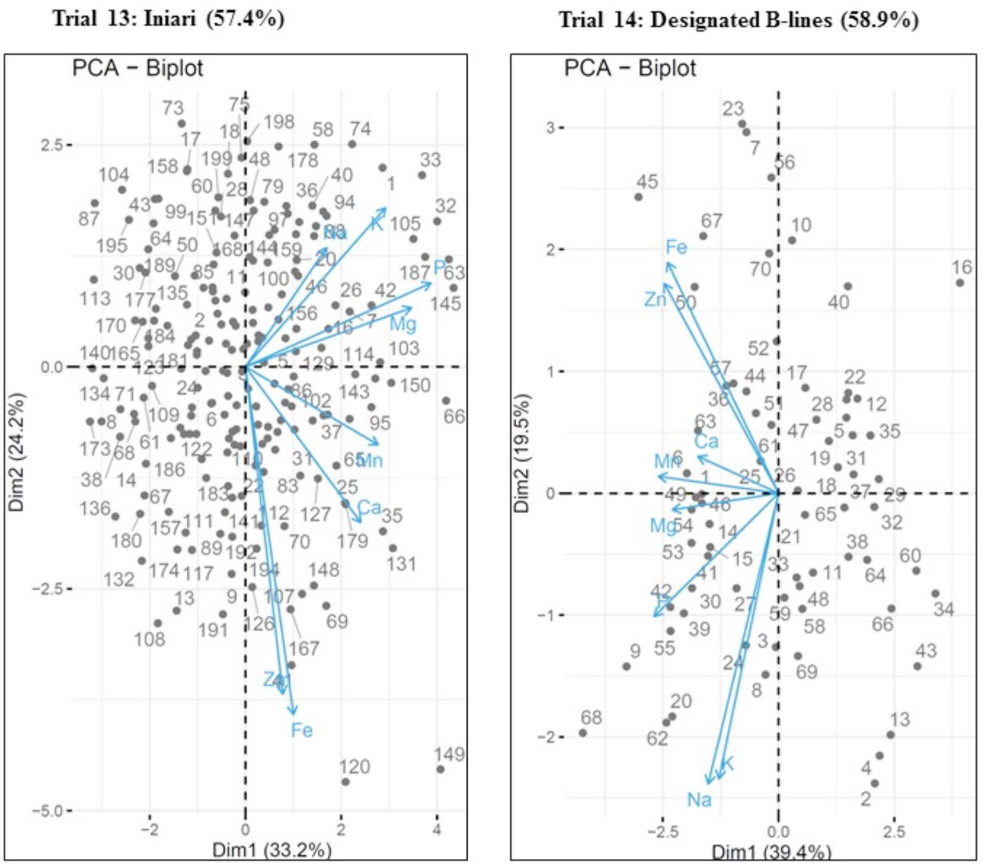

FIGURE 3 | Genotype by trait (GT) biplot of 14 pearl millet trials for grain macronutrient and micronutrient densities.

among these traits is a promising prospect for mainstreaming nutrition traits in pearl millet in the near future.

Phenotypic correlation of pair traits among genotypes is largely dependent on previous breeding objectives for a given trait and likely to be misguided because of the effect of a different trait. Therefore, critical analysis and visualization are required to choose desired traits-based germplasm. This study demonstrated eight nutritional traits association across a different set of genotypes to guide multitrait selection strategy in pearl millet. The genotype (G) by trait (T) biplot is becoming a better tool for depicting correlation among traits across genotypes (35). The relationship among the nutritional traits studied in 14 trials using the GT biplot is shown in **Figure 3**. The GT biplot explained 49.1–63.1% of the total variation in hybrid trials, while it was 57.4–65.8% in parents and germplasm trials, suggesting substantial trait variations explained for each trial and confirming the significant variance in the analysis of variance. Longer vector lengths, mostly observed for Fe, Zn, P, K, and Na, indicate wide variations among test genotypes (in hybrids, parents, and germplasm), whereas the shorter vector length specifies minimal variation among genotypes for other traits in almost all the trials. In such cases, the use of three-dimensional plots may help to visualize the spread variations. It is important to note that the test materials originated from the regular breeding program chiefly targeted for improving productivity traits. The correlation coefficient among traits presented for each trial (**Table 4**) supports the results of the GT biplot with a similar direction. For instance, Fe and Zn were always positively associated in all the trials (**Figure 3**) except for one trial (HT-2), where the association was positive but weak. The positive relationship of both Fe and Zn with Ca and Mn is similar to the correlation matrix. Findings suggest that the breeding for Fe is likely to improve Zn in pearl millet, and interestingly the two nutrients traits can be genetically improved independently from other nutrients. GT biplot delineated the best genotypes as potential sources for one or more desired nutrient traits in pearl millet. A similar pattern of a positive association between Fe and Zn in the GT biplot was reported in pearl millet (49). The GT biplot serves as a quick breeder tool for the selection of nutri-dense entry for each of the nutrients as well as more than one nutrient can be selected for in potential parents (trait donor).

CONCLUSION

This study revealed the potential genetic variation for eight pearl millet grain nutrients coupled with relatively high heritability and significant positive association especially with Fe and Zn content in diverse breeding materials (>900 entries). Released cultivars had low-to-moderate grain mineral variability in the diverse high-yielding backgrounds, suggesting the need for monitoring grain minerals in future cultivars. Parents and germplasm had higher nutrient content; the identified mineral-dense genotypes merit exploration for hybridization to improve grain mineral contents in next-generation breeding progenies and cultivar breeding. The findings of this study indicate that genetic selection for Fe will improve Zn as an associated trait in pearl millet. In addition, the Fe and Zn relationship with other macronutrients and micronutrients suggests increased prospects to achieve multiple nutritional gains in the ongoing HarvestPlus-supported pearl millet biofortification breeding. The results of this study warrant a further systematic multilocation testing to ascertain the magnitude of $G \times E$ interaction concerning micronutrient traits selection accuracy, stability, and inheritance to assist in devising appropriate breeding strategies.

DATA AVAILABILITY STATEMENT

The original contributions presented in the study are included in the article/**Supplementary Files**, further inquiries can be directed to the corresponding author.

AUTHOR CONTRIBUTIONS

MG planned the study and prepared the manuscript. AK and HS recorded the data and supported data analyses. KR and WP contributed the preparation of the manuscript. All the authors read and approved the final version of the manuscript.

FUNDING

This study was supported by special project funding from the HarvestPlus Biofortification Challenge Program of the CGIAR. It was carried out as a part of the CGIAR Research Program (CRP) on Agriculture for Nutrition and Health (A4NH).

ACKNOWLEDGMENTS

The authors greatly acknowledge support from Dr. James Stangoulis, Professor of Plant Science, currently based in Flinders University, Australia, for grain nutrient analysis.

SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fnut.2021.746625/full#supplementary-material

REFERENCES

1. Welch RM, Graham RD. Breeding for micronutrients in staple food crops from a human nutrition perspective. *J Exp Bot.* (2004) 55:353–64. doi: 10.1093/jxb/erh064
2. Mayer JE, Pfeiffer WH, Beyer P. Biofortified crops to alleviate micronutrient malnutrition. *Curr Opinion Plant Biol.* (2008) 11:166–170. doi: 10.1016/j.pbi.2008.01.007
3. Narayan J, Denny J, Nirupama D. Malnutrition in India: status and government initiatives. *J Public Health Policy*. (2019) 40:126–41. 10.1057/s41271-018-0149-5. doi: 10.1057/s41271-018-0149-5
4. Nguyen PH, Scott S, Headey D, Singh N, Tran LM, et al. The double burden of malnutrition in India: Trends and inequalities (2006–2016). *PLoS ONE*. (2021) 16:e0247856. doi: 10.1371/journal.pone.0247856
5. Muthayya S, Rah JH, Sugimoto JD, Roos FF, Kraemer K, et al. The Global Hidden Hunger Indices and maps: An advocacy tool for action. *PLoS ONE.* (2013) 8:e67860. doi: 10.1371/journal.pone.0067860
6. Belay A, Gashu D, Joy EJM, Lark R M, Christopher C, et al. Zinc deficiency is highly prevalent and spatially dependent over short distances in Ethiopia. *Sci Rep.* (2021) 11:6510. doi: 10.1038/s41598-021-85977-x
7. Qaim M, Stein AJ, Meenakshi JV. Economics of biofortification. *Agri Econ.* (2007) 37:119–33. doi: 10.1111/j.1574-0862.2007.00239.x
8. Stein AJ, Nestel P, Meenakshi JV, Qaim M, Sachdev HPS, et al. Plant breeding to control zinc deficiency in India: How cost-effective is biofortification? *Public Health Nutr.* (2007) 10:492–501. doi: 10.1017/S1368980007223857
9. Palanog AD, Calayugan MIC, Descalsota-Empleo GI, Amparado A, Inabangan-Asilo MA, et al. Zinc and iron nutrition status in the Philippines population and local soils. *Front Nutr.* (2019) 6:81. doi: 10.3389/fnut.2019.00081
10. Al-Fartusie FS, Mohssan SN. Essential trace elements and their vital roles in human body. *Indian J Adv Chem Sci.* (2017) 5:127–136.
11. FAO/WHO. *Preliminary report on recommended nutrient intakes. Joint FAO/WHO Expert Consultation on Human Vitamin and Mineral Requirements, FAO, Bangkok, Thailand.* (2000). Geneva, Switzerland: Food and Agricultural Organization of the United Nations and World Health Organization. (accessed on Sept21–30, 1998).
12. Knez M, Stangoulis JCR. Calcium biofortification of crops-challenges and projected benefits. *Front Plant Sci.* (2021) 12:669053. doi: 10.3389/fpls.2021.669053
13. Grusak, M. A., Cakmak, I. Methods to improve the crop-delivery of minerals to humans and livestock. In: Broadley MR., White PJ, editors. *Plant Nutritional Genomics.* (2005). Oxford: Blackwell Publishing. p. 265–86.
14. Sands DC, Morris CE, Dratz EA, Pilgeram AL. Elevating optimal human nutrition to a central goal of plant breeding and production of plant-based foods. *Plant Sci.* (2009) 177:377–89. doi: 10.1016/j.plantsci.2009.07.011
15. Cakmak I, Pfeiffer WH, McClafferty B. Biofortification of durum wheat with zinc and iron. *Cereal Chem.* (2010) 87:10–20. doi: 10.1094/CCHEM-87-1-0010
16. Singh D, Prasanna R. Potential of microbes in the biofortification of Zn and Fe in dietary food grains. *Rev Agron Sustain Dev.* (2020) 40:15. doi: 10.1007/s13593-020-00619-2
17. Grusak MA. Enhancing mineral content in plant food products. *J Am College Nutr.* (2002) 21:178S−83S. doi: 10.1080/07315724.2002.10719263
18. Virk PS, Andersson MS, Arcos J, Govindaraj M, Pfeiffer WH. Transition from targeted breeding to mainstreaming of biofortification traits in crop improvement programs. *Front Plant Sci.* (2021) 12:703990. doi: 10.3389/fpls.2021.703990
19. Kumar S, Hash CT, Thirunavukkarasu N, Singh G, Rajaram V, et al. Mapping quantitative trait loci controlling high iron and zinc content in self and open pollinated grains of pearl millet (*Pennisetum glaucum* (L) R Br). *Front Plant Sci.* (2016) 7:1636. doi: 10.3389/fpls.2016.01636
20. AICRP on Pearl Millet. *Report on the Project Coordinator Review 2019. 54th Annual Group Meeting, ICAR- Indian Agricultural Research Institute. New Delhi.* (2019). p. 1–12.
21. Basavaraj G, Parthasarathy Rao P, Bhagavatula S, Ahmed W. Availability and utilization of pearl millet in India. *J SAT Agri Res.* (2010) 8:1–6.

22. Parthasarathy RP, Birthal PS, Reddy BVS, Rai KN, Ramesh S. Diagnostics of sorghum and pearl millet grains-based nutrition in India International Sorghum and Millets. *Newsletter*. (2006) 46:93–6.
23. Govindaraj M, Rai KN, Cherian B, Pfeiffer WH, Kanatti A, et al. Breeding biofortified pearl millet varieties and hybrids to enhance millet markets for human nutrition. *Agriculture.* (2019) 9:106. doi: 10.3390/agriculture9050106
24. Guo S, Chen Y, Chen X, Chen Y, Yang L, et al. Grain mineral accumulation changes in Chinese Maize Cultivars released in different decades and the responses to nitrogen fertilizer. *Front Plant Sci.* (2019) 10. doi: 10.3389/fpls.2019.01662
25. Rosanoff A. Changing crop magnesium concentrations: impact on human health. *Plant Soil.* (2013) 368:139–53. doi: 10.1007/s11104-012-1471-5
26. Govindaraj M, Rai KN, Shanmugasundaram P. Intra-population genetic variance for grain iron and zinc contents and agronomic traits in pearl millet. *Crop J.* (2016) 04:48–54. doi: 10.1016/j.cj.2015.11.002
27. Pujar M, Govindaraj M, Gangaprasad S, Kanatti A, Shivade H. Genetic variation and diversity for grain iron, zinc, protein and agronomic traits in advanced breeding lines of pearl millet [*Pennisetum glaucum* (L.) R. Br.] for biofortification breeding. *Genet Resour Crop Evol*. (2020) 67:2009–22 doi: 10.1007/s10722-020-00956-x
28. Govindaraj M, Rai KN, Shanmugasundaram P, Dwivedi SL, Sahrawat KL, et al. Combining ability and heterosis for grain iron and zinc densities in pearl millet. *Crop Sci.* (2013) 53:507–17. doi: 10.2135/cropsci2012.08.0477
29. Wheal MS, Fowles TO, Palmer LT. A cost-effective acid digestion method using closed polypropylene tubes for inductively coupled plasma optical emission spectrometry (ICP-OES) analysis of plant essential elements. *Anal Meth.* (2011) 3:2854–63. doi: 10.1039/c1ay05430a
30. Yasmin Z, Paltridge N, Graham R, Huynh B, Stangoulis J. Measuring genotypic variation in wheat seed iron first requires stringent protocols to minimize soil iron contamination. *Crop Sci.* (2014) 54:255–64. doi: 10.2135/cropsci2013.04.0214
31. Gomez KA, Gomez AA. Statistical procedures for agricultural research. 2nd ed (1984). John Wiley and Sons, New York.
32. Hallauer AR, Carena MJ, Miranda Filho JB. *Quantitative Genetics in Maize Breeding (third edition)*. (2010). New York, USA: Springer science, LLC. p. 10013.
33. Al-Jibouri HA, Miler PA, Robinson HF. Genotypic and environmental variance and co-variance in an upland cotton cross of interspecific origin. *Agron J.* (1958) 50:633–6. doi: 10.2134/agronj1958.00021962005000100020x
34. Snedecor GW, Cochran WG. *Statistical Methods, Sixth edition.* (1967). The Iowa State University Press, Ames, IA.
35. Yan W. GGEbiplot: a windows application for graphical analysis of multi-environment trial data and types of two-way data. *Agron J.* (2001) 93:1111–8. doi: 10.2134/agronj2001.9351111x
36. Velu G, Rai KN, Muralidharan V, Kulkarni VN, Longvah T, et al. Prospects of breeding biofortified pearl millet with high grain iron and zinc content. *Plant Breeding.* (2007) 126:182–5. doi: 10.1111/j.1439-0523.2007.01322.x
37. Velu G, Rai KN, Sahrawat KL. Variability for grain iron and zinc content in a diverse range of pearl millet populations. *Crop Improve.* (2008) 35:186–91.
38. Gupta SK, Velu G, Rai KN, Sumalini K. Association of grain iron and zinc content with grain yield and other traits in pearl millet (*Pennisetum glaucum* (L.) R. *Br.). Crop Improve*. (2009) 36:4–7.
39. Govindaraj M, Selvi B, Sudhir KI. Genetic diversity studies in indigenous pearl millet [*Pennisetum glauccum (*L) R *Br*] accessions based on biometrical and nutritional quality traits. *Indian J Plant Genetic Res.* (2011) 24:186–93.
40. Govindaraj M, Rai KN, Shanmugasundaram P, Rao AS. Efficiency of single plant selection for grain iron and zinc density in pearl millet. *Eur J Plant Sci Biotechnol.* (2012) 6:114–7.
41. Velu G. *Genetic Variability, Stability and Inheritance of Grain Iron and Zinc Content in Pearl Millet (Pennisetum glaucum (L.) R*. Br.). Ph.D., Thesis, Tamil Nadu Agricultural University, Coimbatore, India.
42. Arulselvi S, Mohanasundaram K, Selvi B. Genetic analysis of grain quality characters and grain yield in pearl millet *(Pennisetum glaucum (L.) R. Br.). Crop Res*. (2009) 37:161–167.
43. Velu G, Rai KN, Muralidharan V, Longvah T, Crossa J. Gene effects and heterosis for grain iron and zinc density in pearl millet (*Pennisetum glaucum (L) R Br*). *Euphytica.* (2011) 180:251–9. doi: 10.1007/s10681-011-0387-0
44. Rai KN, Govindaraj M, Rao AS. Genetic enhancement of grain iron and zinc content in pearl millet. *Qual Assur Safety Crops Foods.* (2012) 4:119–25. doi: 10.1111/j.1757-837X.2012.00135.x
45. Kanatti A, Rai KN, Radhika K, Govindaraj M, Sahrawat KL, et al. Grain iron and zinc density in pearl millet: combining ability, heterosis and association with grain yield and grain size. *Springerplus.* (2014) 3:763. doi: 10.1186/2193-1801-3-763
46. Kanatti A, Rai KN, Radhika K, Govindaraj M, Rao AS. Genetic architecture of open-pollinated varieties of pearl millet for grain iron and zinc densities. *Indian J Genetics Plant Breed.* (2016) 76:299–303. doi: 10.5958/0975-6906.2016.00045.6
47. Xie X, Hu W, Fan X, Chen H, Tang M. Interactions Between Phosphorus, Zinc, and Iron Homeostasis in Nonmycorrhizal and Mycorrhizal Plants. *Front Plant Sci.* (2019) 10:1172. doi: 10.3389/fpls.2019.01172
48. Govindaraj M, Selvi B, Rajarathinam S. Correlation studies for grain yield components and nutritional quality traits in pearl millet (*Pennisetum glaucum* (L.) R. *Br.) germplasm. World J Agri Sci*. (2009) 5: 686–689.
49. Sanjana Reddy P, Satyavathi CT, Khandelwal V, Patil HT, Gupta PC, Sharma LD, et al. Performance and stability of pearl millet varieties for grain yield and micronutrients in arid and semi-arid regions of India. *Front Plant Sci.* (2021) 12:1–16. doi: 10.3389/fpls.2021.670201

Conflict of Interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

The reviewer HO declared a shared affiliation, with no collaboration, with several of the authors MG, AK, KN, and HS.

Publisher's Note: All claims expressed in this article are solely those of the authors and do not necessarily represent those of their affiliated organizations, or those of the publisher, the editors and the reviewers. Any product that may be evaluated in this article, or claim that may be made by its manufacturer, is not guaranteed or endorsed by the publisher.

frontiers in Sustainable Food Systems

REVIEW
published: 25 February 2022
doi: 10.3389/fsufs.2022.725536

Smart Management of Malnutrition Using Local Foods: A Sustainable Initiative for Developing Countries

Issoufou Amadou[1*†] *and Sitou Lawali*[2†]

[1] *Laboratory of Food Science and Technology, Faculty of Agronomy and Environmental Sciences, Dan Dicko Dankoulodo University of Maradi, Maradi, Niger,* [2] *Laboratory of Socio-Economic Development Study and Research, Faculty of Agronomy and Environmental Sciences, Dan Dicko Dankoulodo University of Maradi, Maradi, Niger*

OPEN ACCESS

Edited by:
Seetha Anitha, International Crops Research Institute for the Semi-Arid Tropics (ICRISAT), India

Reviewed by:
Sapna Langyan, National Bureau of Plant Genetic Resources (ICAR), India
Ananthan R., National Institute of Nutrition, India

***Correspondence:**
Issoufou Amadou
issoufsara@gmail.com

†ORCID:
Issoufou Amadou
orcid.org/0000-0002-9222-4743
Sitou Lawali
orcid.org/0000-0002-6401-4976

Specialty section:
This article was submitted to Nutrition and Sustainable Diets, a section of the journal Frontiers in Sustainable Food Systems

Received: 15 June 2021
Accepted: 17 January 2022
Published: 25 February 2022

Citation:
Amadou I and Lawali S (2022) Smart Management of Malnutrition Using Local Foods: A Sustainable Initiative for Developing Countries. Front. Sustain. Food Syst. 6:725536. doi: 10.3389/fsufs.2022.725536

Malnutrition is one of the major challenges the developing world is currently facing, whether it is caused by climate change, terrorism and conflict, or demographic shifts. Poverty is the main cause of malnutrition in this part of the world, and no progress is possible without the alleviation of poverty to reduce malnutrition. Reducing household vulnerability and increasing household resilience is the pathway to sustainable malnutrition management. Malnutrition has been a major threat to the health and development of children in developing countries, presenting as high levels of micronutrient deficiencies, stunting, and global acute malnutrition. The rates of malnutrition of all forms are above the thresholds accepted by the WHO in some regions. To this end, the resilience program on achieving nutrition in a developing country through at-home learning activities for nutritional rehabilitation and dietary promotion (known as FARN) reported, in this case, successful results from both statements from beneficiaries and non-beneficiaries on the reduction and management of malnutrition in their health centers. FARN activity encourages the consumption of locally available foods not only to eradicate malnutrition but also to protect the ecosystem and sustainable nutrition security. This is much like the saying, "*Give a man a fish and you feed him for a day. Teach a man to fish and you feed him for a lifetime*" to the vulnerable people; parents' knowledge of their child's nutritional status and the use of local-based foods diets showed improvement, which is proof of the impact of the resilience program. It can be concluded that the resilience program through its activities at the level of the selected community significantly affected the factors and degree of persistence of malnutrition and the level of resilience of the populations. Thus, the FARN program showed resounding success in its ability to promote sustainable malnutrition management.

Keywords: malnutrition, FARN, local foods, resilience, vulnerability

INTRODUCTION

Smart food is a concept that teaches others how food should be good for you, good for the planet, and good for the farmer. This requires dedicated effort not just by initially popularizing a couple of smart foods to build value chains for mainstreaming but also by once again focusing on smart foods as staples in developing countries. The concept is meant to provide consumers with the necessary nutrients throughout the entire meal. In agriculture, smart food is good for smallholder farmers because living in areas with high temperatures often means survival with very little water, and there are several more examples of other climate constraints (Martínez-Ballesta et al., 2018; Kane-Potaka, 2019). That means smart food has to be sustainable to be smart.

Vulnerability refers to the inability to withstand the effects of a hostile environment. Vulnerability is the human view of disasters and is the result of the range of economic, social, cultural, institutional, political, and psychological factors that shape people's lives and the environment that they live in. As far as food security is concerned, vulnerability relates to an outcome, such as hunger, food insecurity, or famine. Put differently, vulnerability is the diminished capacity of an individual or group to anticipate, cope with, resist and recover from the impact of a natural or man-made hazard (Thomas et al., 2019; Spielman et al., 2020; Mbuli et al., 2021).

Malnutrition refers to when a person's diet does not provide enough nutrients or the right balance of nutrients for optimal health. Causes of malnutrition include inappropriate dietary choices, a low income, difficulty obtaining food, and various physical and mental health conditions. Malnutrition, in all its forms, includes undernutrition (wasting, stunting, underweight), inadequate vitamins or minerals, being overweight, obesity, and resulting diet-related non-communicable diseases (Phillips et al., 2020; Amadou et al., 2020). In addition, moderate food insecurity can increase the risk of some forms of malnutrition, such as stunting in children, micronutrient deficiencies, or obesity in adults. People experiencing severe food insecurity have run out of food and, at the most extreme, have gone days without eating (Boliko, 2019). The 2021 edition of UNICEF, WHO, and World Bank Group joint child malnutrition estimates for the 2000–2020 period was released. This shows that stunting prevalence has been declining since the year 2000; more than one in five-—49.2 million children under 5—were stunted in 2020, and 45.4 million suffered from wasting. Stunting has declined steadily since 2000, but faster progress is needed to reach the 2030 target. Wasting persists at alarming rates, and the being overweight factor will require a reversal in trajectory if the 2030 target is to be achieved. In addition, about half of all deaths in children under 5 are attributable to undernutrition, which puts children at greater risk of dying from common infections, increases the frequency and severity of such infections, and delays recovery. This joint report represents the most recent global and regional figures, showing that 22.0% of all children under 5 years were stunted in 2020; 13.6 million children under 5 years were affected by wasting in its severe form in 2020, and 5.7% of the same age children in the same were overweight (Akombi et al., 2017; UNICEF, 2018; World Health Organization, 2021; Zagre, 2021). The developmental, economic, social, and medical impacts of the global burden of malnutrition are serious and lasting for individuals and their families, for communities, and for countries. Every country in the world is affected by one or more forms of malnutrition. Combating malnutrition in all its forms is one of the greatest global health challenges. Poverty amplifies the risk of, and risks from malnutrition. People who are poor are more likely to be affected by different forms of malnutrition. Also, malnutrition increases healthcare costs, reduces productivity, and slows economic growth, which can perpetuate a cycle of poverty and ill-health (Adeyeye et al., 2017; Chatindiara et al., 2020).

Eating a healthy diet is not about strict limitations, staying unrealistically thin, or depriving ourselves of the foods we love. Rather, it is about feeling great, having more energy, improving one's health, and boosting one's mood. There exists much conflicting advice, but healthy eating does not have to be overly complicated. Nowadays, any time an expert says something about certain foods being "good," there is another one saying exactly the opposite. Indeed, there are some specific foods or nutrients that have a beneficial effect on mood, while one's overall dietary pattern is most important. Often said processed food may have an impact on health, though the cornerstone of a healthy diet should be eating food that is as close as possible to the way nature made it. Processing food can be the way toward positive benefits such as the removal of antinutrients (Green et al., 2018; Willett et al., 2019). There is a need to balance one's diet with protein, fat, carbohydrates, fiber, vitamins, and minerals to sustain a healthy body. There is no need to eliminate certain categories of food from one's diet, but we should rather select the healthiest options from each category.

A sustainable food system has been defined as a food system that delivers food security and nutrition for all in such a way that the economic, social, and environmental bases to generate food security and nutrition for future generations are not compromised. In other words, a sustainable food system is a type of food system that provides healthy food to people and creates sustainable environmental, economic, and social systems that surround food (Green et al., 2018). Willett et al. (2019) reported that healthy diets from sustainable food systems showed a global shift toward more plant-based foods that would help feed the world's growing population a nutritious and sustainable diet. Various reports have said that typical food production practices can contribute to air pollution, create non-potable water, and cause land erosion among many other consequences contributing to the global climate crisis. Furthermore, sustainable management of agriculture is key to maintaining and revitalizing our environment. Focusing on sustainable modes of food production can be so important such as regenerative agriculture, to benefit the land that's being grown on, where all the system benefits around the managed land (Jhariya et al., 2019; Skaf et al., 2019).

The Secretary General of the UN António Guterres declared during his speech on world environment day on the 5th of June 2021 that "*the man action on the degradation of nature have put 40% of humanity undermining their well-being.*" He furthermore said, "*Luckily, the earth is resilient but she needs our help*." The major deficits in agricultural production due to climate change have a direct effect on the resilience of vulnerable households, and this can lead to migration and constitute a handicap for food security and sustainable agriculture. Climate change poses a threat to health and nutrition. In the future, we will encounter many challenges and opportunities for harnessing these less-mainstream food crops; we should seek to provide strategic recommendations to enable an environment for the promotion, production, marketing, and consumption of these foods, assuring healthy diets for the next generation. Thus, instead of focusing exclusively on food production or access to food, resilient food systems integrate an approach that can tackle both at the same time, reinforcing the food security of poor farmers and consumers in the zone (Mudogo, 2017; Kabore, 2019; Béné, 2020). Indeed, resilience is also at the center of a growing body of research, which attempts to understand what properties make a country, community, or household resilient and to determine the principles and processes that strengthen resilience (Manyena et al., 2019). That is why the approach recently suggested is restoring our ecosystem, which will create jobs, generate revenue, and feed the world. Therefore, this chapter wishes to contribute to the general audience this tool of learning for nutritional rehabilitation homes (FARN) through the use of smart local dietary promotion to manage malnutrition of vulnerable populations in developing countries.

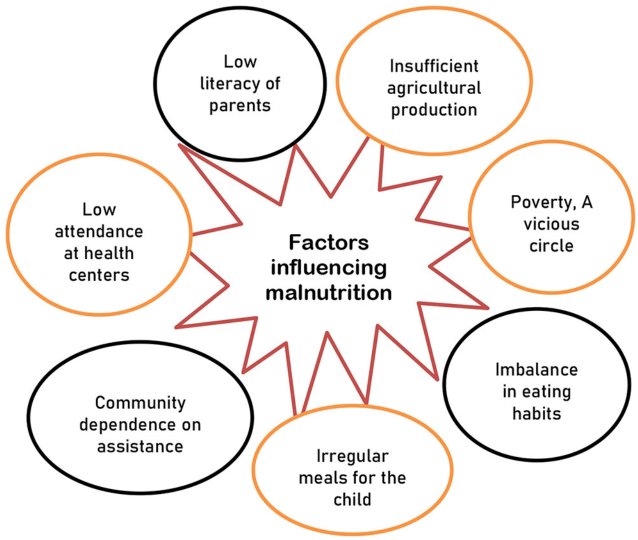

FIGURE 1 | Factors influencing malnutrition.

VULNERABILITY AND REDUCTION OF MALNUTRITION

The people living in rural areas practice agriculture as their main subsistence activity, but this agriculture cannot meet the food needs of households due to the harmful effects of climate change and natural disasters. Poor agricultural production and the lack of a source of income have exposed the majority of the population of these regions to a situation of food vulnerability (Pahlisch, 2019; Spielman et al., 2020). Poor access to agricultural, pastoral, and fishery products has a negative impact on the nutritional status of the population (Chatindiara et al., 2020). Production from agriculture cannot meet household food needs. The irregular rains and attacks from locusts and other crop pests are also responsible for the food situation in the vulnerable communities. In many cases of vulnerability, the food and nutritional situation has always remained at the top of the list, and this is the case of sub-Saharan Africa, which is extremely vulnerable to famine and food crises (Nurhayati and Lubis, 2021).

Research has shown that insufficient food intake in terms of both quantity and quality can tip a child into malnutrition (Boliko, 2019; World Health Organization, 2021). Malnutrition can be caused by poor dietary practices, and this involves a high rate of eating habits that include consuming processed cereal products and a low rate of use of vegetables, fruits, and legumes (Chan et al., 2014; Amadou et al., 2020). This explains the poverty of a population that gives an economic value to these foods. The factors of persistence of food and nutritional security in the regions identified as vulnerable by the surveying system often set up by NGOs and UN organizations (such as the UNDP, FAO, UNICEF, WFP, etc.) and the government are all linked to poverty and ignorance (**Figure 1**).

The fight against all forms of malnutrition requires a large-scale implementation of a package of interventions according to an integrated and multisectoral approach to tackle the immediate and underlying causes of malnutrition, especially in children. This must be achieved through the improvement of family practices essential for the survival and development of the child and better access to health, education, hygiene, and sanitation services on the one hand and the improvement of feeding in infants and young children on the other hand (Onyango et al., 2019; Li et al., 2020; Prieto et al., 2020). Often, this joint program has the objective of strengthening the capacities of the government to monitor and fight against malnutrition and support local interventions aimed at raising public awareness, strengthening capacities and mechanisms to combat insecurity, improve household food sources, and reduce malnutrition (Boliko, 2019; Skaf et al., 2019; Béné, 2020). Action such as introducing activities to increase resilience and nutrition security in some of these vulnerable regions was introduced and showed successful results; such action has had a significant impact, changing the status of households considerably through an increase in the standard of living of families through a reduction in malnutrition (Osabohien et al., 2019; Mary et al., 2020; Lawali et al., 2021).

LEARNING AND NUTRITIONAL REHABILITATION HOMES A NUTRITIONAL SECURITY TOOL FOR RESILIENCE

Nowadays, the term resilience is used for aspects of several disciplines, but it is used more in the field of food security and nutrition, which is a primary topic when discussing development. Indeed, following a series of disasters around the world, resilience is one of the fundamental concepts of Risk and Disaster Management and development. The major challenge that arises is to determine the means of making the system resilient to ensure sustainability for disaster victims (Allam and Jones, 2019; Lemena et al., 2021). The intervention of humanitarian organizations involves various sub-programs, which include the activity of learning and nutritional rehabilitation homes (FARN). FARN is a gathering place in a community or village, where cooking demonstrations and increasing awareness of good nutrition, health, and hygiene take place. FARN activity is encouraging the consumption of locally available foods not only for eradicating malnutrition but also to protect the ecosystem and sustainable nutrition security. It is much like the saying, "*Give a man a fish and you feed him for a day. Teach a man to fish and you feed him for a lifetime.*" Trainer moms, called "Mamans lumière" or light mothers, and beneficiary mothers cook balanced and nutritious meals together; that is, they prepare menus based on locally available foods ingredients. The enlightenment mothers come from a household with modest incomes but with well-nourished and healthy children. In addition, the work involves preventing malnutrition in children under 5 years old, rehabilitating moderately malnourished children within their own communities based on available local foods. Products from farms are the driving force behind FARN's activSities. Upstream, a systemic screening of these children is carried out in the vulnerable target areas before the weekly cooking demonstrations, and home follow-ups and FARN inputs are community-based. In addition, it is at the community level that the screening of malnourished children happens with the help of MUAC Tape (Mid-Upper Arm Circumference). Thus, the people who work for FARN consist of the community relays, light mothers, health workers, mothers of children, fathers of children, and supervisors. In addition, there is positive deviance (positive model) among the FARN mothers that leads to reduction and prevention of malnutrition through behavior changes. Indeed, the FARN activity is meant to impact the malnutrition in the community of the vulnerable populations through the use of recipes based on local products. Furthermore, FARN is a community approach to rehabilitation nutrition and changes in dietary behavior (Houngavou and Masquelier, 2018; Kêdoté et al., 2018).

Impacts of Learning and Nutritional Rehabilitation Homes Activities

The impacts of FARN activity on malnutrition according to the beneficiaries and non-beneficiaries of the resilience program set up by NGOs have seen a clear decrease in malnutrition in the communities tested. The changes were, among others, access to health centers, reduction of malnutrition and birth spacing, formulation of recipes based on local products, prevention of malaria, and family planning. This is due to the fact that nutrition activities concern the entire rural population. The mothers report a clear improvement in the nutritional status of their children following FARN's activities, which use recipes based on local products at home. FARN had an extended positive effect even on the non-programmed tested populations; we noted the development of a community contribution fund initiative to support FARN activity in their communities, which encourages income-generating activities. The use of recipes based on local food products at home had improved significantly the behavior of mothers for the benefit of the nutritional status of their children. It was noticed that a recipe was prepared every week during the FARN activities using the sample recipe guide, and when the demonstrations were taking place, a FARN "Maman lumière" (or light mother) explained the recipe to the mothers. Indeed, a light mother is defined as a mother identified in the community who has successfully fed her children with her limited means using locally available foods. An interview with a FARN witness of a rural community in the Maradi, Niger, said, "Before the opening of this center, we encountered a lot of malnutrition problems that dangerously undermine the growth of our children. Today, thanks to what we have learned, malnutrition has almost disappeared from the village, because it is from local food products that we feed our children." This shows the impact FARN activities have on the community (Amadou et al., 2020; Lawali et al., 2021).

Impacts of Scientific Research on Local Food

Science and technology be a major contribution through the provision of practical solutions. Recently, science and development have promoted local food as a primary block of for the demand of local food. Many practices are optimized and scaled up to advance adaptation throughout the food system. This has occurred mostly in rural areas and developing countries practicing sustainable agriculture through indigenous crops promotions with scientific research evidence (Beddington, 2010; Turner et al., 2018). It is a fact that consumption of healthy and sustainable diets presents major opportunities for the local food system. The global population will grow to around 9 billion by 2050, and this will mean a corresponding increase in the global food demand, putting additional pressure on the food system. The future food cannot bypass local food production and processing, and challenges in resolving food security and nutrition must be solved through research. The result of new and endogenous food has shown its efficiency in increasing the resilience of the local population in many developing countries (Willett et al., 2019; Jia, 2021). Despite climate change and extreme weather challenges, scientific research has come up with solutions to contribute to global food security and tackle malnutrition around the globe, including techniques and technologies from many disciplines, ranging from biotechnology and engineering to newer fields such as nanotechnology (Meyerding et al., 2019). Indeed, during a United Nation's conference on the role of science, technology, and innovation in ensuring food security by 2030, it was stipulated in their second goal that only adopted measures can ensure the proper functioning of food commodity markets and their derivatives and facilitate timely access to market information on items such as food reserves to tackle extreme food price volatility (Jia, 2021; Kane-Potaka et al., 2021).

Evidence of Building Resilience in Communities

The concept of community resilience describes well how a social group can recover from the aftermath of a disaster (Bankoff, 2019). Furthermore, a community can be defined as an entity with geographical borders and as sharing a fate—a destiny.

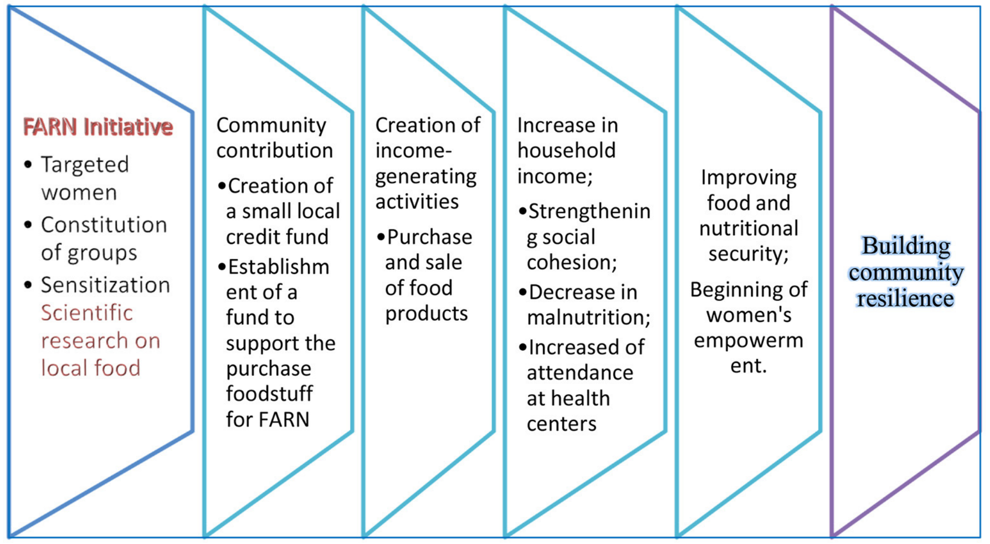

FIGURE 2 | Link between Learning and Nutritional Rehabilitation Homes (FARN) and building resilience.

However, community resilience is not the sum of these parts; it is not because individuals are resilient that the group will be adequately resilient (Townshend et al., 2015). Resilience is considered "the intrinsic capacity of companies, organizations, and communities to regain a state of equilibrium (Allam and Jones, 2019)." When facing a very low production that does not cover the food needs of the household, the population practices adaptation strategies to cover the food needs of the households. According to the surveillance of the vulnerable regions, households generally adapt to the following different strategies to meet their needs: consumption of less preferred foods, reduction of the daily dietary intake, purchase of food on credit, reduction of daily meals in number, borrowing food, leaving more assets than usual, and withdrawing children from school (Caiafa et al., 2019; Mary et al., 2020). Indeed, the causes of disasters are not simply beyond human control and impossible to eliminate through technical solutions only, but it is essential for building resilience to disasters to address socio-economic factors as well as policies that put populations at risk (Meybeck and Gitz, 2017; Manyena et al., 2019; Loconto, 2020). A new framework to build community resilience for children and families was created; a community is said to be resilient when its members have put into practice early and effective actions that can help them to respond to adversity in a healthy manner (Ellis and Dietz, 2017).

To tackle malnutrition, the increase in production helped manage the malnutrition of children from 6 to 59 months of age significantly at the community level through the use of available local food products. Furthermore, the income-generating activities (IGAs) and socioeconomic class change also helped. The IGAs helped due to the community contribution being considered as a strategy developed by the population concerned to ensure their empowerment and to be more resilient. This has resulted not only in improving the living conditions of the households but also contribute to reducing the rates of malnourished children. Such success can be explained by acts of raising awareness and the ability to be autonomous (Abraham et al., 2019). Lemena et al. (2021) underline the importance of the participatory peasant approach during risk assessment in terms of building resilience in the communities. **Figure 2** shows evidence that the learning and nutritional rehabilitation home approach contributed to building resilience in communities.

CONDITIONS FOR THE DURABILITY OF FARN

The High-Level Panel of Experts on Food Security and Nutrition (HLPE) said that "a sustainable food system is a food system that guarantees food security and nutrition for stated everyone without compromising the economic, social and environmental requirements for food security and the nutrition of future generations." Food and agriculture are part of the global concept, driven by consumption, which refers to the integrated implementation of food production and consumption models, respecting the support capacities of natural ecosystems. Agri-food systems thrive within limited and sometimes shrinking resources. The growth of agro-food systems must be inclusive, must target objectives beyond production, including efficiency along the food chain; and should promote sustainable dietary practices and diets. This involves the use of natural resources in an environmentally, economically, socially, and culturally sustainable manner to conserve the ecosystem (Singh and Singh, 2017; Reid et al., 2019).

FARN is a tool for managing malnutrition in communities vulnerable to food and nutrition insecurity crises. In addition to this, there is the problem of hygiene and healthcare of mother and child, which can be caused by dehydration, a heavy expenditure, and a loss of nutrients and energy in the body of the victims. It is recognized that micronutrient deficiencies constitute a public health problem by promoting chronic malnutrition. This type of insecurity is explained not only by the lack of food resources, but it is also linked to the low-level income of households and difficulties to access the basic community resources, such as land, livestock capital, water, cash-generating activities, etc. (Eika et al., 2019; Lawali et al., 2021). Indeed, factors such as the status of women in society and their access to education influence the process of improving children's nutrition (Ogundari and Awokuse, 2018; Sajid et al., 2021). So, to sustain these gains, an educated parent is easily better informed about the care and proper nutrition for their children. This is better for respecting messages of prevention and protection of children against disease and malnutrition as well as for the preservation of a clean and more hygienic environment. This type of parent thinks that health and nutrition education will have to be promoted more widely to guarantee the future of their child (Ruton et al., 2018). Eating is one of the favorite activities of a child right from birth not only because they need it but also because it constitutes a tender moment of exchange with their mother. Breast milk is then the ideal food, but "infant formula" can be chosen. For instance, the activity of FARN in the situation of vulnerability proved to be important for the management of malnutrition in the communities using local food products (Amadou et al., 2020).

Achieving the sustainable development goal of ending hunger and achieving food and nutrition security by 2030 seems difficult, especially following the COVID-19 pandemic (Kansiime et al., 2021). The world population is increasing quite quickly, having a more elevated rate in developing countries, and family farming or traditional agricultural systems are coming under pressure and fail to provide adequate food and income for people (Adhikari et al., 2019; El Bilali et al., 2019). There are several factors that lead to major deficits in agricultural production; yet, climate change has a direct effect on this—a huge trait that led people to migrate, causing a lot of handicaps for food security threatening their health and nutrition. Nowadays, farmers are gradually abandoning the cultivation of their local food crops and replacing these with cash crops such as cotton, mustard, fruits, cocoa, coffee, etc. Adhikari et al. (2019) stated in their study that in Nepal and Bangladesh local crops contributed only to 3 and 7% of their energy intake, respectively. The analysis of local crops showed these to be sustainable foods for the future due to their acceptability by society, their high nutritional values, good and resilient for farming systems. Indeed, they have the potential to improve farmers' income and efficiency for a sustainable economy (Olayide and Alabi, 2018; Eika et al., 2019). The more sustainable the agriculture system the better the availability of local food products for local consumption is; thus, the better the FARN activity is for the sustainable management of malnutrition in the developing countries.

CONCLUSION

The activities carried out by the humanitarian organizations on the resilience-building programs in various developing countries have brought about significant changes to the socio-economic life of the beneficiaries, as their status has changed from vulnerable to less vulnerable. Indeed, the implementation of FARN—the use of local foods—is a model that has proven effective for the prevention of malnutrition in children and the sustainability of food production in these communities. To make this acquired FARN approach a sustainable solution there is a need to revolutionize the local farming systems for a behavior change on the parts of both farmers and consumers, highlighting the importance of the environment and public awareness. However, the ignorance of the population is the first factor that hinders the development of communities on the social level because it has a direct impact on the education of children, attendance at health centers, and the behavior of the rural population. Therefore, we must take into account all the socio-cultural realities in developing countries that will help improve the development of structure activities of resilient vulnerable communities. For this, it is necessary to strengthen the awareness of this type of community in order to lead to a change in favorable behavior to the consumption of local foodstuffs and to popularize FARN on a large scale.

AUTHOR CONTRIBUTIONS

IA and SL designed and planned the work and approved the work and decided to submit it. IA wrote the manuscript. Both of us agree to be accountable for the content of the work.

REFERENCES

Abraham, A., Mohamed-Brahmi, A., and Ngoundo, M. (2019). Resilience of livestock systems in the Sahelian band of Chad (case of the Kanem region): Adaptation tools to climate change Résilience des systèmes d'élevage dans la bande sahélienne du Tchad (cas de la région de Kanem): Outils d'adaptation au changement climatique. *J. New Sci. Sustain. Livestock Manag.* 11, 222–230. Available online at: https://www.jnsciences.org/sustainable-livestock-management/93-volume-11/565-resilience-of-livestock-systems-in-the-sahelian-band-of-chad-case-of-the-kanem-region-adaptation-tools-to-climate-change.html

Adeyeye, S. A. O., Adebayo-Oyetoro, A. O., and Tiamiyu, H. K. (2017). Poverty and malnutrition in Africa: a conceptual analysis. *Nutri. Food Sci.* doi: 10.1108/nfs-02-2017-0027

Adhikari, L., Tuladhar, S., Hussain, A., and Aryal, K. (2019). Are traditional food crops really 'future smart foods?'A sustainability perspective. *Sustainability* 11, 5236. doi: 10.3390/su11195236

Akombi, B. J., Agho, K. E., Merom, D., Renzaho, A. M., and Hall, J. J. (2017). Child malnutrition in sub-Saharan Africa: a meta-analysis of demographic and health surveys (2006-2016). *PLoS ONE* 12, e0177338. doi: 10.1371/journal.pone.0177338

Allam, Z., and Jones, D. (2019). Climate change and economic resilience through urban and cultural heritage: The case of emerging small island developing states economies. *Economies* 7, 62. doi: 10.3390/economies7020062

Amadou, I., Lawali, S., Maman, R. A., and Kolo, M. S. (2020). Facteurs de persistance de la malnutrition dans la région de Maradi au Niger. *J. Appl. Biosci.* 155, 16016–16033. doi: 10.35759/JABs.155.6

Bankoff, G. (2019). Remaking the world in our own image: vulnerability, resilience and adaptation as historical discourses. *Disasters* 43, 221–239. doi: 10.1111/disa.12312

Beddington, J. (2010). Food security: contributions from science to a new and greener revolution. *Philos. Trans. R. Soc. Lond,. B,. Biol. Sci.* 365, 61–71. doi: 10.1098/rstb.2009.0201

Béné, C. (2020). Resilience of local food systems and links to food security– A review of some important concepts in the context of COVID-19 and other shocks. *Food Security* 12, 805–822. doi: 10.1007/s12571-020-01076-1

Boliko, M. C. (2019). FAO and the situation of food security and nutrition in the world. *J. Nutr. Sci. Vitaminol.* 65, S4–S8. doi: 10.3177/jnsv.65.S4

Caiafa, K., Dewey, K. G., Michaelsen, K. F., de Pee, S., Collins, S., Rogers, B. L., et al. (2019). Food aid for nutrition: narrative review of major research topics presented at a scientific symposium held October 21, 2017, at the 21st International Congress of Nutrition in Buenos Aires, Argentina. *Food Nutr. Bull.* 40, 111–123. doi: 10.1177/0379572118817951

Chan, M., Kelly, J., Batterham, M., and Tapsell, L. (2014). A high prevalence of abnormal nutrition parameters found in predialysis end-stage kidney disease: is it a result of uremia or poor eating habits?. *J. Renal Nutr.* 24, 292–302. doi: 10.1053/j.jrn.2014.03.008

Chatindiara, I., Sheridan, N., Kruger, M., and Wham, C. (2020). Eating less the logical thing to do? Vulnerability to malnutrition with advancing age: a qualitative study. *Appetite* 146, 104502. doi: 10.1016/j.appet.2019.104502

Eika, L., Mogstad, M., and Zafar, B. (2019). Educational assortative mating and household income inequality. *J. Polit. Econ.* 127, 2795–2835. doi: 10.3386/w20271

El Bilali, H., Callenius, C., Strassner, C., and Probst, L. (2019). Food and nutrition security and sustainability transitions in food systems. *Food Energy Security* 8, e00154. doi: 10.1002/fes3.154

Ellis, W. R., and Dietz, W. H. (2017). A new framework for addressing adverse childhood and community experiences: The building community resilience model. *Acad. Pediatr.* 17, S86–S93. doi: 10.1016/j.acap.2016.12.011

Green, H., Broun, P., Cook, D., Cooper, K., Drewnowski, A., Pollard, D., et al. (2018). Healthy and sustainable diets for future generations. *J. Sci. Food Agric.* 98, 3219–3224. doi: 10.1002/jsfa.8953

Houngavou, P., and Masquelier, B. (2018). *Participation Communautaire dans la prise en charge et la prévention de la malnutrition chez les enfants: une analyse des perceptions locales des Foyers d'Apprentissage et de Réhabilitation Nutritionnelle (FARN) au sud du Bénin. Faculté des sciences économiques, sociales, politiques et de communication, Université catholique de Louvain*. Available online at : http://hdl.handle.net/2078.1/thesis:16745

Jhariya, M. K., Banerjee, A., Meena, R. S., and Yadav, D. K. (2019). *Sustainable Agriculture, Forest and Environmental Management*. Singapore: Springer. doi: 10.1016/b978-0-12-812134-4.00023-6

Jia, S. S. (2021). Local food campaign in a globalization context: a systematic review. *Sustainability* 13, 7487. doi: 10.3390/su13137487

Kabore, M. (2019). *Lien Entre Résilience Objective Et Résilience Subjective Dans Le Contexte Communautaire. Approche Théorique. (Link Between Objective Resilience and Subjective Resilience in the Community Context. Theoretical Approach.). Approche Théorique. (Link Between Objective Resilience and Subjective Resilience in the Community Context. Theoretical Approach.)*. New York, NY: SSRN. doi: 10.2139/ssrn.3453142

Kane-Potaka, J. (2019). *Study Shows Consumer Acceptance to Diversify Staples With Smart Foods. ICRISAT Happening Newsletter*. Available online at: https://www.icrisat.org/study-shows-consumer-acceptance-to-diversify-staples-with-smart-foods/

Kane-Potaka, J., Anitha, S., Tsusaka, T., Botha, R., Budumuru, M., Upadhyay, S., et al. (2021). Assessing millets and sorghum consumption behavior in urban India: a large-scale survey. *Front. Sustain. Food Syst.* 5, 260. doi: 10.3389/fsufs.2021.680777

Kansiime, M. K., Tambo, J. A., Mugambi, I., Bundi, M., Kara, A., and Owuor, C. (2021). COVID-19 implications on household income and food security in Kenya and Uganda: Findings from a rapid assessment. *World Dev.* 137, 105199. doi: 10.1016/j.worlddev.2020.105199

Kêdoté, N. M., Fassinou, E., Sopoh, G., Capo-chichi, J., and Darboux, A. (2018). Evaluation de la prise en charge des enfants dénutris au niveau communautaire dans la commune de Lalo, Bénin. *Journal africain de pédiatrie et de génétique médicale* 5, 28–32. Available online at: https://journal-fss.uac.bj/pdf/PEDIATRIE/JAP5/4EvaluationdelaPECMAS.pdf

Lawali, S., Amadou, I., Gambo Mahaman, N. D., and Saley, M. (2021). Pesanteurs socioculturelles et leurs impacts sur les activités de développement dans la région de Maradi au Niger. *Int. J. Adv. Res.* 9, 793–804. doi: 10.21474/ijar01/12516

Lemena, H. K., Ralitera, J. C., Salava, J., and Randrianalijaona, M. (2021). Résilience communautaire et participation paysanne à l'évaluation des risques à Madagascar: importance de l'approche participative. *Commun. Technol. Dév.* 56, 1–11. doi: 10.4000/ctd.4021

Li, X., Yadav, R., and Siddique, K. H. (2020). Neglected and underutilized crop species: the key to improving dietary diversity and fighting hunger and malnutrition in asia and the pacific. *Front. Nutr.* 7, 593711. doi: 10.3389/fnut.2020.593711

Loconto, A. (2020). *Systèmes alimentaires durables: Un manuel pour s'y retrouver*. HAL Id : hal-03173213, version 1. doi: 10.4060/ca9917fr

Manyena, B., Machingura, F., and O'Keefe, P. (2019). Disaster Resilience Integrated Framework for Transformation (DRIFT): a new approach to theorising and operationalising resilience. *World Dev.* 123, 104587. doi: 10.1016/j.worlddev.2019.06.011

Martínez-Ballesta, M., Gil-Izquierdo, Á., García-Viguera, C., and Domínguez-Perles, R. (2018). Nanoparticles and controlled delivery for bioactive compounds: outlining challenges for new "smart-foods" for health. *Foods* 7, 72. doi: 10.3390/foods7050072

Mary, S., Shaw, K., Colen, L., and y Paloma, S. G. (2020). Does agricultural aid reduce child stunting?. *World Dev.* 130, p.104951. doi: 10.1016/j.worlddev.2020.104951

Mbuli, C. S., Fonjong, L. N., and Fletcher, A. J. (2021). Climate change and small farmers' vulnerability to food insecurity in cameroon. *Sustainability* 13, 1523. doi: 10.3390/su13031523

Meybeck, A., and Gitz, V. (2017). *L'expérience du Programme FAO/PNUE Pour des Systèmes Alimentaires Durables.* Available online at : https://www.cifor.org/publications/pdf_files/articles/AGitz1702.pdf

Meyerding, S. G., Trajer, N., and Lehberger, M. (2019). What is local food? The case of consumer preferences for local food labeling of tomatoes in Germany. *J. Cleaner Product.* 207, 30–43. doi: 10.1016/j.jclepro.2018.09.224

Mudogo, C. M. (2017). *Vulnerability of Urban Poor Women and Children to the Triple Burden of Malnutrition: A Scoping Review of the Sub-Saharan Africa Environment*. Available online at: https://www.researchgate.net/publication/322499357

Nurhayati, S., and Lubis, R. (2021). E-monitoring the vulnerability of malnutrition of children using the topsis method. *J. Eng. Sci. Technol.* 16, 728–736. Available online at: https://jestec.taylors.edu.my/Vol%2016%20issue%201%20February%202021/16_1_50.pdf

Ogundari, K., and Awokuse, T. (2018). Human capital contribution to economic growth in Sub-Saharan Africa: does health status matter more than education?. *Econ. Anal. Policy* 58, 131–140. doi: 10.1016/j.eap.2018.02.001

Olayide, O. E., and Alabi, T. (2018). Between rainfall and food poverty: Assessing vulnerability to climate change in an agricultural economy. *J. Clean. Prod.* 198, 1–10. doi: 10.1016/j.jclepro.2018.06.221

Onyango, A. W., Jean-Baptiste, J., Samburu, B., and Mahlangu, T. L. M. (2019). Regional overview on the double burden of malnutrition and examples of program and policy responses: African region. *Ann. Nutr. Metab.* 75, 127–130. doi: 10.1159/000503671

Osabohien, R., Matthew, O., Gershon, O., Ogunbiyi, T., and Nwosu, E. (2019). Agriculture development, employment generation and poverty reduction in West Africa. *Open Agri. J.* 13. doi: 10.2174/1874331501913010082

Pahlisch, T. H. (2019). *Essays on vulnerability to climate change and child malnutrition in Southeast Asia* (Doctoral dissertation). Hannover: Institutionelles Repositorium der Leibniz Universität Hannover.

Phillips, W., Doley, J., and Boi, K. (2020). Malnutrition definitions in clinical practice: To be E43 or not to be? *Health Infm. Manag. J.* 49, 74–79. doi: 10.1177/1833358319852304

Prieto, P. A. C., Ramírez, K. M. T., López, S. M., Holguín, J. S., Pineda, D. M., Tomasi, S., et al. (2020). Reduction of chronic malnutrition risk and chronic malnutrition through a public health intervention in children under one year of age in Bogotá, Colombia. *BMC Public Health* 21, 690. doi: 10.21203/rs.3.rs-63504/v1

Reid, A. J., Carlson, A. K., Creed, I. F., Eliason, E. J., Gell, P. A., Johnson, P. T., et al. (2019). Emerging threats and persistent conservation challenges for freshwater biodiversity. *Biological Rev.* 94, 849–873. doi: 10.1111/brv.12480

Ruton, H., Musabyimana, A., Gaju, E., Berhe, A., Grépin, K. A., Ngenzi, J., et al. (2018). The impact of an mHealth monitoring system on health care utilization by mothers and children: an evaluation using routine health information in Rwanda. *Health Policy Plan.* 33, 920–927. doi: 10.1093/heapol/czy066

Sajid, S., Abdullah, N., and Chik, A. R. (2021). Economic growth, female labor force participation and interacting role of education in developing-8 countries: a sustainable development policy perspective. *Psychol. Educ. J.* 58, 5263–5272. doi: 10.17762/pae.v58i1.2082

Singh, R., and Singh, G. S. (2017). Traditional agriculture: a climate-smart approach for sustainable food production. *Energy Ecol. Environ.* 2, 296–316. doi: 10.1007/s40974-017-0074-7

Skaf, L., Buonocore, E., Dumontet, S., Capone, R., and Franzese, P. P. (2019). Food security and sustainable agriculture in Lebanon: an environmental accounting framework. *J. Clean. Prod.* 209, 1025–1032. doi: 10.1016/j.jclepro.2018.10.301

Spielman, S. E., Tuccillo, J., Folch, D. C., Schweikert, A., Davies, R., Wood, N., et al. (2020). Evaluating social vulnerability indicators: criteria and their application to the Social Vulnerability Index. *Natural Hazards* 100, 417–436. doi: 10.1007/s11069-019-03820-z

Thomas, K., Hardy, R. D., Lazrus, H., Mendez, M., Orlove, B., Rivera-Collazo, I., et al. (2019). Explaining differential vulnerability to climate change: a social science review. *Wiley Interdiscip. Rev. Clim. Change* 10, e565. doi: 10.1002/wcc.565

Townshend, I., Awosoga, O., Kulig, J., and Fan, H. (2015). Social cohesion and resilience across communities that have experienced a disaster. *Natural Hazards* 76, 913–938. doi: 10.1007/s11069-014-1526-4

Turner, C., Aggarwal, A., Walls, H., Herforth, A., Drewnowski, A., Coates, J., et al. (2018). Concepts and critical perspectives for food environment research: a global framework with implications for action in low-and middle-income countries. *Global Food Security* 18, 93–101. doi: 10.1016/j.gfs.2018.08.003

UNICEF. (2018). *Levels and Trends in Child Malnutrition*. Available online at: https://www.who.int/nutgrowthdb/2018-jme-brochure.pdf

Willett, W., Rockström, J., Loken, B., Springmann, M., Lang, T., Vermeulen, S., et al. (2019). Food in the Anthropocene: the EAT–Lancet Commission on healthy diets from sustainable food systems. *Lancet* 393, 447–492. doi: 10.1016/s0140-6736(18)31788-4

World Health Organization (2021). *Levels and trends in child malnutrition: UNICEF/WHO/World Bank Group Joint Child Malnutrition Estimates, Key findings of the 2021 edition*. Report, 6 May 2021 Departmental news Reading time: 2 min (497 words). Available online at: https://www.who.int/publications/i/item/9789240025257

Zagre, N. M. (2021). Two imperatives for progress in maternal and child nutrition in Subsaharan Africa: better use of implementation research and decentralized governance in nutrition programmes. *Public Health Nutri.* 1–8. doi: 10.1017/s1368980021001865

Conflict of Interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's Note: All claims expressed in this article are solely those of the authors and do not necessarily represent those of their affiliated organizations, or those of the publisher, the editors and the reviewers. Any product that may be evaluated in this article, or claim that may be made by its manufacturer, is not guaranteed or endorsed by the publisher.

frontiers
in Sustainable Food Systems

ORIGINAL RESEARCH
published: 24 March 2022
doi: 10.3389/fsufs.2022.742909

Dual-Purpose Sorghum: A Targeted Sustainable Crop-Livestock Intervention for the Smallholder Subsistence Farming Communities of Adilabad, India

Krithika Anbazhagan[1], *Marijn Voorhaar*[1], *Jana Kholová*[1]*, *Keerthi Chadalavada*[1], *Sunita Choudhary*[1], *Srikanth Mallayee*[1], *Sivasakthi Kaliamoorthy*[1], *Vincent Garin*[1], *Rekha Baddam*[1], *Kumra Vittal Rao*[2], *Swamikannu Nedumaran*[3] and *Aravazhi Selvaraj*[4]

[1] *Crop Physiology and Modelling, Research Program-Accelerated Crop Improvement, International Crops Research Institute for the Semi-Arid Tropics (ICRISAT), Hyderabad, India,* [2] *Centre for Collective Development (CCD), Adilabad, India,* [3] *Technology Adoption and Impact Analysis, Research Program-Enabling Systems Transformation, International Crops Research Institute for the Semi-Arid Tropics (ICRISAT), Hyderabad, India,* [4] *Agri Business Innovation Platform, Research Program-Enabling Systems Transformation, International Crops Research Institute for the Semi-Arid Tropics (ICRISAT), Hyderabad, India*

OPEN ACCESS

Edited by:
Stefania Grando,
Consultant, Ascoli Piceno, Italy

Reviewed by:
Narendra Kumar,
Indian Institute of Pulses Research (ICAR), India
Sapna Langyan,
National Bureau of Plant Genetic Resources (ICAR), India

***Correspondence:**
Jana Kholová
j.kholova@cgiar.org

Specialty section:
This article was submitted to Nutrition and Sustainable Diets, a section of the journal Frontiers in Sustainable Food Systems

Received: *16 July 2021*
Accepted: *14 February 2022*
Published: *24 March 2022*

Citation:
Anbazhagan K, Voorhaar M, Kholová J, Chadalavada K, Choudhary S, Mallayee S, Kaliamoorthy S, Garin V, Baddam R, Rao KV, Nedumaran S and Selvaraj A (2022) Dual-Purpose Sorghum: A Targeted Sustainable Crop-Livestock Intervention for the Smallholder Subsistence Farming Communities of Adilabad, India. Front. Sustain. Food Syst. 6:742909. doi: 10.3389/fsufs.2022.742909

Sorghum plays an important role in the mixed crop–livestock system of tribal farming communities in Adilabad District, a high climate risk-prone region in India. Currently, the local seed system is limited to landraces and hybrids that are primarily used for domestic grain and fodder purposes. This study aimed to understand the farmers' needs and context, and use this knowledge to deliver relevant, adoptable climate-smart sorghum crop technologies through farmer-participatory approaches (FPAs). We conducted an *ex-ante* survey with 103 farmer households to understand their preferences and constraints concerning sorghum, their staple food-crop. Farmers expressed taste as the most important characteristic, followed by stover yield, grain yield, drought adaptation, and pest resistance. They identified fodder deficit, loss of seed purity in landraces, and lack of diverse sorghum seed options as critical constraints. Therefore, we chose dual-purpose, open-pollinated sorghum varieties suitable for postrainy/*rabi* cultivation as the study site's entry point. Accordingly, sixteen popular *rabi* sorghum varieties were tested at ICRISAT station (2017–18 and 2018–19) for agronomic performance in field conditions under a range of treatments (irrigation and fertilization). The standing crop was also scored by farmer representatives. Additionally, the detailed lysifield study elucidated the plant functions underlying the crop agronomic performance under water stress (plant water use and stay-green score) and an important trait of farmer's interest (relation between stay-green score and *in-vitro* stover digestibility and relation between grain fat and protein content) The selected varieties– Phule Chitra, CSV22, M35-1 and preferred landrace (*Sevata jonna*)–were further tested with 21 farmers at Adilabad (2018–20). Participating farmers from both the trials and focus group discussions voiced their preference and willingness to adopt Phule Chitra and CSV22. This article summarizes how system-relevant crop options were selected for subsistence farmers of Adilabad and deployed using participatory approaches. While varieties are developed for wider adoption, farmers adopt only those suitable for their farm, household, and accessible market. Therefore, we strongly advocate FPA for developing and delivering farmer relevant crop technologies as a vehicle to systematically break crop adoption barriers and create a positive impact on household diets, well-being, and livelihoods, especially for smallholder subsistence farmers.

Keywords: dual-purpose sorghum, farmer-participatory varietal selection, India, landraces, seed production, stover quality, tribal farming community

INTRODUCTION

Sorghum is the world's fifth most important cereal crop, after maize, rice, wheat, and barley (FAOSTAT, 2020). Compared to other major cereals, sorghum requires less water, less external inputs, is more resistant to pests and diseases, and can withstand harsh climatic circumstances (Nagaraj et al., 2013; Balakrishna et al., 2019). The multi-purpose C4 crop plays an essential role in food, feed, and fodder security in dryland agriculture (Upadhyaya et al., 2016; Chapke and Tonapi, 2019). Apart from food, it is also a valuable source of bio-fuel (Appiah-Nkansah et al., 2019). To more than 500 million people in Africa and Asia, sorghum is an integral and irreplaceable dietary staple food (Kumar et al., 2011; Kumar, 2016).

In Asia, India is the main producer of sorghum despite the crop being mostly cultivated by small and marginal farmers in the stress-prone semi-arid regions (Chapke and Tonapi, 2019). In 2019, the country's area under sorghum cultivation was 4.1 million hectares, with a production of 3.5 million tons and a productivity of 849 kg ha^{-1} (Sridhara et al., 2020). Compared to the global average (~1,481 kg ha^{-1}), sorghum productivity in India is rather low, mostly because the crop is often cultivated under rainfed conditions (Yadav et al., 2011; DACNET, 2016; FAOSTAT, 2020). Additionally, heat, water stress (Assefa et al., 2010) and lack of crop management options accessible and affordable to the farmers (Rao et al., 2010; Srivastava et al., 2010) contribute significantly to lower the crop productivity. *Kharif* (rainy) sorghum is primarily used for poultry feed, animal feed industries and alcohol (Patil et al., 2014), whereas *rabi* (postrainy) sorghum is grown for household consumption, fodder purposes, fiber, and fuel (Kumara et al., 2014). In areas where market linkages for sorghum are poor or limited, it is mostly a subsistence crop.

Adilabad district, in Telangana, is one of the areas in India inhabited by a high number of tribal people (**Figures 1A–C**). More than 75% of the district's population lives in rural areas and 35% of the people belong to tribal (farmer) communities (Poshadri et al., 2019). For these farmers, sorghum is the staple food and the primary source of animal fodder (Pandravada et al., 2013). The current regional system offers hybrids that, though cultivated, tend to have poor grain quality and are therefore less preferred for human consumption. The local and improved landraces however, are appreciated for their superior grain quality (bold, white, and with sweeter taste) and hence preferred for household consumption (Nagaraj et al., 2013). These farmer-conserved landraces (Pandravada et al., 2008, 2013), though best suited for human consumption, are not accessible to all, due to lack of formal seed banks and its undetermined seed purity. However, these landraces link closely to their culture, cultivation system, and diet of farming communities and are, thus, of high value (Pandravada et al., 2013; Sivaraj et al., 2016). Unfortunately, the Indian Government's food policies favoring consumption of rice and wheat, together with cultivation of cash crops, has impacted the country's sorghum area under cultivation, crop management (inputs given) and consumption (Srivastava et al., 2010; Pandravada et al., 2013). The decline in sorghum consumption and other coarse cereals, such as pearl millet and finger millet, has substantially reduced the population's iron intake without compensation from other food groups. This is particularly the case in areas where rice replaced the coarse cereals (DeFries et al., 2018). This gradual alteration in dietary habits and cultivation patterns (**Figure 1E**) has resulted in poorer health status among the people (NFHS, 2016; DeFries et al., 2018) and fodder scarcity in the region (Hall et al., 2007). Over 65% of Adilabad's women and children below 5 years are anemic, and more than 35% of the children of that age group are underweight (weight for age) and stunted (height for age) (NFHS, 2016; Poshadri et al., 2019). Several studies have shown that increased consumption of sorghum and millets could reduce anemia and improve the diets of Indian households (Prasad et al., 2016; Phuke et al., 2017; DeFries et al., 2018). Nutritional status of the population in subsistence farming systems is often the outcome of farmers' choices and/or options available and affordable within the socio-economic context. The quality of food consumed directly influences physical health and cognitive abilities, which impact the ability to earn. This further leads to economic losses that impact gross domestic price (GDP) (de la Peña et al., 2018). Besides these health and dietary challenges, the farmers of Adilabad live in an area of India that is prone to high or very high climate risk (Kumar et al., 2018; **Figure 1B**). The district faces major temperature fluctuations, heat, and cold waves and irregular rainfall, which is affecting the crops and threatening the livelihood security of the tribal farming communities. Therefore, cultivation of sustainable climate-smart agro-technologies (e.g., suitable crops), that meet the local farmers' needs and preferences, will be a valuable solution for the communities.

Nagaraj et al. (2013) highlighted the possible changes in policies that would impact the current demand–supply status and food and nutritional security positively, considering sorghum's

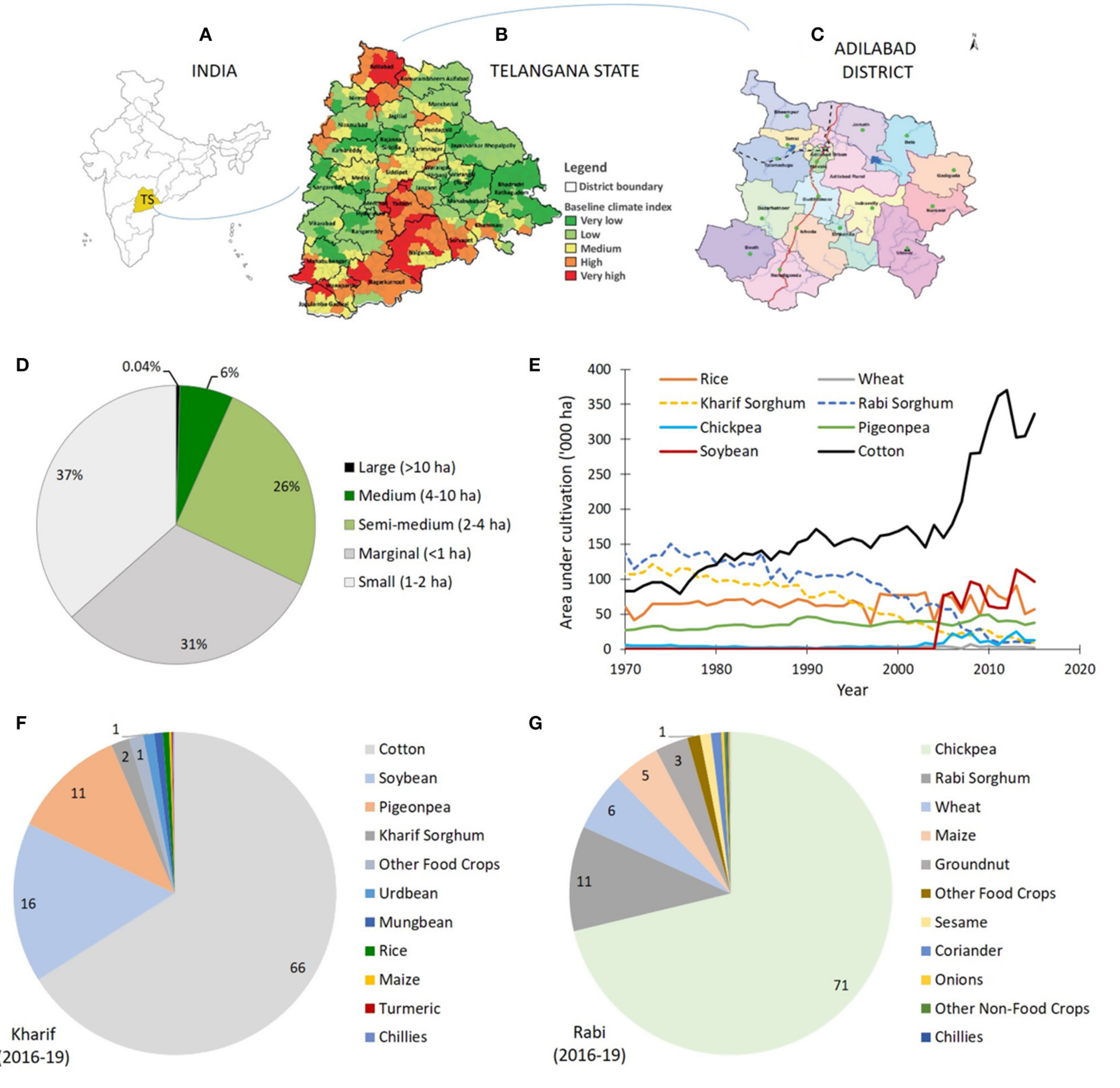

FIGURE 1 | Matrix of figures related to Adilabad district. **(A)** Telangana state in India (Source: https://www.vectorstock.com/royalty-free-vector/telangana-vector-3170092); **(B)** Climate-exposure index for Telangana state (Source: Kumar et al., 2018); **(C)** Mandals in Adilabad district; **(D)** Composition of farmers based on landholding in Adilabad district (2016 data, http://adilabad.telangana.gov.in/keystatistics/); **(E)** Trend of area under cultivation of major crops cultivated in Adilabad (1967–2015 data, http://data.icrisat.org/dld/src/crops.html); **(F)** Major crops being cultivated in Adilabad across rainy (*kharif*); **(G)** Postrainy (*rabi*) cropping seasons (2016–19 data, https://data.telangana.gov.in/dataset/adilabad-district-mandal-wise-crop-areas).

nutritive value (Blümmel et al., 2003; Kumar et al., 2010, 2011) and climate resilience (Kholová et al., 2013; Singh et al., 2014). The renewed global recognition of millets for their nutritional quality and introduction of a minimum support price for sorghum creates a new opportunity to revive sorghum as a competitive cereal crop in India. The Indian Government has committed to double the farmer's income by 2022 (Chand, 2017). For this, a number of policies and programs have been initiated (Paroda, 2018), such as the National Food Security Mission (NFSM), Pradhan Mantri Fasal Bima Yojana (PMFBY) and the National Mission on Sustainable Agriculture (NMSA). Additionally, many state governments (e.g., Odisha, Karnataka, Andhra Pradesh, Tamil Nadu, Sikkim, and Himachal Pradesh) are actively promoting consumption of nutritive coarse grains and legumes through the public distribution system[1] and programs aiming to improve school mid-day meals (https://epds.nic.in/; (Anitha et al., 2019b, 2022)). The Governments are also encouraging the establishment of farmer cooperatives, agri-business setups, and small ventures that create and support value chains for traditional crops (crops other than major cereals, such as rice, maize, or wheat). However, for sorghum to be part of the public distribution system and integrated into school meals, increasing crop yield is necessary (Anitha et al., 2019a,b). Currently, sorghum production in Adilabad District (**Figure 1E**) is insufficient to meet these goals.

To be able to support Adilabad's farmer communities in sustainable sorghum cultivation, it is crucial to see "the bigger picture" and have a systemic approach. Many crop improvement programs mainly focus on productivity gains (Barnes, 2002). Yet, it is widely recognized that adoption of new varieties is still a major problem (Alary et al., 2020), especially among small and marginal farmers. This is often due to a poor definition of the farmers' requirements and not including the socio-economic context (Thiele et al., 2020; Kholová et al., 2021). In that context, farmer participatory variety selection (FPVS) has been demonstrated to be a valuable tool (Ceccarelli, 2015). FPVS actively involves farmers in the selection process which creates a valuable knowledge exchange between all stakeholders and increases the chance of variety adoption (Humphries et al., 2015; Fadda and Van Etten, 2019; Kholová et al., 2021). Also, FPVS offers farmers and researchers the opportunity to detect potential and suitability of the variety in an earlier stage than formal breeding and agricultural extension (Wale and Yalew, 2007; Jiménez et al., 2016).

With this aim in mind, a targeted farmer-participatory study initiated at Adilabad focused on identifying sustainable sorghum options and test its relevance (Schematic diagram shown in **Figure 2**). The objectives of this study encompassed (i) defining the socio-economic context of the existing cropping systems in Adilabad District; (ii) assessing local farmers' preferences and selecting suitable sorghum varieties; and (iii) designing farmer participatory approaches (FPAs) to enable successful transfer of improved sorghum varieties to overcome crop adoption barriers.

METHODOLOGY

An Overview of the Methodology Is Shown in **Figure 3** Describing the Stages of the Study.

Understanding Crop–Livestock System of Adilabad

To understand the farming system in Adilabad, secondary district- and mandal-level data from Central and State Government reports, peer-reviewed publications, and publicly available documents were extracted for collating statistics related to demography, agricultural status and socio-economic metrics (Kumar, 2010; NAIP Project Report, 2012; Sivaraj et al., 2012; Pandravada et al., 2013; Adilabad District Report, 2016; Reddy et al., 2017; Kumar et al., 2018; NITI Aayog Report, 2018; Telangana Districts Profile Report, 2018; Task Force Report, 2019). In addition, an *ex-ante* household survey was conducted with tribal farmer families in Utnoor mandal[2] in the years 2018–20. A total of 103 interviews and a few focus group discussions were conducted with the support of the local Non-Governmental Organization, Centre for Collective Development (NGO, CCD) (**Figure 3**). The survey/interviews covered questions related to demography, socio-economic variables, landholding, livestock management, crop cultivation with an emphasis on sorghum, and dietary patterns.

Selecting Relevant Sorghum Varieties for Post-rainy Cultivation

Two field experiments during postrainy (or *rabi*, Oct–Feb) seasons of 2017–18 (Year 1) and 2018–19 (Year 2) were conducted at ICRISAT research station located in Patancheru, Telangana (latitude 17°30′N; longitude 78°16′E; altitude 549 m). The experimental site with Vertisol (or black soil) of medium depth (∼150 cm) was prepared according to the recommended sorghum cultivation practice (Trivedi, 2008). The experimental layout was a randomized complete block design with irrigation- and nitrogen-based multifactorial treatment as the main factor and genotype as sub-factor, randomized three times in blocks of each factor. The arrangement allowed a density of 15–16 plants m^{-2}. Weather data was recorded using TinyTag Ultra 2® TGU-4500 data loggers (Gemini Dataloggers Ltd, Chichester, UK) placed at the canopy level of the crop.

Sixteen varieties of sorghum were subjected to three agronomic treatments as described in Blümmel et al. (2015): well-watered with high nitrogen input (WWHN); water stressed with high nitrogen input (WSHN); and water stressed with low nitrogen input (WSLN) (**Figure 2**). The well-watered (WW) plots were irrigated every 15 days, whereas water-stressed (WS) plots were irrigated only thrice during the vegetative stage. Plants under high nitrogen (HN) treatment (i.e., WWHN and WSHN) received a basal dosage of ammonium phosphate fertilizer at the rate of 200 kg ha^{-1} and an additional top-dressing of urea at vegetative stage (∼30–40 days after sowing). In contrast, the plants under low nitrogen (LN) treatment (i.e., WSLN) did not receive any fertilizer application throughout the experimentation. They had access to only the residual Nitrogen in the soil. Of the several agronomic traits measured, this article focuses only on stover and grain yield (SY and GY, respectively). Twelve plants per treatment were harvested at physiological maturity for recording yield parameter. Panicle and stover were separated after harvest and dried at 60°C for 72 h.

For selection of varieties for the FPVS, farmers' representatives were invited to score the standing crop in the 2017–18 *rabi* trial. Based on their feedback, SY, and GY, varieties from each treatment were ranked using a selection index with 80% weightage given to GY and 60% weightage to SY for capturing

[1]Public Distribution System (PDS) portal of India. Ministry of Consumer Affairs, Food and Public Distribution. Available online at: https://epds.nic.in/ [Accessed on March 8, 2022].

[2]Administrative unit of a district.

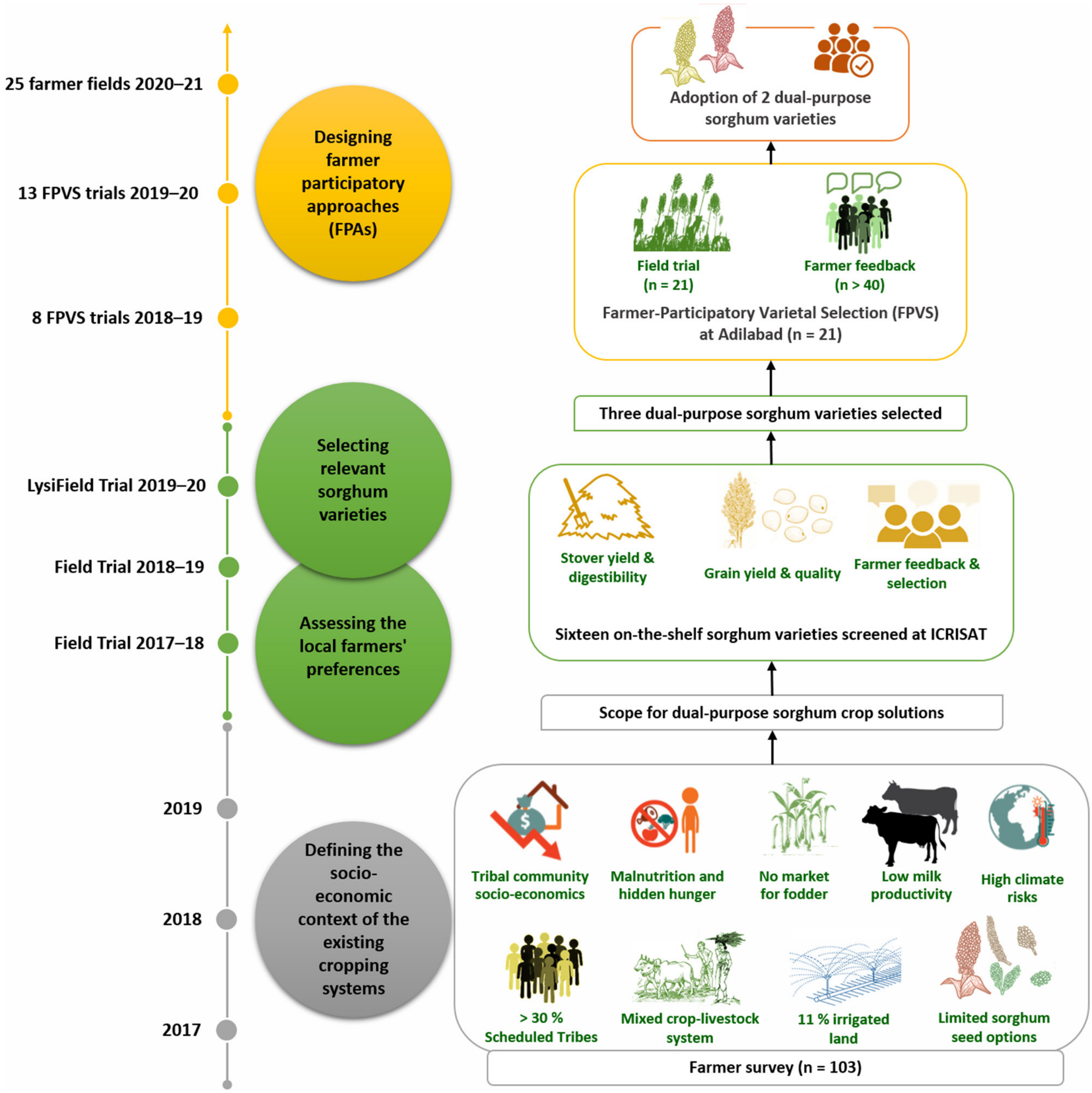

FIGURE 2 | Schematic diagram representing the project objectives, activities, observations and progress across the years.

the dual-purpose functionality of the varieties:

$$Ranking = (0.8 \times SY) + (0.6 \times GY)$$

Assessing Traits Relevant to Crop–Livestock Systems

Based on ranking from the on-station field trial (described above), three dual-purpose varieties– Phule Chitra (IC 523095; Gadakh et al., 2013), CSV22 (IC 552490; Gadakh et al., 2013), and improved landrace M35-1 (IC 552490; Reddy et al., 2009) were selected. These genotypes were tested along with the local landrace, *Sevata jonna*, for agronomic traits under WW and terminal WS conditions during the postrainy season of 2018–19 using high-throughput plant phenotyping platform, LysiField[3] (Vadez et al., 2011) (**Figure 3**). Eight replicates of each genotype were maintained per water treatment in a completely randomized block design factorized for genotypes. The plants were scored for leaf senescence visually from the bottom leaves to the top at 80

[3]http://gems.icrisat.org/lysimetric-facility/

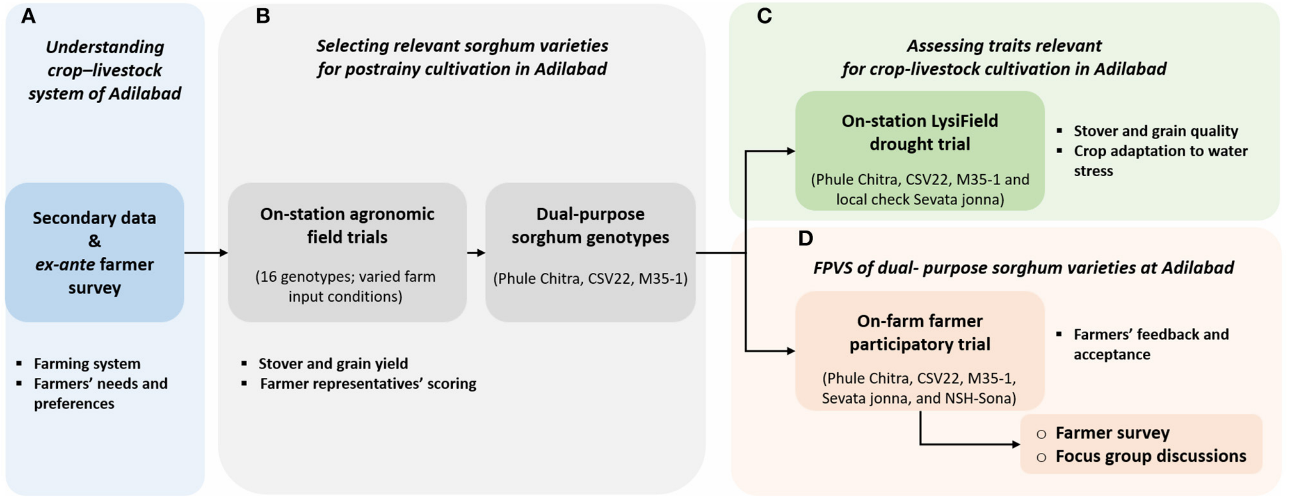

FIGURE 3 | Defined stages of project activities **(A)** Understanding crop–livestock system of Adilabad; **(B)** Selecting relevant sorghum varieties for postrainy cultivation; **(C)** Assessing traits relevant for crop-livestock cultivation; **(D)** FPVS of dual- purpose sorghum varieties at Adilabad.

and 87 days after sowing (DAS) in WS plants. The senescence scores ranged from 10 to 100%. Furthermore, the grain and fodder samples of the four varieties from the LysiField trial were processed for quantifying percentage of protein, fat and *in vitro* digestibility using near-infrared spectroscopy (NIRS; Instrument FOSS® DS2500 with WINSI II software package) and existing robust calibration models (Choudhary et al., 2010; Blümmel et al., 2015).

FPVS of Dual-Purpose Sorghum Varieties

On-farm trials were conducted at Utnoor mandal with the farmers linked to a local NGO (Centre for Collective Development) and farmer producer organization (FPO; Praja Mithra Rythu Federation). The experimental layout had genotypes in adjacent plots of 2.4 × 6 m dimension, i.e., each genotype in four rows of six-meter-long plots with 10 × 60 cm spacing. Farmers were asked to follow their usual management practice. During *rabi* 2018–19, Phule Chitra, CSV22, and M35-1 were cultivated by eight farmers. Based on their feedback, genotypes Phule Chitra and CSV22 were cultivated in trial plots of thirteen farmers in *rabi* 2019–20, along with *Sevata jonna* and local hybrid NSH-Sona for testing suitability of the open-pollinated genotypes compared to local seed options (**Figure 3**). Farmers' willingness to explore seed options and feedback on introduced genotypes (such as grain taste, grain and fodder quality and quantity, disease infestations, bird damage, crop management) were documented through interviews and focus group discussions (**Figure 3**). In addition, farmers were trained for selfing panicles to produce quality seeds using basic bags, thereby lowering their dependence on the market for seed.

Data Analysis

Numeric data from interviews were converted into percentage metrics to assess the preference and distribution of a parameter across the farming community. Quantitative agronomic data were analyzed using unbalanced ANOVA in GenStat® 18th edition (VSN International, Hemel Hempstead, UK) and comparison of means within treatment and across treatments was done using Fisher's unprotected Least Significance Difference (LSD, $P < 0.05$) comparison. As there was a poor plant stand of *Sevata jonna* in the 2018 field trial, the genotype was excluded from the analysis. Grain quality spectral data from FOSS DS2500 were converted to amount of protein and fat per 100 g of seed using winISI software and predefined sorghum model. Narrative analysis method was used for documenting qualitative data from farmer feedback through interviews and focus group discussions.

RESULTS

Secondary Data and *ex-ante* Household Survey

In Adilabad, livelihood of farming communities depends predominantly on mixed crop-livestock systems. Long-term climate analysis shows that all mandals of Adilabad are prone to high or very high climatic risk threatening the livelihood and food security of tribal farming communities (Kumar et al., 2018; **Figure 1B**). About 94% of the farmers own <4 hectares of land. In this group, about 62% of the farmers own <2 hectares of land (i.e., are small and marginal farmers) (**Figure 1D**). Despite the small landholding, the farmers seem to be responsive to market prices and cultivate multiple crops accordingly in both rainy (*kharif*, Jun–Oct) and postrainy (*rabi*, Oct/Nov-Feb/Mar) seasons to meet their domestic and economic needs (**Figures 1F,G**). With strong regional market linkages and stable minimum support price[4] farmers have adopted cash crops (cotton, soybean, pigeonpea, and chickpea) positively over the decades. Consequently, a strong downward trend in the area under cultivation of the region's staple dietary crop, sorghum,

[4]https://farmer.gov.in/mspstatements.aspx

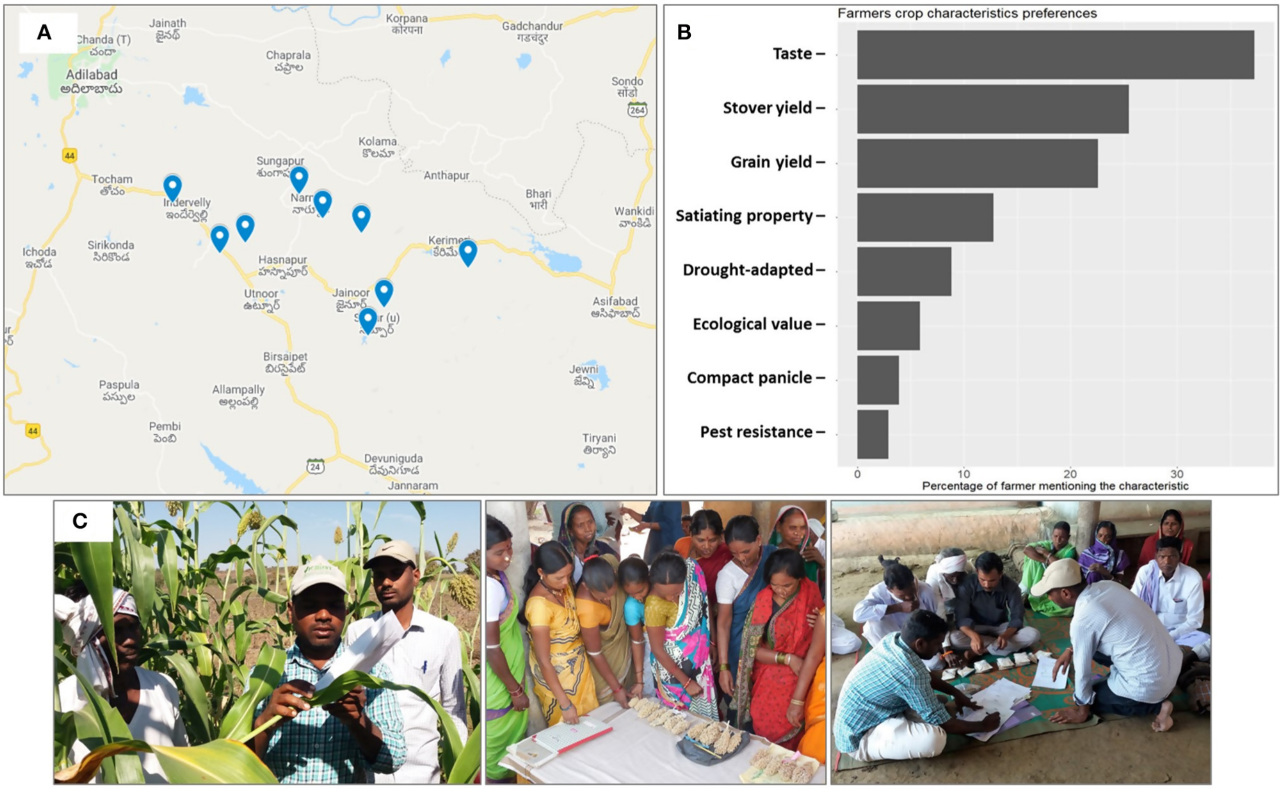

FIGURE 4 | (A) Geo-tagged positions of the villages in Adilabad district where ex ante survey and farmer-participatory varietal selection trials were conducted during 2017–18 and 2018–19; **(B)** Farmers' preferences collated from the 103 farmer interviews and focus group discussions conducted in the years 2018–20; **(C)** Collage of pictures from our participatory work at study site.

is seen from 1975 (0.15 m ha) to 2015 (0.008 m ha) (Adilabad District Report, 2016) (**Figure 1E**).

A total of 103 farm household interviews were conducted in the Utnoor region (**Figures 4A,C**). The farmers expressed that sorghum is an indispensable crop as it is cultivated for both grain and fodder in both *kharif* and *rabi* seasons. Both men and women of the community mentioned that their diets have gradually changed to include rice and wheat over the last 2–3 decades. However, they make an effort to have sorghum in at least one meal per day as it is considered to be more satiating and healthier than rice and wheat. Upon enquiring about farmers' preference for sorghum, taste was the most mentioned trait followed by stover yield, grain yield, drought adaptation, inputs requirements and pest resistance (**Figure 4B**). During the *rabi* cultivation, some farmers grew sorghum in high density to meet their fodder requirement for summer season at the expense of grain.

On-Station Selection of *rabi* Sorghum Varieties for Low Input Systems: Agronomic Trials

Based on the *ex-ante* survey, dual-purpose open-pollinated varieties (OPVs) of sorghum seemed to be a promising entry-point to address both the grain and fodder needs of the region. For testing this hypothesis, fourteen of well-characterized Government released sorghum varieties were tested along with widely popular improved landrace (M35-1) and local landrace (*Sevata jonna*) in the postrainy/*rabi* seasons of 2017–19 in ICRISAT. The on-station field trials at ICRISAT focused on screening the varieties in response to multifactorial treatments of irrigation and nitrogen: WWHN (high-input), WSHN, and WSLN (low-input) conditions. Farmer representatives scored the standing crop for traits such as stover, panicle type, presence of awns, and the taste of grain. They emphasized on varieties coping well-under low input (water stressed, low nitrogen) conditions. **Table 1** summarizes the stover and grain yield along with the metric of ranking recorded in the varieties tested under high-input (WWHN) and low input (WSLN) conditions in *rabi* 2017–18 (data from *rabi* 2018–19 not shown, unpublished).

Consequently, sorghum varieties M35-1, CSV22, and Phule Chitra were selected for further detailed trials on-station and on-field at Adilabad. The on-station trials at ICRISAT focused on screening the quantity and quality traits of the varieties in response to WW and WS conditions. Whereas, the on-farm farmer-participatory varietal selection (FPVS) trials at Utnoor were conducted to assess farmers' willingness to explore seed options and engage with the farming community to understand their preferences and the regional production conditions.

TABLE 1 | Selection of sorghum varieties based on grain and stover yield (g plant^{-1}) tested under low-input (water stressed with low nitrogen input, WSLN) and high-input (well-watered with high nitrogen, WWHN) conditions in postrainy (*rabi)* trial of 2017–18.

Genotypes	Grain yield (g plant^{-1})		Stover yield (g plant^{-1})		Ranking	
	WS LN	WW HN	WS LN	WW HN	WS LN	WW HN
M35-1	20.4	31.1	37.3	46.9	**38.7**	53.0
Phule Revati	18.9	38.7	36.5	55.0	**37.3**	63.9
CSV 22	17.6	31.6	38.1	55.1	**37.0**	58.3
Phule Chitra	16.2	30.5	39.8	50.6	**36.8**	54.8
Phule Vasudha	17.9	29.0	35.6	47.4	**35.7**	51.6
Sevata jonna	15.5	31.1	38.4	46.9	35.4	53.1
CSV 26	17.6	35.1	35.0	55.5	34.7	61.4
Phule Suchitra	16.6	38.9	35.2	53.8	34.4	63.4
Parbhani Moti	13.8	32.5	37.7	52.9	33.9	57.8
CSV 216R	16.2	33.9	32.0	53.7	32.1	59.4
CSV 18	14.4	38.0	32.3	56.7	31.0	64.4
CSV 29R	15.3	31.7	30.5	53.1	30.5	57.2
AKSV 13R	14.0	40.4	31.8	55.7	30.4	65.8
Phule Maulee	14.5	35.6	30.5	48.8	29.9	57.7
CSV 14R	15.4	34.9	28.8	50.0	29.4	57.9
Phule Anuradha	12.9	39.7	26.2	49.3	26.0	61.3
LSD ($P < 0.05$)	*5.4*	*8.4*	*7.8*	*10.0*	-	-
G	*0.318*	*0.129*	*0.020*	*0.543*		
G	*0.325*		*0.107*		-	-
E	*<0.001*		*<0.001*			
G × E	*0.032*		*0.163*		-	-

Ranking was calculated with 80% weightage prescribed to grain yield and 60% weightage to stover yield. Output of unbalanced ANOVA and Fisher's unprotected Least Significance Difference (LSD; $P < 0.05$) comparison of means within and across treatments for SY and GY is presented in the table.
LN, low Nitrogen; HN, high Nitrogen; WS, water stressed; WW, well-watered conditions.
Top five ranking varieties suitable for low-input conditions are highlighted in bold font.

Critical Assessment of Traits Relevant to Crop–Livestock Systems: LysiField Drought Trial

The LysiField trial conducted on-station in 2019–20 aimed to dissect the impact of drought on agronomic and quality traits (SY, GY, leaf senescence, protein and fat content of grains, and *in vitro* organic matter digestibility of stover) (**Figures 5–7**). Harvest index, an important criterion in crop selection representing the percentage of grain yield to total aerial biomass ranged from 10–34 to 24–47% in WS and WW plants, respectively (**Supplementary Figure 1A**). A very tight relationship between grain yield and HI was observed in WS plants ($r^2 = 0.90$, $n = 18$), unlike the WW plants ($r^2 = 0.42$, $n = 17$) (**Supplementary Figure 1A**). In WW plants, the residual of seed weight not explained by HI, correlated significantly with stover weight ($r^2 = 0.89$; $n = 17$; **Supplementary Figure 1B**). Leaf senescence in WS plants was scored at 80 DAS and 87 DAS. As most of WS plants had 100% leaf senescence by 87 DAS, we depended on the 80 DAS score for analysis. SY and GY showed contrasting relationship trends with leaf senescence (r^2 SY = 0.17; r^2 GY = −0.41) (GY; **Figure 5**). The inverse of leaf senescence was interpreted as stay-green score. Among the 4 genotypes, M35-1 had the highest stay-green score at 80 DAS (51%) followed by CSV22 (30%), Sevata jonna (26%), and Phule Chitra (19%) (data not shown).

Under WW and WS conditions, the *in vitro* organic matter digestibility (IVOMD) of stover ranged from 51 to 61%. No significant genotypic variation for IVOMD was observed among the varieties tested. Also, no significant relationship between stover yield and the IVOMD under WW ($r^2 = 0.06$; $n = 11$) and WS ($r^2 = 0.30$, $n = 8$) conditions was observed (**Supplementary Figure 2**). However, a positive relationship between leaf senescence at 80 DAS and IVOMD was found in WS plants ($r^2 = 0.62$, $n = 7$) (**Figure 6**). In WS plants, seeds with higher protein content had lower fat content as opposed to WW plants (WS $r^2 = -0.73$, $n = 8$; WW $r^2 = 0.52$, $n = 6$; **Figure 7**).

Capturing Farmers' Feedback and Knowledge Exchange Through FPVS

The twenty-one farmer-participatory trials conducted during 2018–20 in collaboration with the local NGO offered enriching knowledge exchange between the project partners. The farmers

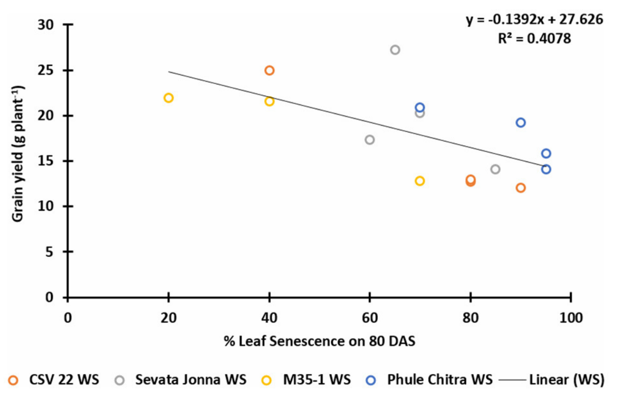

FIGURE 5 | Trait relationship between grain yield (g plant^{-1}) and leaf senescence at 80 DAS in water stressed (WS) plants of four sorghum varieties tested in 2019–20 lysimetric trial. Crop varieties are color coded; Open circles (O) indicate WS plants; solid line (–) indicated relationship between the traits.

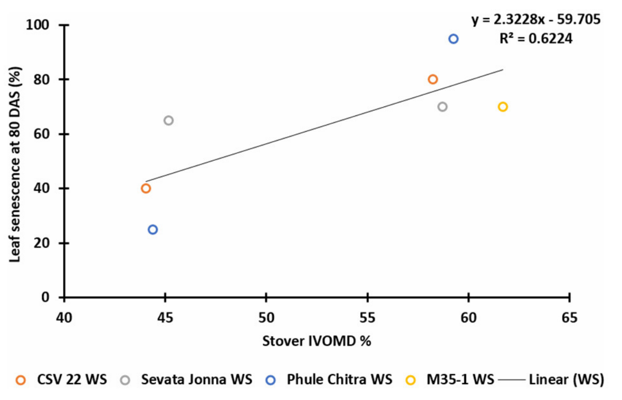

FIGURE 6 | Trait relationship between leaf senescence at 80 DAS and stover quality (*in vitro* organic matter digestibility, IVOMD) in water stressed (WS) plants of four sorghum varieties tested in 2019–20 lysimetric trial. Crop varieties are color coded; Open circles (○) indicate WS plants; solid line (—) indicated relationship between the traits.

compared the harvested material for several parameters: morphological traits (e.g., presence of awns, panicle compactness, secondary branching of panicle rachis), agronomic traits (e.g., plant height, stover yield, pest resistance, and water requirement) and quality traits (e.g., hardness and taste of seed, and taste of cooked product (*roti*[5] and *upma*[6]). Focus group discussions with over 40 farmers and results of the FPVS feedback survey showed a high variability in farms, crop management, farmers' preference and willingness of farmers to explore. Most farmers preferred Phule Chitra over CSV22, for its long panicle, thick stems, seed set, and seed boldness.

Several farmers expressed their positive attitude toward the newly introduced varieties and are planning to continue cultivation:

[5] Flattened bread.
[6] Thick porridge with seasoning.

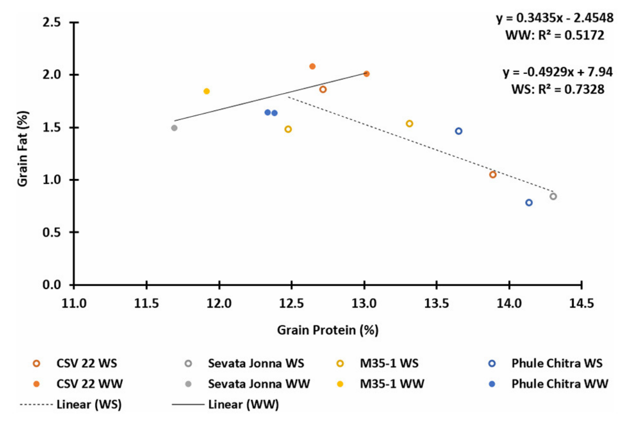

FIGURE 7 | Quality trait relationship between grain fat and protein content in water stressed (WS) and well-watered (WW) plants across four genotypes tested in 2019–20 lysimetric trial. Crop varieties are color coded; Full circles (•) indicate WW plants; Open circles (O) indicate WS plants; solid line (—) indicates relationship in WW plants; dashed line (––) indicates relationship in WS plants.

Farmer 1 [F1], male, from Saleguda: "CSV22 and Phule Chitra suit my land. I have saved seeds from the harvest for next sowing."
F2, male, from Narnoor: "I'm planning to grow both varieties. I was surprised by the fodder yield under minimum irrigation."
F3, male, from Keslaguda: "I prefer Phule Chitra, because it has long panicles and gives more seeds. I will keep the harvested seeds, so I can sow them again next season."
F4, male, Soyamguda: "I like Phule Chitra for its long panicles, thick stems and bold seeds. The panicle type and seed set of CSV22 is also good."
F5, male, Narnoor: "I prefer CSV22 over Phule Chitra, because of the type of panicles. The amount of seeds per panicle was good."

According to the participants of the focus group discussions, the taste of the food prepared with Phule Chitra was comparable to that of their local landrace. Some concerns were shown regarding the sweetness of the grains of both varieties, which attracted birds during the seed maturation stage. This required farmers to spend more time in the field and to install bird scaring devices. By contrast, the bent compact panicles of the landraces tend to reduce damage by birds (**Figure 8**).

DISCUSSION

Relevant Sorghum Crop Choices for Adilabad

Adilabad is one of the many underdeveloped districts in India that is tackling poverty, poor health, education, and basic infrastructure deficits (NITI Aayog Report, 2018). The region has a high proportion of indigenous populations (>30%) belonging to Scheduled Castes and Scheduled Tribes that face complex socio-economic and political limitations. The livelihood status of farming communities in this region is further challenged by climate risks. Ceccarelli (2015) stated that crop interventions meeting farmers' needs and preferences are vital for its adoption

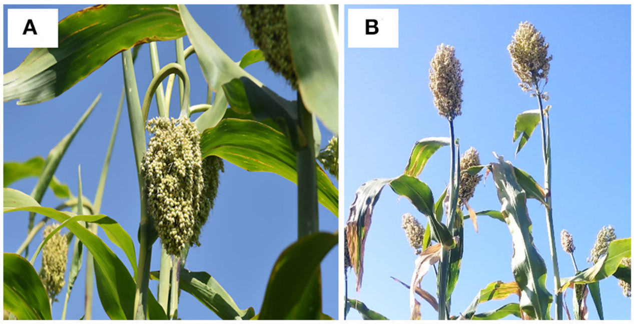

FIGURE 8 | Representative image of the panicle of **(A)** local sorghum landrace *Sevata jonna* and **(B)** open-pollinated dual-purpose sorghum variety Phule Chitra.

and integration in the existing system. During our *ex-ante* survey with the farming community, three key constraints were highlighted: (i) fodder scarcity as one of the important concerns for the farming community; (ii) the limitation of available sorghum seed option to landraces and hybrids; and (iii) the loss of seed purity in landraces over the years. Our initial interactions with the farmers at Utnoor focused on reviving landraces (Rajani, 2018) that hold special cultural and dietary value to the locals (Pandravada et al., 2013; Paltasingh and Paliwal, 2014). These shortcomings indicate that training farmers to maintain purity of seeds and increasing seed options would benefit the sorghum food system among these communities (**Figure 3C**; Voorhaar and Anbazhagan, 2019). In addition, open-pollinated varieties (OPVs) would drastically reduce farmers' dependence on the market for hybrid seeds. Therefore, we explored possible crop options that could fit and benefit the farmers.

Several reports suggest that dual-purpose varieties with superior grain and stover traits can increase overall farm productivity, particularly in (semi)-arid regions with mixed crop-livestock systems where fodder is scarce (Rao and Hall, 2003; Sharma et al., 2010; Erenstein et al., 2011). Ceccarelli (1996) explicitly highlighted the importance of selecting varieties in unfavorable or limited conditions prior to deployment as most of the varieties are bred through cycles of well-managed high input selection trials. In this study, the on-station trials at ICRISAT were conducted under both high- and low-input conditions for quantifying the agronomic traits (stover yield, grain yield, harvest index and stay-green trait) and quality traits (stover digestibility, grain protein and fat) across the dual-purpose varieties–Phule Chitra, CSV22, M35-1 and *Sevata jonna*. Rao and Hall (2003) stated that yield benefits and quality of produce are not the only selection criteria for farmers for varietal adoption. Similarly, the participating farmers ranked taste first followed by stover yield, grain yield, and adaptability to drought, panicle compactness and pest resistance. They also mentioned several morphological and crop traits (as mentioned in results section) that were considered while selecting the varieties from existing the sorghum elite collection.

Of the agronomic traits measured in the on-station trials, we would like to highlight the stay-green trait as it is closely linked with continued photosynthetic capacity of the crop in the post-flowering stage and adaptation to terminal drought (Prasad et al., 2014). Quality traits assessed using near-infrared spectroscopy (NIRS) indicated that stover digestibility, unlike grain protein and fat content, was not severely affected by water stress. As seen in **Figure 6**, a positive relationship ($r^2 = 0.62$) between with stover digestibility (IVOMD) and leaf senescence was observed. This is an important visual trait as every 1% increase in digestibility of sorghum stover can result in 6–8% increase in milk productivity in livestock (Kristjanson and Zerbini, 1999; Zerbini and Thomas, 2003; Hall et al., 2004). Increasing milk production in the region will enhance the nutrition of women and children, and enhance household income through the sale of surplus quantity fodder and milk. Further, NIR-based grain quality analysis showed an inverse relationship between protein and fat content in WS plants (**Figure 7**). Srivastava (2018) documented that smallholder farmers of Adilabad, commonly consume what they cultivated and conserve a portion of their harvested seeds for next season sowing. In the study site, farmers often stored their harvested seeds in jute/plastic bags or earthen pots at home. As sorghum grains tend to get rancid upon storage, seed types that have lower fat and higher protein would be suitable for the smallholder farmer household consumption.

Combining Farmers' and Researchers' Knowledge

Genotype–environment (G × E) interactions are complex and they get further complicated with crop management and resources accessible during cultivation (G × E × M). Farming communities manage these complexities in the best way possible with the options they have access to. Introducing new OPVs through farmer-participatory methods was helpful in testing if the varieties met the farmers' requirements (taste and stover) (Voorhaar and Anbazhagan, 2019). Although our FPVS trials are still ongoing (25 farmers participated in 2020–21 and more to be included in 2021–22), until now, the varieties Phule Chitra and CSV22 have been well-received and adopted by the participating sorghum farmers. In the absence of formal seed banks, the responsibility of conserving seeds lies on the community itself. During our study, farmers were trained to use selfing article bags to maintain seed purity and enable farmers to produce their own seeds (landraces or the OPVs) independently. In addition to the efforts to revive landraces, the participating sorghum farmers' overall response to Phule Chitra and CSV22's was positive. Several farmers informed that they will continue to practice panicle selfing to maintain seed purity, cultivate *Sevata jonna* for household consumption and OPVs Phule Chitra and CSV22 in high density for fodder. A farmer from Chintakara village continued to cultivate CSV22 and Phule Chitra beyond the 2017–18 FPVS, proactively performed selfing and shared the harvested seeds with other farmers of his village. Although this appears as a singular activity, within close-knit communities such as the tribal community in the study area, knowledge transfer through demonstration, experience, and word-of-mouth plays a significant role. Based on the outcome of the study, conservation of landraces and integration of OPVs, can be a valuable short-term solution to strengthen the sorghum seed system in Adilabad.

CONCLUSION

Building further on the farmer participatory approach, a detailed socio-economic analysis along with a scaled-up varietal intervention project with impact assessment is planned for 2021–24. We envision that bringing nutritionally-dense, dual-purpose sorghum into the cultivation system can improve health, wellbeing and livelihood of the tribal farming community. With improved household income and quality of diet, physical health and cognitive development of the community will change for the better. The "Nutri-Food Basket" and "Giri Poshana" projects at Adilabad show that food-based interventions supplementing existing diets, help in reducing underweight among children and tackling anemia in women Padmaja and Kavitha (2017), Padmaja et al. (2018). In addition, by increasing the regional production of sorghum grain and fodder, several crop value chains and market linkages can be created with farmer producer organizations and cooperatives boosting the local economy and farm income. With the support of already existing Government policies/initiatives (such as the National Food Security Mission and National Mission on Sustainable Agriculture), the surplus grain can be channeled into the Government's procurement system. This could be a game-changing market-pull for increasing sorghum cultivation, breaking the threshold of subsistence farming in several households, and replacing rice in the mid-day school meal program and public distribution system.

In conclusion, for increasing the varietal adoption and plant breeding efficiency, we emphasize the importance of (i) FPAs to understand farming community's context and specific needs, (ii) on-station selection methodologies that reflect different cultivation conditions, and (iii) knowledge and technology exchange between farmers and researchers to consider traits beyond yield and productivity gains.

DATA AVAILABILITY STATEMENT

The datasets presented in this study can be found in online repositories. The names of the repository/repositories and accession number(s) can be found below: doi: 10.21421/D2/LBSXTT.

ETHICS STATEMENT

The farmer participants provided written informed consent to participate in this study. Also, a prior informed consent was obtained from the participants, both farmers and researchers, for the publication of any potentially identifiable images or data included in this article.

AUTHOR CONTRIBUTIONS

KA and MV: on-field experimentation at Adilabad, designing farmers' survey, interviewing farmers at Adilabad, data collation and analysis, and drafting of manuscript. JK: research ideation, project planning and management, experimentation on-station and on-field, interviewing farmers at Adilabad, and reviewing of manuscript. KC: grain and stover quality assessment and data collation. SC: project planning and management, on-field experimentation and interviewing farmers at Adilabad, data collation and analysis, and reviewing of manuscript. SM: experimentation on-station at ICRISAT and on-field at Adilabad, lysimetric trial on-station, interviewing farmers at Adilabad, and data compilation. SK: lysimetric trial on-station and on-field experimentation and interviewing farmers at Adilabad, and data collation and analysis. VG: on-field experimentation at Adilabad, interviewing farmers, and data analysis. RB: lysimetric trial on-station, on-field experimentation, designing farmers' survey and interviewing farmers at Adilabad, data analysis, and coordinating project activities. KR: networking, organizing on-field farmer-participatory trials, and project activity coordination in Adilabad. SN: designing farmers' survey, Adilabad agri-market insights and strategizing relevant intervention, and reviewing of manuscript. AS: networking on-ground at Adilabad, insights on project operations, and marketable interventions. All authors contributed to the article and approved the submitted version.

FUNDING

This research work was sourced from ongoing projects funded by USAID grant for Climate Resilient Sorghum (2013–18), ACIAR for improving post-rainy sorghum varieties to meet the growing grain and fodder demand in India (2015–18), CGIAR's Crop to End Hunger (CtEH) initiative, and CGIAR Research Program grant for Grain Legumes and Dryland Cereals (CRP-GLDC; 2018–2022). SK was supported by the Global Challenge Research Fund (GCRF)/Biotechnology and Biological Sciences Research Council (BBSRC)-funded project Transforming India's Green Revolution by Research and Empowerment for Sustainable food Supplies (TIGR^{2}ESS; BB/P027970/1; 2018–22).

ACKNOWLEDGMENTS

This work was supported extensively by the research team of Crop Physiology and Modelling (www.gems.icrisat/team/). Special note of thanks to Mr. Vishwanath Mannally, Mr. Amrutha Kumar, Ms. Premalatha Teegalnagaram and Ms. Jayalakshmi Ambhati for their assistance in the activities related to crop management and grain quality assessment. We would like to thank the partner NGO, Centre for Collective Development, and their staff in Utnoor mandal for their support and coordination of activities on-ground. We would like to acknowledge Dr. Michael Blümmel from International Livestock Research Institute (ILRI) for his valuable inputs and Dr. Thannammal Ravichandran for her inputs, enthusiasm and participation in the project activities at Utnoor.

SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fsufs.2022.742909/full#supplementary-material

Supplementary Figure 1 | (A) Trait relationship between grain yield (g plant^{-1}) and harvest index (HI); and **(B)** regression plot between the residual of grain yield not explained by HI vs. stover yield (g plant^{-1}) in water stressed (WS) and well-watered (WW) plants across four genotypes tested in 2019–20 lysimetric trial. Crop varieties are color coded; Full circles (•) indicate WW plants; Open circles (◯) indicate WS plants; solid line (—–) indicates relationship in WW plants; dashed line (—–) indicates relationship in WS plants.

Supplementary Figure 2 | Trait relationship between stover quality (IVOMD, %) and stover yield (g plant^{-1}) in water stressed (WS) and well-watered (WW) plants across four genotypes tested in 2019–20 lysimetric trial. Crop varieties are color coded; Full circles (•) indicate WW plants; Open circles (◯) indicate WS plants; solid line (—–) indicates relationship in WW plants; dashed line (—–) indicates relationship in WS plants.

REFERENCES

Adilabad District Report (2016). Available online at: https://adilabad.telangana.gov.in/document-category/district-profile/ (accessed: March 20, 2021).

Alary, V., Yigez, A. Y., and Bassi, F. M. (2020). Participatory farmers-weighted selection (PWS) indices to raise adoption of durum cultivars. *Crop Breed. Genet. Genom.* 2, 1–20. doi: 10.20900/cbgg20200014

Anitha, S., Givens, D. I., Subramaniam, K., Upadhyay, S., Kane-Potaka, J., Vogtschmidt, Y. D., et al. (2022). Can feeding a millet-based diet improve the growth of children?—A systematic review and meta-analysis. *Nutrients* 14:225. doi: 10.3390/nu14010225

Anitha, S., Govindaraj, M., and Kane-Potaka, J. (2019a). Balanced amino acid and higher micronutrients in millets complements legumes for improved human dietary nutrition. *Cereal Chem.* 97, 74–84. doi: 10.1002/cche.10227

Anitha, S., Kane-Potaka, J., Tsusaka, T. W., Tripathi, D., Upadhyay, S., Kavishwar, A., et al. (2019b). Acceptance and impact of millet-based mid-day meal on the nutritional status of adolescent school going children in a peri urban region of Karnataka State in India. *Nutrients* 11:2077. doi: 10.3390/nu11092077

Appiah-Nkansah, N. B., Li, J., Rooney, W., and Wang, D. (2019). A review of sweet sorghum as a viable renewable bioenergy crop and its techno-economic analysis. *Renew. Energy* 143, 1121–1132. doi: 10.1016/j.renene.2019.05.066

Assefa, Y., Staggenborg, S. A., and Prasad, P. V. V. (2010). Grain sorghum water requirement and responses to drought stress: a review. *Crop Manag.* 9, 1–11. doi: 10.1094/CM-2010-1109-01-RV

Balakrishna, D., Vinodh, R., Madhu, P., and Avinash, S. (2019). "Tissue culture and genetic transformation in Sorghum bicolor," in *Breeding Sorghum for Diverse end Uses*, eds C. Aruna, K. B. R. S. Visarada, B. V. Bhat, V. A. Tonapi (Cambridge: Woodhead Publishing, Elsevier), 115–130. doi: 10.1016/B978-0-08-101879-8.00007-3

Barnes, A. P. (2002). Publicly funded UK agricultural R and D and 'social' total factor productivity. *Agric. Econ.* 27, 65–74. doi: 10.1111/j.1574-0862.2002.tb00105.x

Blümmel, M., Deshpande, S., Kholová, J., and Vadez, V. (2015). Introgression of staygreen QTLs for concomitant improvement of food and fodder traits in *Sorghum bicolor*. *Field Crops Res.* 180, 228–237. doi: 10.1016/j.fcr.2015.06.005

Blümmel, M., Zerbini, E., Reddy, B. V. S., Hash, C. T., Bidinger, F. R., and Khan, A. A. (2003). Improving the production and utilization of sorghum and pearl millet as livestock feed: progress towards dual-purpose genotypes. *Field Crops Res.* 84, 143–158 doi: 10.1016/S0378-4290(03)00146-1

Ceccarelli, S. (1996). "Positive interpretation of genotype by environment interactions in relation to sustainability and biodiversity," in *Plant Adaptation and Crop Improvement*, eds M. Cooper, G. L. Hammers (Wallingford/Hyderabad/ Manila: CAB International/ ICRISAT/IRRI), 467–486.

Ceccarelli, S. (2015). Efficiency of plant breeding. *Crop Sci*. 55, 87–97. doi: 10.2135/cropsci2014.02.0158

Chand, R. (2017). *Doubling Farmers' Income: Rationale, Strategy, Prospects and Action Plan*. NITI Policy Paper no.1/2017, National Institution for Transforming India, New Delhi. Available online at: https://www.niti.gov.in/writereaddata/files/document_publication/DOUBLING%20FARMERS%20INCOME.pdf) (accessed: March 20, 2021).

Chapke, R. R., and Tonapi, V. A. (2019). Adoption and socio-economic benefits of improved post-rainy sorghum production technology. *Agric. Res.* 8, 270–278. doi: 10.1007/s40003-018-0359-x

Choudhary, S., Hash, C. T., Sagar, P., Prasad, K. V. S. V., and Blümmel, M. (2010). "Near infrared spectroscopy estimation of pearl millet grain composition and feed quality," in *Proceedings of the 14th International Conference on NIRS Spectroscopy, Bangkok, November*, eds S. Saranwong, S. Kasemsumran, W. Thanapase, P. Williams (Chichester: IM Publications), 87–90.

DACNET (2016). *Department of Agriculture Cooperation*, Government of India. Available online at: https://eands.dacnet.nic.in/PDF/Glance-2016.pdf (accessed: March 20, 2021).

de la Peña, I., Garrett, J., and Gelli, A. (2018). *Nutrition-sensitive value chains from a smallholder perspective: A framework for project design*. Rome: International Fund for Agricultural Development (IFAD). Available online at: https://www.ifad.org/en/web/knowledge/-/publication/research-series-issue-30-nutrition-sensitive-value-chains-from-a-smallholder-perspective-a-framework-for-project-design (accessed on March 20, 2021).

DeFries, R., Chhartre, A., Davis, K. F., Dutta, A., Fanzo, J., Ghosh-Jerath, S., et al. (2018). Impact of historical changes in coarse cereals consumption in India on micronutrient intake and anemia prevalence. *Food Nutr. Bull.* 39, 377–392. doi: 10.1177/0379572118783492

Erenstein, O., Samaddar, A., Teufel, N., and Blümmel, M. (2011). The paradox of limited maize stover use in India's small holder crop-livestock systems. *Exp. Agric.* 47, 677–704. doi: 10.1017/S0014479711000433

Fadda, C., and Van Etten, J. (2019). "Generating farm-validated variety recommendations for climate adaptation," in *The Climate-Smart Agriculture Papers*, eds T. S. Rosenstock, A. Nowak, E. Girvetz (Cham: Springer), 127–138. doi: 10.1007/978-3-319-92798-5_11

FAOSTAT, Food and Agriculture Organization of the United Nations. (2020). Available online at: https://www.fao.org/faostat/en/#data/QCL (accessed on March 20, 2021).

Gadakh, S. R., Shinde, M. S., and Gaikwad, A. R. (2013). Postrainy season sorghum varieties developed at All India Coordinated Sorghum Improvement Project, MPKV, Rahuri. *SAT J.* 11, 1–2.

Hall, A., Blümmel, M., Thorpe, W., Bidinger, F. R., and Hash, C. T. (2004). Sorghum and pearl millet as food-feed-crops in India. *Anim. Nutr. Feed Technol.* 4, 1–15.

Hall, A., Sulaiman, R. V., and Bezkorowajny, J. P. (2007). *Reframing Technical Change: Livestock Fodder Scarcity Revisited as Innovation Capacity Scarcity – A Conceptual Framework*. Nairobi, Kenya: ILRI and UNU/MERIT. Available online at: https://hdl.handle.net/10568/277 (accessed: March 20, 2021).

Humphries, S., Rosas, J. C., Gómez, M., Jiménez, J., Sierra, F., Gallardo, O., et al. (2015). Synergies at the interface of farmer–scientist partnerships: agricultural innovation through participatory research and plant breeding in Honduras. *Agric. Food Secur.* 4:27. doi: 10.1186/s40066-015-0046-0

Jiménez, D., Dorado, H., Cock, J., Prager, S. D., Delerce, S., Grillon, A., et al. (2016). From observation to information: data-driven understanding of on farm yield variation. *PLoS ONE.* 11:e0150015. doi: 10.1371/journal.pone.0150015

Kholová, J., McLean, G., Vadez, V., Craufurd, P., and Hammer, G. L. (2013). Drought stress characterization of post-rainy season (rabi) sorghum in India. *Field Crops Res.* 141, 38–46. doi: 10.1016/j.fcr.2012.10.020

Kholová, J., Oldrich Urban, M., Cock, J., Arcos, J., Arnaud, E., Aytekin, D., et al. (2021). In pursuit of a better world: crop improvement and the CGIAR, *J. Exp. Bot.* 72, 5158–5179. doi: 10.1093/jxb/erab226

Kristjanson, P. M., and Zerbini, E. (1999). *Genetic enhancement of sorghum and millet residues fed to ruminants: An ex-ante assessment of returns to research: ILRI Impact Assessment Series No. 3*. Nairobi: International Livestock Research

Institute. Available online at: https://hdl.handle.net/10568/497 (accessed on March 11, 2021).

Kumar, A. A. (2016). “Botany, taxonomy and breeding,” in *The Sorghum Genome: Compendium of Plant Genomes*, eds. S. Rakshit and Y. H. Wang (Cham: Springer), 27–45. doi: 10.1007/978-3-319-47789-3_2

Kumar, A. A., Reddy, B. V. S., Sahrawat, K. L., and Ramaiah, B. (2010). Combating micronutrient malnutrition: Identification of commercial sorghum varieties with high grain iron and zinc. *J. SAT Agric. Res.* 8, 1–5.

Kumar, A. A., Reddy, B. V. S., Sharma, H. C., Hash, C. T., Rao, P. S., Ramaiah, B., et al. (2011). Recent advances in sorghum genetic enhancement research at ICRISAT. *Am.J. Plant Sci.* 2, 589–600. doi: 10.4236/ajps.2011.24070

Kumar, K. A. (2010). *Local Knowledge and Agricultural Sustainability: A Case Study of Pradhan Tribe in Adilabad District (Working Paper No. 81)*. Begumpet: Centre for Economic and Social Studies.

Kumar, S., Murthy, D. K., Gumma, M., Khan, E., Khatri-Chhetri, A., Aggarwal, P., et al. (2018). *Towards climate-smart agricultural policies and investments in Telangana*. Telangana: CGIAR Research Program on Climate Change, Agriculture and Food Security. Available online at: https://hdl.handle.net/10568/90627 (accessed on March 11, 2021).

Kumara, C. D., Bantilan, M. C. S., Rajalaxmi, A., Reddy, B. V. S., Borikar, S. T., Kumar, A. A., et al. (2014). *Development and Diffusion of Sorghum Improved Varieties in India: Impact on Growth and Stability in Yield* (Working Paper Series no. 50). Patancheru: International Crops Research Institute for the Semi-Arid Tropics.

Nagaraj, N., Basavaraj, G., Rao, P. P., Bantilan, M. C. S., and Haldar, S. (2013). Sorghum and pearl millet economy of India: future outlook and options. *Econ. Polit. Wkly.* 48, 74–81.

NAIP Project Report (2012). *Harmonizing Biodiversity Conservation and Agricultural Intensification Through Integration of Plant, Animal and Fish Genetic Resources for Livelihood Security in Fragile Ecosystems*. Available online at: http://www.nbpgr.ernet.in:8080/NAIP-GEF_3/index_naip-gef_3.html (accessed: January 10, 2021).

NFHS (2016). *National Family Health Survey of Adilabad District 2015–16*. Available online at: http://rchiips.org/nfhs/FCTS/TG/TG_FactSheet_532_Adilabad.pdf (accessed: January 10, 2021).

NITI Aayog Report (2018). *Adilabad District Report*. Available online at: https://www.niti.gov.in/niti/hi/best-practices/district-wise-statistics?term_node_tid_depth=188 (accessed: October 10, 2020).

Padmaja, R., Gebremarium, H., and Kavitha, K. (2018). *Talking nutrition with adolescents in tribal regions of Telangana*. Available online at: http://gldc.cgiar.org/talking-nutrition-with-adolescents-in-tribal-regions-of-telangana/ (accessed on March 7, 2022).

Padmaja, R., and Kavitha, K. (2017). *Food basket of sorghum, millet and legume products to address malnutrition in Telangana, India*. ICRISAT Happenings. Available online at: https://www.icrisat.org/food-basket-of-sorghum-millet-and-legume-products-to-address-malnutrition-in-telangana-india/ (accessed on March 7, 2022).

Paltasingh, T., and Paliwal, G. (2014). Tribal population in India: regional dimensions and imperatives. *J. Reg. Dev. Plan.* 3, 27–36. Available online at: https://ideas.repec.org/a/ris/jrdpin/0032.html (accessed on March 20, 2021).

Pandravada, S. R., Sivaraj, N., Kamala, V., Sunil, N., Sarath Babu, B., and Varaprasad, K. S. (2008). "Agri-biodiversity of Eastern Ghats - Exploration, collection and conservation of crop genetic resources," in Proceedings of the National Seminar on Conservation of Eastern Ghats. (Hyderabad: EPTRI), 11-12. Available online at: http://www.apenvis.nic.in/All%20PDF%20Files/COMMON/Andhra%20Pradesh.pdf (accessed on March 20, 2021).

Pandravada, S. R., Sivaraj, N., Sunil, N., Jairam, R., Prasanthi, Y., Chakrabarty, S. K., et al. (2013). *Sorghum landraces* patronized by tribal communities in Adilabad district, Andhra Pradesh. *Indian J. Tradit. Knowl.* 12, 465–471.

Paroda, R. S. (2018). *Strategy for Doubling Farmers' Income (Strategy Paper)*. New Delhi: Trust for Advancement of Agricultural Sciences. doi: 10.1079/9781786395177.0274

Patil, J. V., Reddy, S. P., Prabhakar, A., Umakanth, V., Gomashe, S., and Ganapathy, K. N. (2014). History of post-rainy season sorghum research in India and strategies for breaking the yield plateau. *Indian J. Genet. Plant Breed.* 74, 271–2285. doi: 10.5958/0975-6906.2014.00845.1

Phuke, R. M., Anuradha, K., Radhika, K., Jabeen, F., Anuradha, G., Ramesh, T., et al. (2017). Genetic variability, genotype × environment interaction, correlation, and GGE biplot analysis for grain iron and zinc concentration and other agronomic traits in RIL population of sorghum (*Sorghum bicolor* L. *Moench). Front. Plant Sci.* 8:712. doi: 10.3389/fpls.2017.00712

Poshadri, A., Kumar, P. Y., Kumar, S. M., Charan, S. G., Raghuveer, M., and Devi, R. A. (2019). Backyard kitchen gardening for nutritional security in tribal areas of Adilabad district, India. *Int. J. Chem. Stud.* 7, 4502–4504.

Prasad, R., Benhur, D., Kommi, K., Madhari, R., Rao, V. M., and Patil, J. V. (2016). Impact of sorghum supplementation on growth and micronutrient status of school going children in southern India: a randomized trial. *Indian J. Pediatr.* 83, 9–14. doi: 10.1007/s12098-015-1782-7

Prasad, R., Shivay, Y. S., and Kumar, D. (2014). Agronomic biofortification of cereal grains with iron and zinc. *Adv. Agron.* 125, 55–91. doi: 10.1016/B978-0-12-800137-0.00002-9

Rajani, K. (2018). *Preserving Legacy Grains: The Persa Jonna story*. Agribuzz-ICRISAT Happenings Newsletter. No. 1755, 22 June 2018. Available online at: https://www.icrisat.org/preserving-legacy-grains-the-persa-jonna-story/

Rao, D. B., Seetharama, A., Suresh, A., Sreekanth, M., Reddy Nirmal, K., and Rao, S. V. (2010). *Dynamics of Value and Trade Channels of Sorghum in India.* Rajendranagar: Directorate of Sorghum Research.

Rao, P. P., and Hall, A. (2003). Importance of crop residues in crop-livestock systems in India and farmers' perceptions of fodder quality in coarse cereals. *Field Crop Res.* 84, 189–198. doi: 10.1016/S0378-4290(03)00150-3

Reddy, M. T., Pandravada, S. R., Sivaraj, N., Kamala, V., Sunil, N., and Dikshit, N. (2017). Classification and characterization of landscapes in the territory of Adilabad district, Telangana, Deccan Region, India. *OA Lib. J.* 4:e3745. doi: 10.4236/oalib.1103745

Reddy, S., Reddy, B. V. S., and Kumar, A. (2009). M 35-1 derived sorghum varieties for cultivation during the postrainy season. *J. SAT Agric Res.* 7:4.

Sharma, K., Pattanaik, A. K., Anadan, S., and Blümmel, M. (2010). Food-feed crop research: a synthesis. *Anim. Nutr Feed Technol.* 10, 1–10.

Singh, Y. P., Nayak, A. K., Sharma, D. K., Gautam, R. K., Singh, R. K., Singh, R., et al. (2014). Farmers' participatory variety selection: a suitable crop improvement approach for 21st century. *Agroecol. Sustain. Food Syst.* 38, 427–444. doi: 10.1080/21683565.2013.870101

Sivaraj, N., Elangovan, M., Kamala, V., Pandravada, S. R., Pranusha, P., and Chakrabarty, S. K. (2016). Maximum entropy (Maxent) approach to sorghum landraces distribution modelling. *Indian J. Plant Genet. Resour.* 29, 16–21. doi: 10.5958/0976-1926.2016.00004.8

Sivaraj, N., Pandravada, S. R., Jairam, R., Sunil, N., Varaprasad, K. S., Ramesh, P., et al. (2012). An indigenous cost-effective innovation for bird scares by the Gond tribe in Adilabad district, Andhra Pradesh. *Indian J. Tradit. Knowl.* 11, 714–718.

Sridhara, S., Ramesh, N., Gopakkali, P., Das, B., Venkatappa, S. D., Sanjivaiah, S. H., et al. (2020). Weather-based neural network, stepwise linear and sparse regression approach for rabi sorghum yield forecasting of Karnataka, India. *Agron* 10:1645. doi: 10.3390/agronomy10111645

Srivastava, A., Kumar, N. S., and Aggarwal, P. K. (2010). Assessment on vulnerability of sorghum to climate change in India. *Agric. Ecosyst. Environ.* 138, 160–169. doi: 10.1016/j.agee.2010.04.012

Srivastava, V. K. (2018). The National Committee report on tribal people. *Soc. Change* 48, 120–130. doi: 10.1177/0049085717743843

Task Force Report (2019). *Agriculture Challenges and Way Forward (Submitted to NITI Aayog), Government of Telangana, Agriculture and Cooperation Department, 47.* Available online at: http://niti.gov.in/writereaddata/files/Telangana_Report_1.pdf (accessed: April 11, 2021).

Telangana districts profile report (2018). Available online at: https://data.telangana.gov.in/dataset/adilabad-district-mandal-wise-crop-areas (accessed: March 10, 2021).

Thiele, G., Dufour, D., Vernier, P., Mwanga, R. O. M., Parker, M. L., Schulte Geldermann, E., et al. (2020). A review of varietal change in roots, tubers and bananas: consumer preferences and other drivers of adoption and implications for breeding. *Int. J. Food Sci. Technol.* 56, 1076–1092. doi: 10.1111/ijfs.14684

Trivedi, T. P. (2008). *Handbook of Agriculture*. New Delhi, India: Directorate of Information and Publications of Agriculture, Indian Council of Agricultural Research.

Upadhyaya, H. D., Dwivedi, S. L., Wang, Y. H., Vetriventhan, M., Ciampitti, I., and Prasad, V. (2016). "Sorghum genetic resources", in *Sorghum*, eds I. Ciampitti, V. E. Prasad (Washington, DC: American Society of Agronomy and Crop Science Society of America, Inc.), 1–27. doi: 10.2134/agronmonogr58.2014.0056.5

Vadez, V., Krishnamurthy, L., Hash, C. T., Upadhyaya, H. D., and Borrell, A. K. (2011). Yield, transpiration efficiency, and water-use variations and their interrelationships in the sorghum reference collection. *Crop Pasture Sci.* 62, 645–655. doi: 10.1071/CP11007

Voorhaar, M., and Anbazhagan, K. (2019). *A Farmer's Choice: Tribal Communities in Adilabad Select Their Preferred Sorghum Varieties*. Agribuzz-ICRISAT Happenings. Available online at: https://www.icrisat.org/tribal-communities-in-adilabad-select-their-preferred-sorghum-varieties/ (accessed: March 20, 2021).

Wale, E., and Yalew, A. (2007). Farmers' variety attribute preferences: implications for breeding priority setting and agricultural extension policy in Ethiopia. *Afr. Dev. Rev.* 19, 379–396. doi: 10.1111/j.1467-8268.2007.00167.x

Yadav, J., Ojha, A. K., George, C., Sajeevan, R., Singh, G., Bhargav, H., et al. (2011). *All India Report on Agriculture Census 2010–2011*. Available online at: https://agricoop.nic.in/sites/default/files/air2010-11complete.pdf (accessed: March 20, 2021).

Zerbini, E., and Thomas, D. (2003). Opportunities for improvement of nutritive value in sorghum and pearl millet residues in South Asia through genetic enhancement. *Field Crop Res.* 84, 3–15. doi: 10.1016/S0378-4290(03)00137-0

Conflict of Interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's Note: All claims expressed in this article are solely those of the authors and do not necessarily represent those of their affiliated organizations, or those of the publisher, the editors and the reviewers. Any product that may be evaluated in this article, or claim that may be made by its manufacturer, is not guaranteed or endorsed by the publisher.

frontiers in Nutrition

ORIGINAL RESEARCH
published: 24 March 2022
doi: 10.3389/fnut.2021.791392

A Natural Low Phytic Acid Finger Millet Accession Significantly Improves Iron Bioavailability in Indian Women

Bellam H. Rajashekar Reddy[1‡], Prashanth Thankachan[1‡], Masoami Hatakayama[2,3], Netravati Hiremath[4], Diego Moretti[5†], Yellodu A. Nanjareddy[6], Mathi B. Thumilan[6], Ramapura L. Ravikumar[7], Shamprasad Phadnis[7], Beena Bose[1], Lucy Poveda[3], Geetha Kalaiah[4], Michael B. Zimmermann[5], Kentaro K. Shimizu[2], Ralph Schlapbach[3], Anura V. Kurpad[1,8§] and Sheshshayee M. Sreeman[6*§]

[1] Division of Nutrition, St. John's Research Institute, St. John's National Academy of Health Sciences, Bengaluru, India, [2] Department of Evolutionary Biology and Environmental Studies, University of Zurich, Zurich, Switzerland, [3] Functional Genomics Center Zurich, Zurich, Switzerland, [4] All India Coordinated Research Project (Foods and Nutrition), University of Agricultural Sciences, Bengaluru, India, [5] Laboratory for Human Nutrition, Institute of Food, Nutrition and Health, ETH Zurich, Zurich, Switzerland, [6] Department of Crop Physiology, University of Agricultural Sciences, Bengaluru, India, [7] Department of Biotechnology, University of Agricultural Sciences, Bengaluru, India, [8] Department of Physiology, St. John's Medical College, St. John's National Academy of Health Sciences, Bengaluru, India

OPEN ACCESS

Edited by:
Seetha Anitha, International Crops Research Institute for the Semi-Arid Tropics (ICRISAT), India

Reviewed by:
Matthew John Milner, National Institute of Agricultural Botany (NIAB), United Kingdom
Hengjia Ni, Institute of Subtropical Agriculture, Chinese Academy of Sciences (CAS), China

***Correspondence:**
Sheshshayee M. Sreeman
msshesh1@uasbangalore.edu.in

†Present address:
Diego Moretti, Health Department, Swiss Distance University of Applied Sciences (FFHS), Brig, Switzerland

‡These authors have contributed equally to this work
§These authors have contributed equally to this work and share last authorship

Specialty section:
This article was submitted to Nutrition and Sustainable Diets, a section of the journal Frontiers in Nutrition

Received: 08 October 2021
Accepted: 12 November 2021
Published: 24 March 2022

Citation:
Reddy BHR, Thankachan P, Hatakayama M, Hiremath N, Moretti D, Nanjareddy YA, Thumilan MB, Ravikumar RL, Phadnis S, Bose B, Poveda L, Kalaiah G, Zimmermann MB, Shimizu KK, Schlapbach R, Kurpad AV and Sreeman SM (2022) A Natural Low Phytic Acid Finger Millet Accession Significantly Improves Iron Bioavailability in Indian Women. Front. Nutr. 8:791392. doi: 10.3389/fnut.2021.791392

Iron deficiency and anemia are common in low- and middle-income countries. This is due to a poor dietary iron density and low iron absorption resulting from the high inhibitory phytic acid content in cereal and millet-based diets. Here, we report that a naturally occurring low phytic acid finger millet accession (571 mg 100 g^{-1}), stable across three growing seasons with normal iron content (3.6 mg 100 g^{-1}), increases iron absorption by 3-folds in normal Indian women. The accessions differing in grain phytic acid content, GE 2358 (low), and GE1004 (high) were selected from a core collection of 623 accessions. Whole genome re-sequencing of the accessions revealed significant single nucleotide variations segregating them into distinct clades. A non-synonymous mutation in the *EcABCC* phytic acid transporter gene between high and low accessions could affect gene function and result in phytic acid differences. The highly sensitive dual stable-isotope erythrocyte incorporation method was adopted to assess the fractional iron absorption. The low phytic acid accession resulted in a significantly higher iron absorption compared with the high phytic acid accession (3.7 vs. 1.3%, $p < 0.05$). The low phytic acid accession could be effective in preventing iron deficiency in regions where finger millet is habitually eaten. With its low water requirement, finger millet leaves low environmental footprints and hence would be an excellent sustainable strategy to mitigate iron deficiency.

Keywords: finger millet, grain phytic acid, iron deficiency anemia (IDA), bioavailability, stable isotope

INTRODUCTION

The prevalence of iron deficiency (ID) and iron deficiency anemia (IDA) is high in many parts of the world (1). For example, in India, the prevalence of all-cause anemia in women of reproductive age (WRA) was reported to be 53% in a national survey (2). Globally, the most significant contributor to anemia is thought to be the deficiency of iron (3). In

India, where the daily diet has a low iron density of about 8.5 mg 1,000 kcal^{-1} (4), a mild risk of ID could occur with cereal based diets, which may not deliver the complete daily iron requirement of 15 mg day^{-1} in WRA (5) and 18 mg day^{-1} in adolescent children (6). Since the net iron absorbed is the product of the iron content of food and its bioavailability, either of these (or both) could be altered when trying to improve the iron availability to humans. Thus, one strategy is to increase the iron density in staple food crops either by conventional breeding, biotechnology techniques, or agronomic approaches (7). However, this does not address iron absorption and could even lower it. For example, in common beans (8) and pearl millet (9), the net iron absorbed from iron biofortified varieties was higher compared to control varieties. However, the fractional iron absorption remained the same (in biofortified pearl millet) but was lower (in biofortified common beans) compared to their respective controls. Other strategies to improve iron density, such as food iron fortification, are also likely to have a low impact since the absorption of iron from fortified staple foods (that are high in inhibitors) in efficacy trials has been uniformly low, ranging from about 1–2% (10–12). It is known that delivering high doses of relatively non-bioavailable iron to the intestinal mucosa can result in higher hepcidin levels that reduce iron absorption by up to 50% (13), and therefore the strategy of increasing iron density of grains, while more subtle in approach than supplementation, might still eventually be limited in success in the long term. There may also be a downside in the delivery of more iron into the body, whether it is absorbed from the intestine or not, since unabsorbed iron has a negative impact on the gut microbiome (14), while increased absorbed iron can increase the risk of many chronic diseases through a variety of effects (15).

The other, and perhaps safer and better, strategy in the prevention of ID is to improve iron absorption. The usual monotonous cereal-based and generally vegetarian diet are inhibitory due to the high content of iron absorption inhibitors such as phytic acid and polyphenols, and the low content of absorption enhancers like vitamin C. Phytic acid (*myo*-Inositol-1,2,3,4,5,6-hexa*kis*phosphate) a phosphorous storage molecule in plant seeds and ubiquitous to eukaryotic cells (16). During seed development, phytic acid is accumulated in the seed and readily chelates mineral cations like Mg, Fe, K, Cu, and Zn to form mixed salts known as phytate or phytin, which act as an antioxidant in the seed (17, 18). From the perspective of the plant, these mixed salts of phytic acid act as a store for phosphorous, inositol, and mineral cations (19). These stored nutrients are broken down and retrieved during the seed germination process by the enzymatic action of phytase (20). Monogastric animals, including humans, lack phytase in their gastrointestinal tract, and this makes the phytate bound nutrients unavailable for absorption (21). One way to overcome these inhibitory effects is by increasing diet diversity that includes increased intake of fruits that contain vitamin C, which is a facilitator of iron absorption. Vitamin C overcomes the inhibitory effect of phytate or polyphenols on iron absorption when available in an appropriate molar ratio to the meal iron content (4, 22). Another key strategy for increasing iron absorption from diets is by reducing the dietary phytic acid content (23), through the introduction of the phytase enzyme into grain flours (24), but requires specific conditions for effective enzymatic activity (25). This strategy will be effective only if most phytate is removed. For instance, in one study, 95% dephytinization of test meals made with biofortified beans resulted in an increase in fractional iron absorption by 51% compared to control (8). Though these methods are effective, they are expensive and possibly unsustainable. It might appear that the more sensible and sustainable option is to improve iron absorption by finding or developing low phytic acid cereal or millet grains for farming. Strategies to reduce the grain phytic acid (GPA) content of grain crops include either the development of low GPA mutants by altering the biosynthetic pathway and transport genes (26), or the identification and selection of natural variants with a low GPA content (27, 28).

This study evaluated how iron absorption could be improved in finger millet, which is culturally popular and habitually consumed in India (29) as well as in other parts of the world (30). It is rich in minerals like iron and calcium and has a high content of dietary fiber (31). However, although its iron content is thought to be high, its bioavailability is lower than other cereals (4.6% in ragi, 8.3% in white rice, and 11.2% in whole wheat *Atta* flour measured by isotopic meal-based iron absorption studies in women with ID) (32), due to its phytic acid and tannin content. Therefore, this study aimed to select a natural and consistently low GPA content finger millet accession from a collection of 623 accessions that represented their global diversity (33). Along with the phenotypic characteristics, allelic variations in the GPA biosynthetic pathway and transporter gene(s), from whole genome sequences of this accession, were compared with those of a selected high GPA accession. Finally, the translational potential of the low GPA content accession in promoting iron absorption was measured in human subjects using stable isotopes of iron (34).

MATERIALS AND METHODS

Development of a Diversity Panel From a Core Collection of 623 Finger Millet Accessions

A "core" collection consisting of 623 finger millet accessions representing global diversity (33) was acquired from the All India Coordinated Research Project on small millets, GKVK campus, Bangalore, India. These were grown in a field experiment during the first production season of 2015, at the University of Agricultural Sciences (UASB), Bangalore (13.05°N, 77.34°E). Phenotyping for 18 quantitative traits was carried out and trait values were recorded following guidelines defined by International Board for Plant Genetic Resources (IBPGR, 1985) (35). The detailed crop production activities and the measurement of phenotypic traits are described in **Supplementary Text A**. For GPA measurement, bulk seed samples of each accession were dried in a hot air oven at 60°C for 48 h and ground to a fine flour by using a ball mill and stored in an airtight container until analysis. The GPA content in each of the core 623 finger millet accessions was measured in triplicates using a modified high throughput Wade colorimetric assay (36). A common finger millet accession (MR-6) was used to monitor inter assay variation and GPA values were accepted when the CV was <5% between the assays.

Molecular diversity analysis of the 623 core accessions was performed by using previously reported 35 simple sequence repeat (SSR) markers (37) (**Supplementary Table 1**) from finger millet. Protocol for genomic DNA isolation, specific reaction conditions to perform polymerase reactions, and marker scoring are described in **Supplementary Text B**.

The phenotypic and molecular data of the 623 accessions (2015) was analyzed using POWERCORE 1.0 Software (38) to develop a "diversity panel" of 350 finger millet accessions, representing the wide diversity present in the original core panel. The accessions from the diversity panel were grown again in production season 2016 at UASB as described earlier. In the 350 accessions grown, 75 showed poor germination and low crop stand. Therefore, these accessions were not considered for GPA analysis. To ascertain if the remaining 275 accessions were representative of 350 "diversity panel" accessions, the means, and variances of all 19 traits (including GPA) between 350 and 275 accessions were comparable.

Selection of Finger Millet Accessions With High and Low GPA Content From the Diversity Panel

A two-step approach was undertaken for the final selection of low and high GPA content accessions for human testing. First, 19 accessions were selected in a stratified manner which is evenly distributed for GPA across the 275 diversity panel. Second, the GPA content was rigorously tested through a validating measurement at an external laboratory (Human Nutritional Laboratory, ETH, Zurich). This validating analysis used the modified Makower method (39) with certified wheat bran as quality control (phytic acid concentration range: 4.16–5.42 g 100 g^{-1}). The iron content of the 19 selected accessions was also quantified at the Human Nutritional Laboratory, ETH, Zurich, using an Atomic Absorption spectrophotometer (AAS) (39).

Finally, from the selected 19 accessions, one high (GE 1004) and one low type (GE 2358), which showed consistently high and low GPA content in both production seasons, as well as consistent GPA values between the analytical laboratories, were selected for evaluation of iron bioavailability in human feeding trials. The selected two finger millet accessions were grown during the production season 2017 at UASB on a larger area of land (between July and November) to produce the quantity of seed required (∼5 kg each) for the human iron bioavailability study.

Identification of Allelic Variations in GPA Biosynthetic Pathway and Transport Genes

The whole genomes of the selected 19 accessions, were re-sequenced on the Illumina HiSeq4000 platform (Illumina, Inc., California, USA) by 150 bp paired-end libraries with an average 10X coverage of the genome. The sequenced reads were aligned to the updated PR202 reference sequence (**Supplementary Text C**) by BWA (v0.7.17) with trimming and filtering low quality reads by Trimmomatic (v 0.36) pre-processing. The aligned reads were analyzed and classified into A and B sub-genomes by EAGLERC (v1.1.1) software (40). For each of the 19 accessions, SNP calling was performed independently for the two sub-genomes by GATK (v4.1.2.0) which revealed 7,732,239 and 8,743,397 SNPs, respectively. False positive SNPs were removed by aligning the sequences of selected 19 accessions with the reference sequence of PR 202. Common SNPs in 19 accessions were considered false and omitted from further analysis.

To identify SNPs associated with GPA content, 18 genes involved in GPA biosynthetic pathway and transport (26) were selected from the rice genome database, IRGSP-1. BLAST comparison of these 18 genes with the draft genome sequence of PR202 was performed. Homologous sequences of these 18 genes among the 19 accessions were aligned to identify SNPs using GATK (v4.1.2.0). To assess the genetic diversity/relatedness across 19 accessions, the SNPs identified were subjected to Neighbor joining (NJ) tree and Principal component analyses (PCA) using VCF-Kit (ver. 0.2.9) and PLINK (ver. 1.90beta6.21). *In silico* analysis and protein structure prediction were performed to validate the SNP's between high and low GPA accessions (**Supplementary Text D**).

Measurement of Iron Absorption in Humans From Low and High GPA Content Finger Millet Accessions

From the 2017 season, seeds of the selected accessions (GE2358; GE1004) were ground to a fine flour using a custom-made Teflon coated grinder equipped with titanium blades (Cingularity, Bengaluru) to avoid any external iron contamination. The flour was stored at 4°C until the test meals were prepared. A culturally acceptable unleavened flat bread (*Ragi roti*) was made from flour and used as a test meal. The recipe was standardized in the metabolic kitchen of the Division of Nutrition at St. John's Research Institute, Bengaluru (**Supplementary Text E**). The nutrient composition of the test meal is presented in (**Supplementary Table 2**) and was within the macronutrient requirements for a breakfast meal that provided one-fourth of daily energy and protein requirement of healthy sedentary women (41) and the remaining nutritional requirements of subjects were met through their habitual dietary intake (as lunch, dinner, and one snack).

A total of 20 healthy young women were screened from the students and junior staff population of UAS, Bengaluru, for the study. This study site was chosen as the preparation of *Ragi roti* is a commonly consumed breakfast/lunch meal in this population. About 10 subjects with no reported chronic medical illnesses, with no infection (C-reactive protein, CRP <10 mg L^{-1}), who were not pregnant or lactating, with normal iron status (Hb > 12 g dL^{-1}, serum ferritin >15 μg L^{-1}) were included in the study. Those who were taking vitamin or mineral supplementation within the last month prior to the study were excluded. The study protocol was approved by the Institutional Ethical Committee at St. John's Medical College, Bengaluru, India (IERB No:87/2015), registered at the Clinical Trials Registry of India as CTRI/2020/01/02267, and informed written consent was obtained from all subjects.

On the baseline collection day (Day 1), participants reported to the study location at 8 AM after an overnight fast. Their height and weight were measured using standardized procedures followed by a basal venous blood sample (6 mL) collection in an EDTA vacutainer (2 mL, Becton Dickenson, NJ, USA) and serum collection tube (4 mL, Becton Dickenson, NJ, USA). Whole blood (2 mL collected in a plain vacutainer) for analysis of the basal Fe isotopic enrichment in Hb, was stored at −80○C. Serum (1.5 mL) obtained after refrigerated centrifugation of whole blood at 3,500 rpm (Eppendorf, 5810 R, Germany) was stored at −20○C until analysis.

A crossover study design using each millet type (high and low GPA content) was followed for measuring iron absorption. The absorption is measured as a daily single meal protocol, that is, the iron absorbed from a single test meal. The test meals were administered each morning for 6 consecutive days, and subjects randomly received either high or low GPA test meals for three days each in succession. Just prior to intake by the subject, the test meals containing low or high GPA content finger millet were extrinsically labeled with $^{57}FeSO_4$ or $^{58}FeSO_4$, respectively. The dose of $^{57}FeSO_4$ and $^{58}FeSO_4$ were prepared as described elsewhere (22). The isotopic label added to the meal was equivalent to ∼33% of the meal's native iron content. The dose of isotope label administered was gravimetrically determined and evenly dispensed onto the test meal, just before administration. The subjects were instructed to refrain from food and drink consumption until 3 h after a meal, post which they continued their habitual dietary intake. For the remaining 5 days, the same test meal administration protocol was followed, except that on days 4–6, the alternate GPA content test meal was used, with the alternate Fe isotope. On Day 20, that is, 14 days after the last test meal administration, a fasted venous blood sample (6 mL) was collected from the subjects, aliquoted, and stored as described earlier. The concentrations of Hb, serum ferritin, and CRP were measured on day 1 and day 20 (**Supplementary Text F**). All measurements were performed in duplicate.

The shift in isotopic ratios of $^{57}Fe/^{56}Fe$ and $^{58}Fe/^{56}Fe$ in Hb of the blood samples was analyzed in duplicate as described by Walczyk et al. (42). After mineralization, chromatographic separation, and extraction of iron from blood samples, the iron isotopic composition of the samples was determined by NTIMS (Triton, Thermo, Bremen, Germany) with a multicollector system (**Supplementary Text G**). The amount of circulating isotope label was calculated on the basis of the shift in the isotopic ratios and the amount of circulating iron in the blood. Calculations were based on principles of dilution, and the non-monoisotopic nature of the labels was taken into consideration (42). Circulating iron was calculated on the basis of blood volume and Hb concentration and 80% incorporation of the absorbed iron into erythrocytes was assumed (34). The observed shift in iron isotope ratios was converted to fractional iron absorption using standard algorithms (42).

STATISTICAL ANALYSIS

The data are presented as mean ±SD. The comparison of the 18 traits between the core collection ($n = 623$), diversity panel ($n = 350$), and the successfully grown set ($n = 275$) was performed by analysis of variance (ANOVA) followed by *post-hoc* Newman–Keuls tests. To assess the level of diversity captured in the diversity panel from the core collection, mean difference (MD%), coincidence rate (CR%), and variable rate (VR%) were calculated as described elsewhere (43). Shannon and Weaver's (44) diversity index (H') were used to measure and compare the phenotypic diversity for each trait in the core collection and diversity panel. Data for GPA content for both seasons was tested for normality using the Shapiro-Wilk test. Genetic, phenotypic, and environmental variances and their CV were estimated as described elsewhere (45, 46). GPA content was correlated between seasons and labs by Pearson correlation. The agreement between the analytical laboratory estimates of GPA content was evaluated by the Bland–Altman method (47). For the human iron absorption study, the number of subjects ($n = 10$) had 80% power to detect a significant difference of 50% in iron absorption between the two accession test meals. The paired student's t-test was used to test differences between the log transformed iron absorption values from the low and high GPA content accessions and reconverted for reporting. Statistical analyses were conducted using SPSS software (version 17.0) and differences were considered significant at $p < 0.05$.

RESULTS

Development of Finger Millet Diversity Panel

The mean, range, and variance of the 19 traits of the 623 accessions of the core collection are given in **Table 1**. Out of the 35-simple sequence repeat (SSR) markers screened for molecular characterization of the core collection, 8 primer pairs showed polymorphism. Both phenotypic and molecular diversities were analyzed by POWERCORE, from which the diversity panel of 350 accessions was assembled representing the original diversity of the core collection. Of the 19 traits, the mean values of 14 traits were not significantly different ($p > 0.05$) between the diversity panel and the core collection. The mean difference (MD%) of traits, between the core collection and diversity panel, were within the acceptable range of <20% and the coincidence rate (CR%) of the latter was 99.3 ± 0.5 for all traits (**Supplementary Table 3**). The mean Shannon weaver diversity index (H') for the 19 traits in the core collection (6.34 ± 0.09) and diversity panel (5.75 ± 0.10) was comparable (**Supplementary Table 3**). When analyzed for their GPA content (**Supplementary File 1**), the core collection showed

TABLE 1 | Comparison of mean, range, and variance for 19 quantitative traits in finger millet core collection ($n = 623$) and diversity panel ($n = 350$).

Sl No.	Trait	Range		Mean			Variance	
		$n = 623$	$n = 350$	$n = 623$	$n = 350$	*p*-value	$n = 623$	$n = 350$
1	Ear head emergence (days)	45.0–79.0	45.0–79.0	59.9	60.3	0.27	39.9	40.2
2	Plant height (cm)	45.0–146.0	45.0–146.0	94.4	95.5	0.34	278.6	329.2
3	Productive tiller (no plant^{-1})	1.4–8.4	1.4–8.4	3.9	4.0	0.15	1.3	1.6
4	Unproductive tiller (no plant^{-1})	0.1–2.0	0.1–2.0	0.5	0.5	0.12	0.1	0.1
5	Productive tiller ratio (%)	60.0–100.0	60.0–100.0	90.4	90.0	0.44	48.5	55.3
6	Leaf number (no plant^{-1})	11.9–96.0	12.2–96.0	36.7	38.4	0.05	156.6	194.5
7	Leaf area (cm^2 plant^{-1})	268.2–4685.1	277.1–4685.1	1256.4	1349.0	0.03*	297747.1	406625.8
8	Specific leaf weight (mg cm^{-2})	0.5–10.0	0.5–10.0	6.0	6.0	0.49	1.0	1.3
9	Leaf dry weight (g plant^{-1})	1.7–22.4	1.8–22.4	7.4	8.0	0.02*	9.8	12.8
10	Leaf area index	0.9–15.6	0.9–15.6	4.2	4.5	0.03*	3.3	4.5
11	Stem dry weight (g plant^{-1})	6.9–104.7	6.9–104.7	32.1	34.2	0.04*	169.4	223.7
12	Mean ear head weight (g ear^{-1})	0.7–12.4	0.7–12.4	4.9	5.0	0.37	4.0	4.9
13	Ear head weight (g plant^{-1})	2.9–53.9	2.9–53.9	18.7	19.7	0.07	65.6	87.5
14	Seed yield (g plant^{-1})	2.0–43.4	2.0–43.4	13.7	14.4	0.11	38.3	51.2
15	Total dry matter (g plant^{-1})	11.5–181.1	11.5–181.1	58.6	62.5	0.03*	517.1	695.7
16	Threshing (%)	41.9–90.9	42.1–89.7	72.9	72.4	0.31	57.4	63.8
17	Test weight (g 1,000 seeds^{-1})	1.0–3.6	1.0–3.6	2.5	2.4	0.79	0.2	0.2
18	Harvest Index	0.05–0.45	0.05–0.45	0.2	0.2	0.59	0.0	0.0
19	Grain phytic acid (mg 100 g^{-1})	488.6–951.0	497.1–951.0	738.0	727.9	0.10	8463.5	8269.2

Significantly different at $p < 0.05$ based on ANOVA and post-hoc Newman-Keuls test.

TABLE 2 | Descriptive statistics for GPA content in finger millet accessions across generations.

Sl No.	Parameters	Year 2015	Year 2015	Year 2016
		$n = 623$	$n = 275$	$n = 275$
1	Mean (mg 100 g^{-1})	738	721	658
2	Minimum (mg 100 g^{-1})	489	497	478
3	Maximum (mg 100 g^{-1})	951	903	887
4	Standard deviation (SD)	92	90	80
5	Standard error mean (SEM)	9	9	12
6	Coefficient of variation (CV)	12.5	12.4	12.2
7	*p*-value (between accessions)*	<0.001	<0.001	<0.001

ANOVA was performed for GPA content and was significant at $p < 0.05$.

significant variability between accessions (ANOVA, $p < 0.001$). The mean GPA content of the core collection (production season 2015) was 738 ± 92 mg 100 g^{-1}, with the lowest content being 489 mg 100 g^{-1} and the highest being 951 mg 100 g^{-1} (**Table 2**).

For the diversity panel, a subset of 275 accessions of the original selection (350 accessions) was successfully grown in the second production season 2016; these 275 accessions represented the diversity panel for a further selection of low and high GPA content accessions. There were no significant differences between the selected set of 350 and the successfully grown set of 275 accessions in all 19 traits studied (**Supplementary Table 4**). The GPA content of the 275 accessions is presented in **Supplementary File 1**. Analysis of variance for GPA content revealed significant variability between accessions ($p < 0.001$). The mean GPA content was 658 ± 80 mg 100 g^{-1} with the lowest content being 478 mg 100 g^{-1} and the highest being 887 mg 100 g^{-1} (**Table 2**). Analysis of genetic determinants of GPA showed values of phenotypic coefficient of variation of 12.68 and 12.40 and genotypic coefficient of variation of 12.50 and 11.98, respectively, for the two production seasons (**Table 3**). The estimates for heritability (h^2) and genetic advance over mean for GPA content were 97.2 and 25.4, respectively, during production season 2015, and 93.4 and 24.0, respectively, for production season 2016 (**Table 3**). The GPA content among the 275 accessions was significantly and positively correlated between the two production seasons of 2015 and 2016 ($r = 0.688$, $p < 0.001$, **Figure 1**) indicating consistency of the trait. However, the mean GPA content differed significantly ($p < 0.001$) between the two production seasons.

Selection of High and Low GPA Finger Millet Accessions

In the final selection of a low GPA content accession (and a high GPA content accession as control) for the measurement of iron bioavailability in humans, 19 accessions were selected from the diversity panel, to represent its range of GPA content. In these 19 accessions, the GPA content was confirmed at an external laboratory using the Makower method of estimation. The GPA values from the Wade method at UAS Bengaluru, and the Makower method at ETH Zurich correlated significantly ($r^2 = 0.773$, $p < 0.001$, **Supplementary Figure 1**). A Bland–Altman analysis of differences in the GPA content between the two methods showed a systematic difference of 68 mg 100 g^{-1} seed, while the random difference was ±100 mg 100 g^{-1} (**Supplementary Figure 2**). There was no significant correlation between the magnitude of the GPA content and the difference between the methods.

The GPA content of the selected 19 accessions from the diversity panel was examined for its stability during the two growing seasons of 2015 and 2016. From these selected 19 accessions, two contrasting GPA accessions which showed a consistently low (GE 2358) and high (GE 1004) GPA content were selected. The variation across the two seasons was <10% for the two selected accessions **(Supplementary Table 5)**. The two selected accessions were grown again during the production season 2017 to produce adequate grains for human feeding trials to measure iron bioavailability. The stability of the GPA content of these selections between the three growing seasons was good and varied by a mere 36.4 mg 100 g^{-1} for GE 2358 and 67.1 mg 100 g^{-1} for GE 1004. In addition, the GPA content for these two accessions differed by <1% in the inter-laboratory analytical comparison. Thus, GPA content estimates for the final selection of low and high GPA accessions are reported as estimated by the Makower method. The low GPA accession, GE 2358, had 571 mg 100 g^{-1} while the high GPA accession, GE 1004, had 757 mg phytic acid in 100 g of grains. The iron content of these two accessions was 3.6 and 2.8 mg 100 g^{-1} in GE 2358 and GE 1004, respectively, and their phytate:iron molar ratios were 13.5:1 and 23.1:1, respectively. Thus, the low GPA accession also had a slightly higher iron content. In terms of grain yield during the 2016 production season, the low GPA content accession, GE 2358, had a slightly lower grain

TABLE 3 | Estimates of genetic parameters for GPA content in finger millet core collection and diversity panel.

Sl No	Genetic parameters	Year 2015	Year 2015	Year 2016
		***n* = 623**	***n* = 275**	***n* = 275**
1	GV	8493.1	7855.1	6199.5
2	PV	8738.6	8097.4	6637.7
3	EV	245.5	242.4	438.2
4	GCV	12.5	12.3	12.0
5	PCV	12.7	12.5	12.4
6	ECV	2.1	2.2	3.2
7	H^2	97.2	97.0	93.4
8	GAM (%)	25.4	25.0	23.9

GV, PV, and EV, Genetic phenotypic and environmental variances; GCV, PCV, and ECV, Genetic, phenotypic and environment coefficient of variation; H^2, broad sense heritability; GAM, Genetic advance over mean.

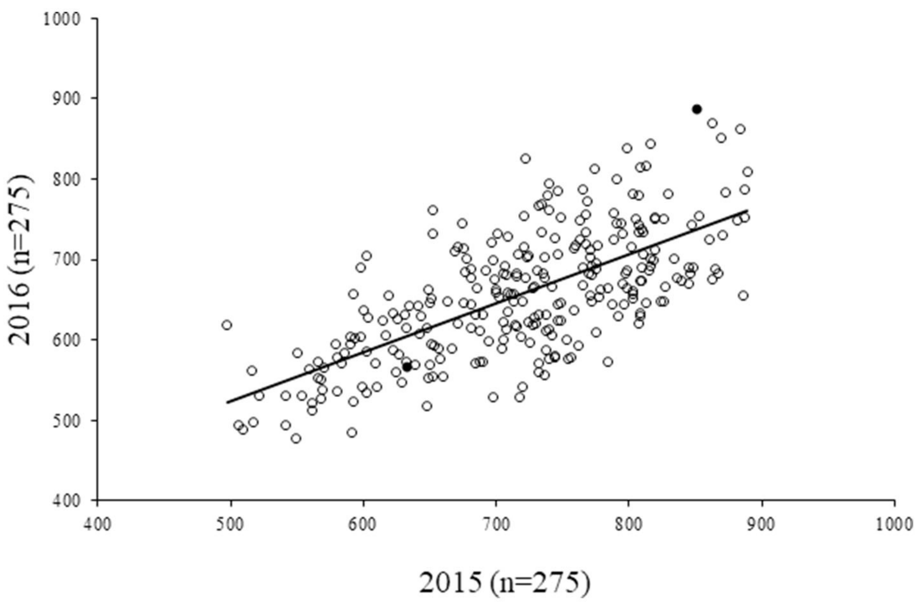

FIGURE 1 | Correlation of GPA content in 275 finger millet accessions between two seasons (2015 and 2016). $r^2 = 0.472$; $r = 0.688$, $p < 0.001$. Filled circles represent the accessions that were eventually selected for human iron absorption studies.

yield (by 9.9%) compared to the current Indian market variety (GPU 28).

Allelic Variations in the GPA Biosynthesis and Transport Genes Between the High and Low GPA Finger Millet Accessions

The 18 selected genes from the rice genome database (IRGPSV) when compared through a BLAST revealed 66 matching sequences across both A and B sub-genomes of finger millet. Of the 18 genes, 16 had at least two copies in either A or B sub-genomes (i.e., at least five copies in the whole genome), and five genes had five copies in the two sub-genomes of PR 202 (**Supplementary Table 6**). Annotation of the entire sequences of the 66 matches among 19 accessions revealed an average of 25.7 and 25.2 SNPs per accession in the entire gene sequence and an average of 0.78 and 0.76 SNPs per gene, on A and B sub-genomes, respectively. Further, we detected 123 and 186 SNPs on the coding regions (exons) among the selected 19 accessions. The average SNP in coding regions of each gene was 0.2 and 0.3, respectively, on the A and B sub-genomes. These genic SNPs segregated the 19 selected accessions into distinct clades as revealed by a Neighbor joining (NJ) tree. The low and high GPA accessions were separated with a significant genetic distance (**Figure 2A**). The mean GPA values of the accessions in the two clades differed significantly ($p = 0.04$) in both production seasons (**Supplementary Table 7**). Principal component analysis of genic SNPs from A sub-genomes was scattered and did not separate contrast accessions (**Figure 2B**), while SNPs from B sub-genome distinctly separated two selected (GE 1004 and GE 2358) contrasting accessions (**Figure 2C**). The first two principal components explained 21.8% and 16.27% of the total variance respectively from the A sub-genome. GE 2358 (Low GPA) with coordinates −0.359, 0.227 and GE1004 (high GPA) with coordinates 0.310, 0.110 represented distinct differences. Similarly, the two principal components from the B sub-genome explained 41.17 and 8.58% of the total variance. GE 2358 with coordinates −0.151, −0.116, and GE 1004 with coordinates 0.396, 0.00376 again reiterated the genetic diversity between the selected accessions. From the analysis of both sub-genomes, GE 2358 was found to be governed by negative alleles while the high GPA accession, GE 1004, was governed by positive alleles, hence creating the differences in the observed GPA values.

Though there were several SNPs among the 19 accessions, we concentrated on SNPs in only the two contrasting accessions (Ge 1004 and GE 2358). Among all the SNPs one non-synonymous variant was found at the 1408th position of the nucleotide sequence of gb14539 the putative ATP-Binding cassette transporter-C family gene (phytate transporter gene *EcABCC*). The high GPA accession, GE 1004 had Guanine replaced with Cytosine in the low GPA accession GE 2358. This substitution changed the amino acid at the 470th position from aspartic acid in GE 1004, which is conserved among monocot crop species, to histidine in GE 2358 (**Figure 3**). A secondary structural analysis of the protein suggested that this mutation resulted in a change at the beta sheet region of the cytosol-facing ABC transporter domain (**Supplementary Figure 3**).

Measurement of Iron Absorption From Low and High GPA Content Finger Millet Accessions

For the human iron absorption study, the recruited women's age was between 20 and 30 y, with a body mass index between 18.9 and 25.0 kg m^{-2}. Anthropometric data and baseline iron status are presented in **Table 4**. All subjects had normal Hb levels (13.7 $\pm$ 0.8 g dL^{-1}) with mean $\pm$ SD serum ferritin of 25.2 $\pm$ 10.1 μg L^{-1} and with no infection or inflammation (mean $\pm$ SD CRP: 1.6 $\pm$ 2.1 mg L^{-1}). Because of the differing iron content of the two accessions, the iron content of the 3 test meals fed to the subjects for each accession, was slightly higher for the low GPA content GE 2358 (10.0 mg in total) than for the high GPA content GE 1004 (8.1 mg in total). The Fe isotopes (^{57}Fe and ^{58}Fe) were dispensed (1 mg of iron) onto the test meals (in total 3 mg of iron for 3 meals), thus constituting ∼33% of the total iron in the meals. No subject reported any adverse reaction to the isotope feeding protocol during the study. The atom percent excess of the isotope labels of ^{57}Fe and ^{58}Fe at the end of the 14-day incorporation period in red blood cells was 0.27 and 1.02%, respectively.

The fractional iron absorption from the low GPA test meal and high GPA test meal was significantly different ($p < 0.05$, **Figure 4**), at 3.7% (geometric mean, range: 2.0 to 7.4%) for the low GPA accession (GE 2358) in comparison to 1.3% (geometric mean, range: 0.1 to 5.5%) for the high GPA accession (GE 1004). Thus, the iron bioavailability was 2.9-fold higher on average with the low GPA accession meal intake. The low and high GPA test meals which contained 3.3 and 2.7 mg iron, contributed 0.122 mg (range: 0.066 to 0.243 mg) and 0.035 mg (range: 0.02–0.149 mg) respectively to daily physiological iron requirement (1.2 mg/d) after correcting for fractional iron absorption. For each accession, iron absorption had a negative but not significant correlation with serum ferritin for GE 2358 ($r^2 = 0.38$, slope $= -0.108$ decrease in iron absorption per unit increase in serum ferritin, $p = 0.056$) and for GE 1004 ($r^2 = 0.15$, slope $= -0.060$ decrease in iron absorption per unit increase in serum ferritin, $p = 0.275$). The negative slope indicates that subjects with a lower iron status (even within the normal range of serum ferritin) had a higher iron absorption (**Figure 5**).

DISCUSSION

Public health approaches to ID and IDA have involved the chemical iron fortification of foods, but unless there is adequate diet diversification, the absorption of fortified iron remains low (10), with low impact. There have also been global initiatives to biofortify crops, however, most of these have focused on increasing iron density (7, 48, 49), but have seen low to modest success (50, 51). A more effective strategy is to improve iron absorption from grains through the reduction of dietary inhibitors like phytic acid. This should be seen in conjunction with the revival and encouragement of the consumption of millets. With their relatively high iron, calcium, and fiber content (31), millet crops also have low input costs and water requirement (30) and have a sustainable, small

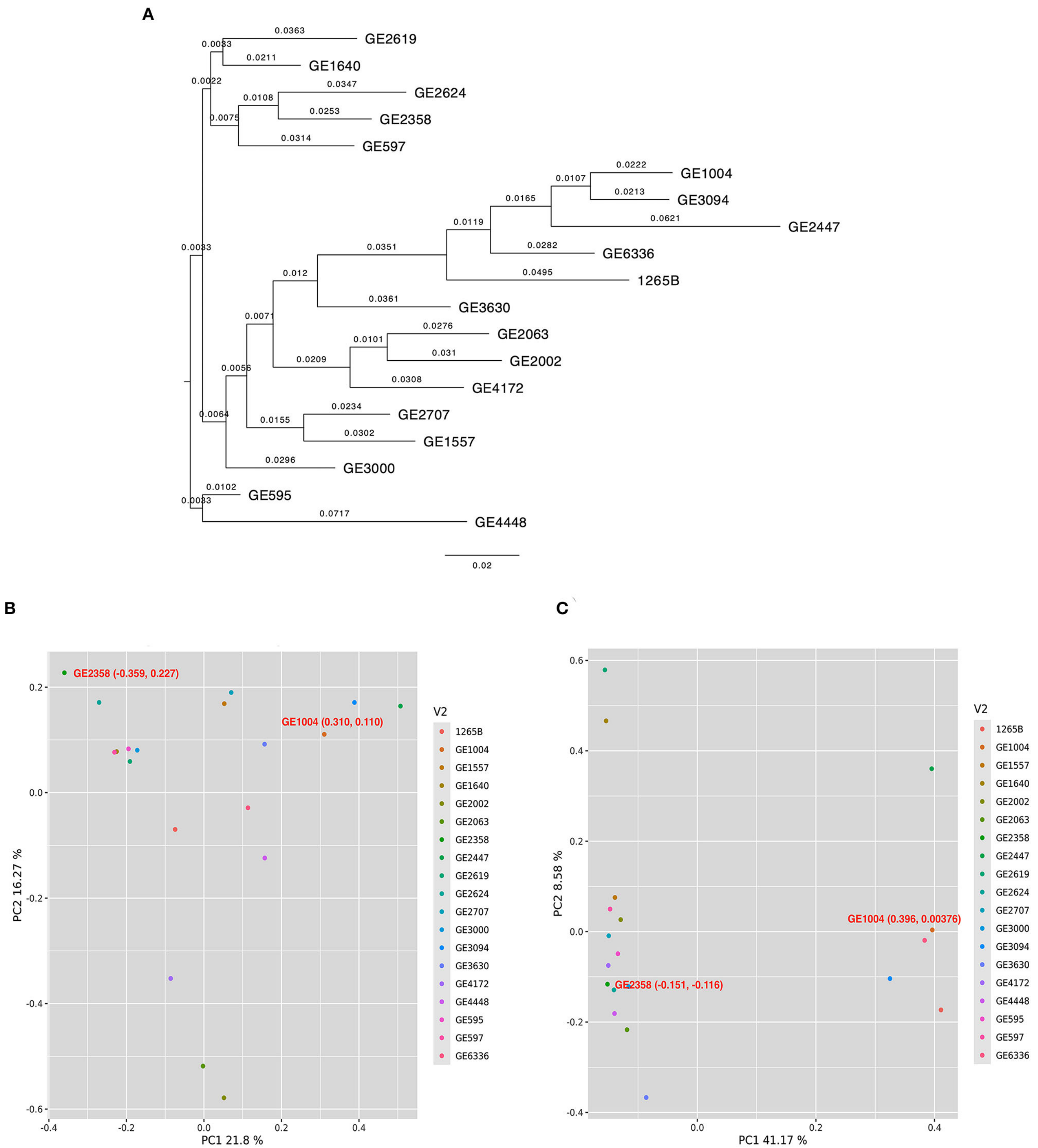

FIGURE 2 | Diversity and principal component analysis for 19 finger millet accessions. **(A)** Neighbor joining tree to assess the genetic relatedness among 19 finger millet accessions. Principal component analysis for 19 finger millet accessions with identified SNPs across 66 matching sequences for A sub-genome **(B)** and B sub-genome **(C)**. Filled circles with different colors in scattered plots indicate the different accessions.

ecological footprint. A further ecological benefit of reducing GPA content of the feed in non-ruminant (e.g., poultry) nutrition accrues from the reduced excretion of undigested phosphorus, with a possible impact of these reduced losses, through water run-off from farms, on the resultant eutrophication of water bodies (52).

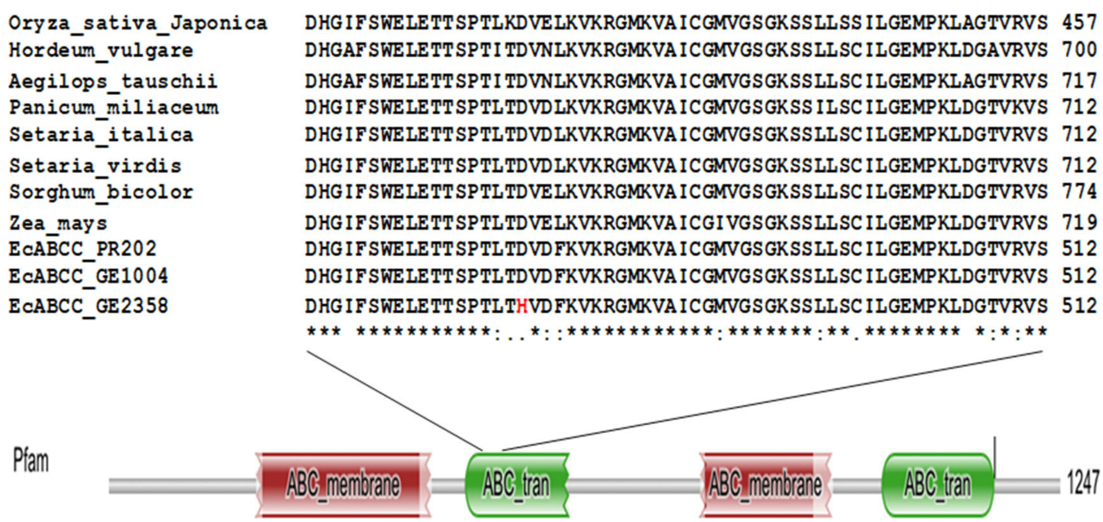

FIGURE 3 | Multiple sequence alignment of deduced amino acid sequence of *EcABCC* from GE2358, GE1004, PR202, and monocot crop species and domain search analysis of *EcABCC*. The amino acid indicated in red color is replaced amino acid in low GPA accession.

In this study, a natural low GPA finger millet accession with adequate iron content, and stability of GPA content across growing seasons, was selected from a large germplasm collection with natural variation. This selection was hypothesized to increase native iron absorption. When measured in humans against a high GPA content finger millet contrast, using an accurate stable isotope method, showed an almost 3-fold increase in iron absorption. This increase in iron absorption is most likely due to the difference in phytic acid content (24%); while the iron content also was higher in the low GPA accession (by 24%), it is unlikely to increase iron absorption by this much. For example, an increase in the iron content of biofortified beans, without a change in native phytic acid content, did not result in higher iron absorption (8), and the iron fortification of foods has also shown little or no increase in iron absorption (10–12).

In selection and plant breeding programs, the existence of natural variations for any specific trait is a prerequisite. Since India is a secondary center for the diversity of finger millet, this study included a diverse core collection (33) that consisted of 623 accessions developed from 5940 accessions of finger millet from 23 countries, representing geographical regions and biological races from the entire collection. This core collection was extensively phenotyped in the first production season and the stability of GPA was evaluated using a smaller, but representative set of 275 accessions in the second production season. Finally, through a careful analysis of the iron and GPA content of a further selected 19 accessions, an accession that had a low GPA content was selected, along with a high GPA control, and further evaluated for their ability to breed true in a third production season. This approach also had the benefit of circumventing the deep concerns, including the social ramifications of farmer autonomy around crop genetic modification strategies.

TABLE 4 | Baseline characteristics of the subjects.

Sl No.	Parameter	Mean ± SD (range)
1	Age (y)	21.6 ± 3.0 (20–30)
2	Height (m)	1.6 ± 0.1 (1.4–1.6)
3	Weight (kg)	50.5 ± 4.2 (41.7–58.4)
4	BMI (kg M^{-2})	20.5 ± 1.5 (18.9–23.0)
5	Hemoglobin (g dL^{-1})	13.7 ± 0.8 (12.3–15.0)
6	Serum ferritin (μg L^{-1})	25.2 ± 10.1 (11.7–40.7)
7	CRP (mg L^{-1})	1.6 ± 2.1 (0.1–6.5)

BMI, Body Mass Index; CRP, C-reactive protein.

Genetic modification strategies for reducing the GPA content operate through the identification of induced mutations in conventional varieties, or through the genetic engineering of enzymatic genes or transporters involved in phytic acid biosynthesis and storage, to yield *low phytic acid* (*lpa*) mutants. In several crops, *lpa* mutants have been identified, including maize, rice, wheat, barley, common bean, and soybean (53) by induced mutagenesis. Any reduction in GPA content is expected to have a negative impact on important plant phenological developments such as reduced germination, seedling survival, and increased susceptibility to stresses leading to reduced seed yield (53). Transgenic approaches have also been tried, for example, embryo-specific silencing of the multidrug resistance associated protein ABC transporter gene resulted in reductions in phytic acid content by 68–87% in maize and 37–90% in soybean (54). The much larger reduction of GPA content in transgenic lines renders them as an ideal material to examine the negative effects of low GPA content. But, this may perhaps be delayed or not permitted under the present restrictions on cultivation

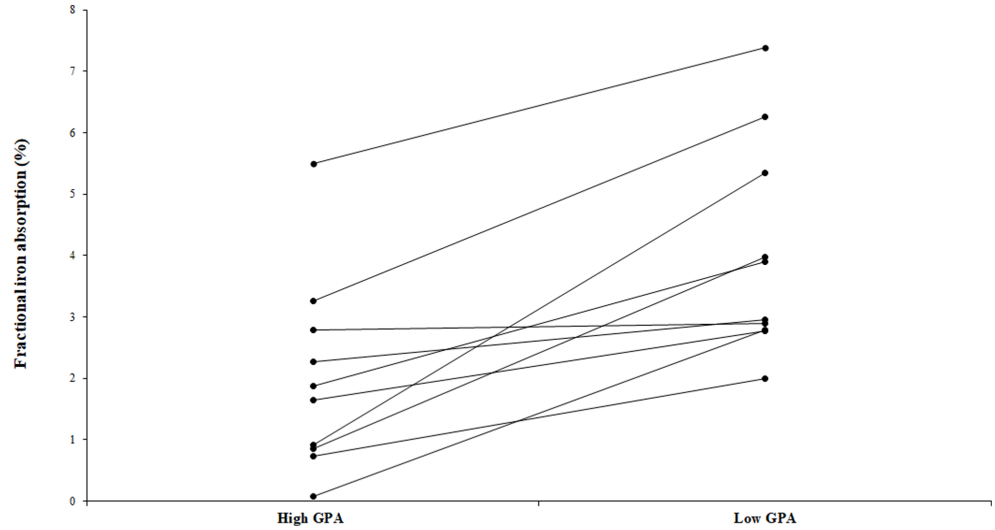

FIGURE 4 | Fractional iron absorption from the low GPA and high GPA finger millet meals (*ragi roti*, $n = 10$). Both low and high GPA meals were administered to the same subject. Data points representing iron absorption of the same subject for high and low GPA test meals are connected with lines to illustrate the trend.

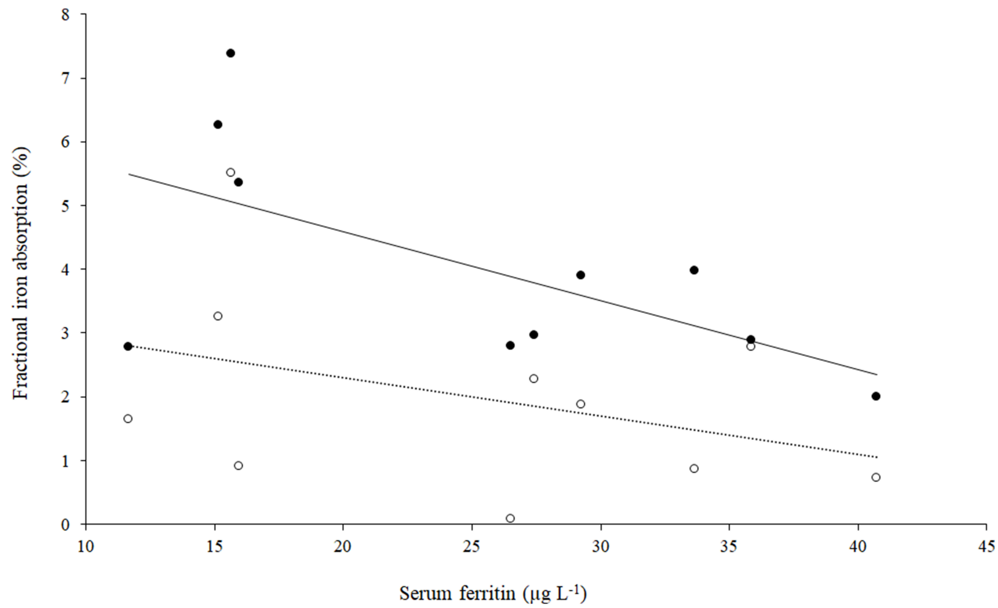

FIGURE 5 | Scatter plot of iron absorption against serum ferritin in low GPA accession GE 2358 and high GPA accession GE 1004 ($n = 10$). Filled circles: Low GPA content finger millet accession; $r^2 = 0.38$, $p = 0.056$, iron absorption = (−0.108 × serum ferritin) + 6.75. Open circles: High GPA content finger millet accession. $r^2 = 0.15$, $p = 0.275$, iron absorption = (−0.060 × serum ferritin) + 3.50.

or consumption of genetically modified food grains in most countries, especially in India.

The selected low GPA accession in the present study did not have reduced germination and seedling establishment and yielded only 10% less than the most widely cultivated finger millet variety in the region (GPU 28). These observations suggest that a marginal 30% reduction in GPA content may not alter seedling vigor. From a farmers' sustainability perspective, the selection of accessions that naturally accumulate a low content of phytic acid, with a small yield penalty, seems a promising strategy. Since the variation in GPA values was naturally encountered, these contrasts formed an excellent set of accessions to examine iron absorption through clinical trials using human subjects. The two selected accessions were distinct even in their genomic sequences evidenced through SNP analysis using the available whole genome sequence information. Though there are 18 well-characterized genes involved in biosynthesis and transport/storage of phytic acid, Myo-inositol PO_4 synthase (*MIPS*), that catalyzes the first step in phytic acid biosynthesis, that is, Glu 6-PO_4 to Myo-inositol PO_4, and the gene coding for the ABCC transporter that transports phytic acid to the protein storage vesicles (PSV) has been suggested as rate limiting steps (26). Hence, sequence variations in MIPS and ABC transporter genes were analyzed. Interestingly, there were no sequence variations in the MIPS gene among the 19 accessions and we found one nonsynonymous SNP in the ABCC transporter gene at the 1408 position. This variation resulted in an amino acid change from aspartic acid to histidine in the transporter domain that could affect the function of *EcABCC* phytate transporter protein. In support of this, changes in amino acid from glutamic acid to lysine, which represents a similar group change of amino acids, in the transmembrane domain of *Pvmrp1* (Phaseolus) (55) were associated with reduced phytic acid accumulation in protein storage vacuoles in which phytic acid is also stored.

Even though the iron absorption increased almost 3-fold from the low GPA content accession, the absolute values observed in the present study were relatively low. It is important to note that the present study subjects had a normal iron status and Hb, where iron absorption will be down regulated by the adequate body iron status. In women with ID, an earlier iron absorption study from a market-variety finger millet with a GPA content of 650 mg/100 g, eaten in the form of a steamed ball, showed that the iron absorption was higher, at 4.6% (32). An additional reason for the higher iron absorption in the earlier study could have been the study design with a single meal with a higher isotopic iron dose, which could result in higher isotopic iron absorption (32). The present study design was careful to avoid this by using three meals, such that the added isotopic iron was much lower, resembling the phytate: iron molar ratios present in a habitually consumed meal. The iron absorption findings in the normal subjects of this study can be extrapolated to a population with ID where the absorption is likely to be upregulated. Serum ferritin concentrations have a close inverse relation with iron absorption (56), such that observed iron absorption values can be corrected to a value of serum ferritin concentration present in a population with a high IDA prevalence. In a large survey of women in the Indian state of Uttar Pradesh (57), the geometric mean of inflammation adjusted serum ferritin concentrations was 15.9 $\mu g\ L^{-1}$. When corrected (56, 58) to a ferritin value of 15 $\mu g\ L^{-1}$, the iron absorption for the two accessions in this study was 6 vs. 2% (low GPA vs. high GPA) and will be higher as the iron status declines.

Finger millet was chosen for this study as it is a drought hardy crop species and leaves a lower water footprint on diets; indeed, finger millet is even seen as a "superfood" and a nutraceutical food. From an iron transaction view, the low GPA accession (with an iron content of 3.6 mg 100 g^{-1}) when consumed in two meals of 100 g each, could contribute 0.3 mg iron daily to the physiological requirement. In contrast, the high GPA variety would provide only a third of this amount of iron. Improving diet diversity to increase the intake of absorption enhancers such as vitamin C, or behavioral modifications such as limiting the intake of inhibitory polyphenol rich beverages like tea with a meal (22, 59) are useful complementary strategies. From a food system perspective, the modest increase in iron absorption with a reduction in GPA content aligns with the principle that public health nutrition initiatives should necessarily be restrained to avoid the risks of over-nutrition or other unseen risks. Introducing such seeds into the seed market takes time, but once done, cannot be easily reversed.

Yield is a major concern of farmers, and while the yield of the low GPA accession was slightly (10%) lower than the market variety of finger millet, a village-level evaluation of the acceptability of a theoretically higher priced, but more iron-effective finger millet in Karnataka, India, showed that people were willing to pay more for millet that they felt had a health benefit (60). It is likely that with effective outreach and extension, a seed with a higher health benefit might fetch a higher price, offsetting the slightly lower yield. Further evidence of benefit from the selected low GPA content accession, through longer term feeding trials, is also required.

CONCLUSION

This study leveraged the natural variation in GPA content in among the germplasm to identify a low GPA finger millet accession with improved iron absorption, without genetic modification. This accession can either be developed into a cultivar or used as a trait-donor genotype in focused breeding programs to further improve popular varieties of finger millet.

DATA AVAILABILITY STATEMENT

The datasets presented in this study can be found in online repositories. This data can be found at the "DDBJ" data base here: https://ddbj.nig.ac.jp/resource/bioproject/PRJDB8131 and at: https://ddbj.nig.ac.jp/resource/bioproject/PRJDB10731.

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by the Institutional Ethical Committee at St. John's Medical College, Bengaluru, India (IERB No.: 87/2015). The patients/participants provided their written informed consent to participate in this study.

AUTHOR CONTRIBUTIONS

BR, SS, AK, DM, and MZ conceptualized the entire research. BR, NH, YN, RR, MT, SP, GK, and SS were involved in screening, cultivation, GPA analysis, and final selection of the low GPA accession. BR, MH, LP, KS, RS, and SS were involved in sequencing and *in silico* protein structure analysis. PT, BR, BB, and AK were involved in the human testing of iron absorption. All authors contributed to the preparation and editing of the manuscript.

FUNDING

This research was supported by Indo-Swiss collaboration in Biotechnology Program (BT/IC-2/ISCB/Phase-IV/03/RAGI/2014; Department of Biotechnology, Government of India and the Swiss Agency for Development and Cooperation, Switzerland), and Swiss National Science Foundation Sinergia project (Number: 183578). BR acknowledges the Science and Engineering Research Board (SERB), Department of Science and Technology, Government of India for awarding the National Postdoctoral Fellowship. File No. PDF/2016/000639.

ACKNOWLEDGMENTS

The authors gratefully acknowledge the assistance of Harish B, Charles Milton, and Sindhu Kashyap in fieldwork, laboratory analyses, human studies, and manuscript preparation. The authors gratefully acknowledge Dr. B. Gopal, Indian Institute of Science, Bangalore, for the help in model prediction and structure analysis. We would like to thank Ms. Aki Morishima, Dr. Rie Shimizu-Inatsugi at the University of Zurich for the sample preparation of reference genome improvement, Dr. Sirisha Aluri and Ms. Catharine Aquino at the Functional Genomics Center Zurich for the library preparation and discussion, Dr. Tony C. Y. Kuo for help and useful discussion in genome assembly, and all subjects for their voluntary participation in this study.

SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fnut.2021.791392/full#supplementary-material

REFERENCES

1. Balarajan Y, Ramakrishnan U, Özaltin E, Shankar AH, Subramanian SV. Anaemia in low-income and middle-income countries. *Lancet*. (2011) 378:2123–35. doi: 10.1016/S0140-6736(10)62304-5
2. Indian Institute for Population Sciences (IIPS) and MoHFW. *National Family Health Survey—4* (2017). Available online at: http://rchiips.org/nfhs/pdf/NFHS4/ India.pdf (accessed January 15, 2020).
3. Kassebaum NJ, GBD 2013 Anemia Collaborators. The global burden of anemia. *Hematol Oncol Clin North Am*. (2016) 30:247–308. doi: 10.1016/j.hoc.2015.11.002
4. Nair KM, Iyengar V. Iron content, bioavailability & factors affecting iron status of Indians. *Indian J Med Res.* (2009) 130:634–45.
5. Ghosh S, Sinha S, Thomas T, Sachdev HS, Kurpad AV. Revisiting dietary iron requirement and deficiency in Indian women: implications for food iron fortification and supplementation. *J Nutr*. (2019) 149:366–71. doi: 10.1093/jn/nxy283
6. Ghosh S, Sinha S, Shivakumar N, Thomas T, Sachdev HS, Kurpad AV. Daily iron requirements in healthy Indian children and adolescents. *Indian Pediatr*. (2019) 56:551–5. doi: 10.1007/s13312-019-1552-0
7. Bouis HE, Saltzman A. Improving nutrition through biofortification: a review of evidence from HarvestPlus, 2003 through 2016. *Glob Food Sec.* (2017) 12:49–58. doi: 10.1016/j.gfs.2017.01.009
8. Petry N, Egli I, Gahutu JB, Tugirimana PL, Boy E, Hurrell R. Phytic acid concentration influences iron bioavailability from biofortified beans in Rwandese women with low iron status. *J Nutr*. (2014) 144:1681–7. doi: 10.3945/jn.114.192989
9. Cercamondi CI, Egli IM, Mitchikpe E, Tossou F, Zeder C, Hounhouigan JD, et al. Total iron absorption by young women from iron-biofortified pearl millet composite meals is double that from regular millet meals but less than that from post-harvest iron-fortified millet meals. *J Nutr*. (2013) 143:1376–82. doi: 10.3945/jn.113.176826
10. Swaminathan S, Ghosh S, Varghese JS, Sachdev HS, Kurpad AV, Thomas T. Dietary iron intake and anemia are weakly associated, limiting effective iron fortification strategies in India. *J Nutr*. (2019) 149:831–9. doi: 10.1093/jn/nxz009
11. Andersson M, Thankachan P, Muthayya S, Goud RB, Kurpad AV, Hurrell RF, et al. Dual fortification of salt with iodine and iron: a randomized, double-blind, controlled trial of micronized ferric pyrophosphate and encapsulated ferrous fumarate in southern India. *Am J Clin Nutr*. (2008) 88:1378–87. doi: 10.3945/ajcn.2008.26149
12. Moretti D, Zimmermann MB, Muthayya S, Thankachan P, Lee TC, Kurpad AV, et al. Extruded rice fortified with micronized ground ferric pyrophosphate reduces iron deficiency in Indian schoolchildren: a double-blind randomized controlled trial. *Am J Clin Nutr*. (2006) 84:822–9. doi: 10.1093/ajcn/84.4.822
13. Moretti D, Goede JS, Zeder C, Jiskra M, Chatzinakou V, Tjalsma H, et al. Oral iron supplements increase hepcidin and decrease iron absorption from daily or twice-daily doses in iron-depleted young women. *Blood*. (2015) 126:1981–9. doi: 10.1182/blood-2015-05-642223
14. Paganini D, Zimmermann MB. The effects of iron fortification and supplementation on the gut microbiome and diarrhea in infants and children: a review. *Am J Clin Nutr*. (2017) 106:1688S—93. doi: 10.3945/ajcn.117.156067
15. Simcox JA, McClain DA. Iron and diabetes risk. *Cell Metab*. (2013) 17:329–41. doi: 10.1016/j.cmet.2013.02.007
16. Cosgrove DJ. Inositol hexakis phosphates. Inositol Phosphates. In: Cosgrove DJ, editor. *Their Chemistry, Biochemistry, and Physiology*. Amsterdam (1980). p. 26–43.
17. Raboy V, The biochemistry and genetics of phytic acid synthesis. In: MorreÂD, Boss W, Loewus F, editors. *Inositol Metabolism in Plants*. New York, NY: Wiley-Liss (1990). p. 55–76.
18. Graf E, Epson KL, Eaton JW. Phytic acid: a natural antioxidant. *J Biol Chem.* (1987) 262:11647–50. doi: 10.1016/S0021-9258(18)60858-0
19. Frossard E, Bucher M, Mächler F, Mozafar A, Hurrell R. Potential for increasing the content and bioavailability of Fe, Zn and Ca in plants for human nutrition. *J Sci Food Agric*. (2000) 80:861–79. doi: 10.1002/(SICI)1097-0010(20000515)80:7<861::AID-JSFA601>3.0.CO;2-P
20. Raboy V. myo-Inositol-1, 2, 3, 4, 5, 6-hexakisphosphate. *Phytochemistry.* (2003) 64:1033–43. doi: 10.1016/S0031-9422(03)00446-1
21. Fairweather-Tait S, Hurrell RF. Bioavailability of minerals and trace elements: members of EC flair concerted Action No. 10: measurements of micronutrient absorption and status. *Nutr Res Rev.* (1996) 9:295–324. doi: 10.1079/NRR19960016
22. Thankachan P, Walczyk T, Muthayya S, Kurpad AV, Hurrell RF. Iron absorption in young Indian women: the interaction of iron status with the influence of tea and ascorbic acid. *Am J Clin Nutr*. (2008) 87:881–6. doi: 10.1093/ajcn/87.4.881
23. Gibson RS, Raboy V, King JC. Implications of phytate in plant-based foods for iron and zinc bioavailability, setting dietary requirements, and formulating programs and policies. *Nutr Rev*. (2018) 76:793–804. doi: 10.1093/nutrit/nuy028
24. Troesch B, Jing H, Laillou A, Fowler A. Absorption studies show that phytase from Aspergillus niger significantly increases iron and zinc bioavailability from phytate-rich foods. *Food Nutr Bull*. (2013) 34:S90–101. doi: 10.1177/15648265130342S111

25. Nielsen AV, Nyffenegger C, Meyer AS. Performance of microbial phytases for gastric inositol phosphate degradation. *J Agr Food Chem*. (2015) 63:943–50. doi: 10.1021/jf5050469
26. Raboy V. Approaches and challenges to engineering seed phytate and total phosphorus. *Plant Sci*. (2009) 177:281–96. doi: 10.1016/j.plantsci.2009.06.012
27. Dai F, Wang J, Zhang S, Xu Z, Zhang G. Genotypic and environmental variation in phytic acid content and its relation to protein content and malt quality in barley. *Food Chem*. (2007) 105:606–11. doi: 10.1016/j.foodchem.2007.04.019
28. Wang F, King JD, Rose T, Kretzschmar T, Wissuwa M. Can natural variation in grain P concentrations be exploited in rice breeding to lower fertilizer requirements?. *PLoS ONE*. (2017) 12:e0179484. doi: 10.1371/journal.pone.0179484
29. Makkar S, Minocha S, Swaminathan S, Kurpad AV. A policy perspective millets in the Indian plate. *Econ Polit Wkly*. (2019) 54.
30. Saleh AS, Zhang Q, Chen J, Shen Q. Millet grains: nutritional quality, processing, and potential health benefits. *Compr Rev Food Sci Food Saf*. (2013) 12:281–95. doi: 10.1111/1541-4337.12012
31. Longvah T, Anantan I, Bhaskarachary K, Venkaiah K, Longvah T. *Indian Food Composition Tables*. Hyderabad: National Institute of Nutrition, Indian Council of Medical Research (2017).
32. Kalasuramath S, Kurpad AV, Thankachan P. Effect of iron status on iron absorption in different habitual meals in young south Indian women. *Indian J Med Res*. (2013) 137:324–30.
33. Upadhyaya HD, Sarma ND, Ravishankar CR, Albrecht T, Narasimhudu Y, Singh SK, et al. Developing a mini-core collection in finger millet using multilocation data. *Crop Sci*. (2010) 50:1924–31. doi: 10.2135/cropsci2009.11.0689
34. Davidsson L. In Assessment of Iron Bioavailability in Humans Using Stable Iron Isotope Techniques. IN: *IAEA Human Health Series No. 21.* Austria: IAEA Human Health Series; 21 (2012).
35. International Board for Plant Genetic Resources. *Descriptors for Finger Millet*. Rome: IBPGR Secretariat (1985).
36. Gao Y, Shang C, Maroof MS, Biyashev RM, Grabau EA, Kwanyuen P, et al. A modified colorimetric method for phytic acid analysis in soybean. *Crop Sci*. (2007) 47:1797–803 doi: 10.2135/cropsci2007.03.0122
37. Dida MM, Ramakrishnan S, Bennetzen JL, Gale MD, Devos KM. The genetic map of finger millet, Eleusine coracana. *Theor Appl Genet*. (2007) 114:321–32. doi: 10.1007/s00122-006-0435-7
38. Kim KW, Chung HK, Cho GT, Ma KH D, Chandrabalan Gwag JG, et al. PowerCore: a program applying the advanced M strategy with a heuristic search for establishing core sets. *Bioinformatics*. (2007) 23:2155–62. doi: 10.1093/bioinformatics/btm313
39. Hurrell RF, Juillerat MA, Reddy MB, Lynch SR, Dassenko SA, Cook JD. Soy protein, phytate, and iron absorption in humans. *Am J Clin Nutr*. (1992) 56:573–8. doi: 10.1093/ajcn/56.3.573
40. Kuo T, Frith MC, Sese J, Horton P. EAGLE: explicit alternative genome likelihood evaluator. *BMC Med Genomics*. (2018) 11:1–0. doi: 10.1186/s12920-018-0342-1
41. ICMR-NIN. *Experts Group on Nutrient Requirement for Indians, Recommended Dietary Allowances (RDA), and Estimated Average Requirements (EAR)-2020*. Hyderabad (2020).
42. Walczyk T, Davidsson L, Zavaleta N, Hurrell RF. Stable isotope labels as a tool to determine the iron absorption by Peruvian school children from a breakfast meal. *Fresenius J Anal Chem*. (1997) 359:445–9. doi: 10.1007/s002160050608
43. Hu J, Zhu J, Xu HM. Methods of constructing core collections by stepwise clustering with three sampling strategies based on the genotypic values of crops. *Theor Appl Genet*. (2000) 101:264–8. doi: 10.1007/s001220051478
44. Shannon CE, Weaver W. *The Mathematical Theory of Communication*. Urbana: University of Illinois Press (1949). p. 96.
45. Robinson HF, Comstock RE, Harvey PH. Estimates of heritability and the degree of dominance in corn. *Agron J*. (1949) 41:353–9. doi: 10.2134/agronj1949.00021962004100080005x
46. Burton GW, Devane DE. Estimating heritability in tall fescue (Festuca arundinacea) from replicated clonal material 1. *Agron J*. (1953) 45:478–81. doi: 10.2134/agronj1953.00021962004500100005x
47. Bland JM, Altman DG. Measuring agreement in method comparison studies. *Stat Meth Med. Res*. (1999) 8:135–60. doi: 10.1191/096228099673819272
48. Genc Y, Humphries JM, Lyons GH, Graham RD. Exploiting genotypic variation in plant nutrient accumulation to alleviate micronutrient deficiency in populations. *Trace Elem Med Biol*. (2005) 18:319–24. doi: 10.1016/j.jtemb.2005.02.005
49. Waters BM, Sankaran RP. Moving micronutrients from the soil to the seeds: genes and physiological processes from a biofortification perspective. *Plant Sci*. (2011) 180:562–74. doi: 10.1016/j.plantsci.2010.12.003
50. Sperotto RA, Ricachenevsky FK, de Abreu Waldow V, Fett JP. Iron biofortification in rice: it's a long way to the top. *Plant Sci*. (2012) 190:24–39. doi: 10.1016/j.plantsci.2012.03.004
51. Finkelstein JL, Haas JD, Mehta S. Iron-biofortified staple food crops for improving iron status: a review of the current evidence. *Curr Opin Biotech*. (2017) 44:138–45. doi: 10.1016/j.copbio.2017.01.003
52. Bohn L, Meyer AS, Rasmussen SK. Phytate: impact on environment and human nutrition. A challenge for molecular breeding. *J Zhejiang Univ Sci B*. (2008) 9:165–91. doi: 10.1631/jzus.B0710640
53. Raboy V. The ABCs of low-phytate crops. *Nat Biotech*. (2007) 25:874–5. doi: 10.1038/nbt0807-874
54. Shi J, Wang H, Schellin K, Li B, Faller M, Stoop JM, et al. Embryo-specific silencing of a transporter reduces phytic acid content of maize and soybean seeds. *Nat Biotech*. (2007) 25:930–7. doi: 10.1038/nbt1322
55. Panzeri D, Cassani E, Doria E, Tagliabue G, Forti L, Campion B, et al. A defective ABC transporter of the MRP family, responsible for the bean lpa1 mutation, affects the regulation of the phytic acid pathway, reduces seed myo-inositol and alters ABA sensitivity. *New Phytol*. (2011) 191:70–83. doi: 10.1111/j.1469-8137.2011.03666.x
56. Cook JD, Dassenko SA, Lynch SR. Assessment of the role of nonheme-iron availability in iron balance. *Am. J. Clin. Nutr*. (1991) 54:717–22. doi: 10.1093/ajcn/54.4.717
57. Larson L, Thomas T, Kurpad AV, Martorell R, Hoddinott J, Swaminathan S, et al. Anemia in women and children in Uttar Pradesh: a path analysis of the associations between nutritional, environmental, infectious, and genetic determinants. In: *Poster presented at the Annual Meeting of the American Society of Nutrition*. Boston, MA (2018). Available online at: https://doi.org/10.1093/cdn/nzy039
58. Available online at: https://www.who.int/vmnis/indicators/serumferritin.pdf (accessed Feb 24, 2020).
59. Ahmad Fuzi SF, Koller D, Bruggraber S, Pereira DI, Dainty JR, Mushtaq S. A 1-h time interval between a meal containing iron and consumption of tea attenuates the inhibitory effects on iron absorption: a controlled trial in a cohort of healthy UK women using a stable iron isotope. *Am J Clin Nutr*. (2017) 106:1413–21. doi: 10.3945/ajcn.117.161364
60. Sakamma S, Umesh KB, Girish MR, Ravi SC, Satishkumar M, Bellundagi V. Finger millet (*Eleusine coracana* L. Gaertn.) production system: status, potential, constraints and implications for improving small farmer's welfare. *J Agric Sci*. (2018) 10:162–79. doi: 10.5539/jas.v10n1p162

Conflict of Interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

frontiers | Frontiers in Nutrition

TYPE Review
PUBLISHED 06 December 2022
DOI 10.3389/fnut.2022.1050099

Check for updates

OPEN ACCESS

EDITED BY
Ramadjita Tabo,
International Crops Research Institute for the Semi-Arid Tropics, Mali

REVIEWED BY
Filipa S. Reis,
Centro de Investigação de Montanha (CIMO), Portugal
Yichen Hu,
Chengdu University, China

*CORRESPONDENCE
T. H. Fernandes
profcattitofernandes@gmail.com

SPECIALTY SECTION
This article was submitted to Nutrition and Sustainable Diets, a section of the journal Frontiers in Nutrition

RECEIVED 21 September 2022
ACCEPTED 08 November 2022
PUBLISHED 06 December 2022

CITATION
Bell V, Silva CRPG, Guina J and Fernandes TH (2022) Mushrooms as future generation healthy foods. *Front. Nutr.* 9:1050099.
doi: 10.3389/fnut.2022.1050099

Mushrooms as future generation healthy foods

V. Bell [1], C. R. P. G. Silva [2], J. Guina [3] and T. H. Fernandes [4,5]*

[1]Faculty of Pharmacy, University of Coimbra, Pólo das Ciências da Saúde, Coimbra, Portugal, [2]Department of Health and Social Care, School of Health and Care Management, Arden University, Coventry, United Kingdom, [3]Instituto Superior de Estudos Universitários de Nampula (ISEUNA), Universidade a Politécnica, Nampula, Mozambique, [4]CIISA—Centre for Interdisciplinary Research in Animal Health, Faculty of Veterinary Medicine, Associate Laboratory for Animal and Veterinary Sciences (AL4AnimalS), University of Lisbon, Lisbon, Portugal, [5]Centro de Estudos Interdisciplinares Lurio (CEIL), Lúrio University, Nampula, Mozambique

The potential of edible mushrooms as an unexploited treasure trove, although rarely included in known food guidelines, is highlighted. Their role in shielding people against the side effects of an unhealthy stylish diet is reviewed. Mushrooms complement the human diet with various bioactive molecules not identified or deficient in foodstuffs of plant and animal sources, being considered a functional food for the prevention of several human diseases. Mushrooms have been widely used as medicinal products for more than 2,000 years, but globally the potential field of use of wild mushrooms has been untapped. There is a broad range of edible mushrooms which remain poorly identified or even unreported which is a valuable pool as sources of bioactive compounds for biopharma utilization and new dietary supplements. Some unique elements of mushrooms and their role in preventative healthcare are emphasized, through their positive impact on the immune system. The potential of mushrooms as antiviral, anti-inflammatory, anti-neoplastic, and other health concerns is discussed. Mushrooms incorporate top sources of non-digestible oligosaccharides, and ergothioneine, which humans are unable to synthesize, the later a unique antioxidant, cytoprotective, and anti-inflammatory element, with therapeutic potential, approved by world food agencies. The prebiotic activity of mushrooms beneficially affects gut homeostasis performance and the balance of gut microbiota is enhanced. Several recent studies on neurological impact and contribution to the growth of nerve and brain cells are mentioned. Indeed, mushrooms as functional foods' nutraceuticals are presently regarded as next-generation foods, supporting health and wellness, and are promising prophylactic or therapeutic agents.

KEYWORDS

fungi nourishment, bioactive elements, healthcare prevention, functional foods, pharmanutrients

Introduction

The global food system is very complex and influenced by many different inputs, including farming, economics, politics, environment, transport, storage, and consumers, and it must entail long-term dimensions of sustainability. A healthy, resilient, and sustainable food system supporting a growing poor population in a changing environment, aggravated by pandemics and wars, requires structural changes and innovations (1).

Agriculture and food security are the basis to support human health and dietary patterns by improving agricultural diversity and providing the basis for a balanced healthy diet. The occurrence of both under-nutrition and over-nutrition in the same community, even in the same household, is common probably due to unsound diets, economic globalization, or diseases (2).

Few food and nutrient guidelines were established by a handful of agencies to provide consumers and health practitioners with evidence-based recommendations on nutrient and dietary intakes associated with low risk of nutritional deficiencies and diet-related chronic diseases. However, there is little health research on diet quality based on the standard diet consumed and universal micronutrient supplementation (3, 4).

Mushrooms are edible fungus and have been widely used as medicinal products in China, Japan, and Korea. In other countries, only in the past few decades, special attention has been given to dietary supplements as sources to improve health and wellness. The nutritional role of mushroom products, as indirect probiotics, as direct prebiotics, or as both (synbiotics), is justified by their influence on the inflammation process and the gut microbiota through their contents of β-glucans, enzymes, and secondary metabolites (5).

Gut microbiota have different and specific profiles from different regions and populations, which need to be studied in order to match and determine their nutrient requirements as well as the widespread anti-biotic-resistant infections by its over- or misuse (6).

Several fungi bioactive compounds are not found or deficient in human food items of plant and animal origin, are known to support good health and wellbeing and are used as prophylactics for various human diseases. Mushrooms are now recognized as a source of nutraceuticals in nutrient balancing, strengthening the human immune system, enhancing natural body resistance, and lowering proneness to disease (7).

In this review, we underline the potential of edible mushrooms, as novel food sources and surprisingly rarely included in dietary guidelines, as an unexploited gold mine and one of the greatest untapped food resources to provide income for growing and poorer populations, and a role in shielding people against the side effects of an unhealthy stylish diet.

Mushrooms

Fungi are eukaryotic organisms that comprise microorganisms such as yeasts and molds, as well as mushrooms and fungi producing macrostructures yielding spores. Mushrooms as macro-fungi are the root of a multitude of compelling secondary metabolites produced in the portion of soil found adjacent to plant roots as self-defense as a response to biotic or non-living factors of stress (8).

The fungus mycelium creates an intercellular network growing between the endodermis single layer of cells bordering the cortex of a plant's root, but not penetrating them, sharing nutrients and water between them. Interaction or symbiosis, as a function of resource allocation, emerges as one of the essential characteristics of life, alongside metabolism and reproduction (9).

The mushroom supplies the plant with water and minerals (e.g., phosphorus and nitrogen) taken from the soil, whereas the plant provides fungi with carbon substrates and energy derived from photosynthesis (10). Mushrooms are indispensable to human life and essential for the environment as major decomposers of organic matter namely in forests and recyclers in nature ecosystems.

Mycorrhizae, known as root fungi, benefit from mutual dependence symbiotic associations entrenched between certain soil fungi and most vascular plants. Ectomycorrhizal fungi (Ascomycetes and Basidiomycetes) at root tips protect plants against pathogens, improve nutrient arrest, remediate heavy metal contamination, and avoid extreme soil pollution (11).

Phenotype-based approaches have been used to establish mushroom diversity. The global distribution of mushrooms has not been yet completely surveyed, identified, and classified under the latest protocols and technological improvements that genome sequencing offers for promising alternatives to DNA barcoding (12).

Mushrooms engage central roles in dynamic and complex communities of plant, animal, and microorganisms, decaying dead humus and interacting with evergreens, being key controllers of carbon and nutrient cycling for the maintenance of biodiversity.

Truffles, underground macro-fungi from arid- or semi-arid ecosystems, are expensive food commodities, greatly appreciated for both their culinary and medicinal properties and for enhancing the capacity of their host plant roots to resist dry spells (13).

They have been used since the Paleolithic period (ca. 2.5 million years ago to 10,000 B.C.), where their application has been historically related to spiritualism. Globally, and despite a lot of speculation, there are some 3–6 million species of fungi, but only some 3–8% have been accurately and precisely identified (14).

Only some 25 species have been perceived as edible and intensively cultivated (15), while only few species are the most produced and consumed edible mushroom worldwide: *Agaricus bisporus* (white mushroom, 22%), followed by *Lentinus edodes* (shiitake, 19%), *Pleurotus* spp. (oyster mushroom, 15%), and *Flammulina velutipes* (enoki mushroom, 11%) (16).

With the application of a collection of new molecular methods to search for biomarkers in the genome and proteome, it is now possible to identify mushrooms previously considered non-existent and detail their low-molecular-weight (e.g., glycosphingolipids, biochrome quinones, and polyphenolic isoflavones) (17) and high-molecular-weight compounds (e.g., different glucans, glycopeptides, and ribonucleoprotein complexes) (18).

Biochemical and nutrient composition of the main industrialized mushrooms is well-known; however, for many wild and under-exploited edible fungi, detailed nutritive data are limited or even non-existent. This is problematic in low-income settings where they are collected for food, as income, and constitute a valuable resource for food security.

Many research studies (19) have determined the safeguarding health outcomes of wholesome mushrooms to protect or treat various chronic diseases (Figure 1).

Most edible mushrooms, like most food items, have a limited probability of surplus dosage or toxicity and, along with negligible side effects, are perfect candidates for developing novel dietary supplements and therapeutics (20).

Here, up-to-date information is given on the current and factual knowledge on edible mushroom composition, antioxidants, and bioactive elements, enhancing their use in some disorders, and for innovative biotechnological, medicinal, and ecological applications (21). Their application in alternative or complementary folk or traditional medicine is out of the present scope.

Nutritive value of mushrooms

Many different mushroom species have diversified compositions and nutritional values (22). Common mushrooms provide micronutrients and minimal energy being outstanding suppliers of crude protein, several essential minerals, complex polysaccharides, fat free but with essential unsaturated fatty acids (>75%), vitamins B (B2 riboflavin, B9 folate, B1 thiamine, B5 pantothenic acid, and B3 niacin), and secondary metabolites (23).

Presently, there is growing attention on using mushrooms in the development of natural functional dietary supplements and biotherapeutics, as they may modulate the immune system and act as anti-inflammatory through their high content of antioxidants (24, 25).

For millennia, humans picked and ingested mushrooms for their taste and curative properties, unaware of their nutritive value. Raw fresh mushrooms contain a high concentration of water (85–95%) influencing the specific heat, a property of food materials needed for analysis and design processes involving heat transfer.

Vegans refrain from consuming animal products and may lack vitamin B12 responsible to maintain the myelin surrounding nerve cells, mental ability, red blood cell formation, and the breaking down of fatty and amino acids to produce energy. Vitamin B12 is generally low across most varieties of mushrooms and completely absent from plant sources and has a close relationship with folate, both depending on one another to work properly. Nevertheless, shiitake mushrooms contain the highest amount of vitamin B12 at 5.6 micrograms per 100 g DM (26).

Bioactive molecules of mushrooms

One of the great assets of fungi and yeasts is their unsophistication. As a eukaryotic organism, with larger and more complex ribosomal protein subunits, its cell biology often relies on fewer factors and fewer complex regulations (27).

The prevailing and vast list of secondary metabolites in mushrooms (e.g., lectins, phenolic carboxylic acids, coenzymes, triterpenoids, and among many other bioactive compounds) may oversee the activation of cellular phagocytes, cytotoxic T cells, and anti-body-mediated immunity, consequently expanding protection to disorders (4).

Mushrooms' performance on intestinal health is *via* specific bioactive molecules, namely oligo- and polysaccharide β-glucans (PSP—polysaccharide peptide or PSK—polysaccharide-krestin) and nucleotides (4). Most of these bioactive compounds function as biological response modifiers (BRM) which are immunomodulation substances that stimulate the body's response to infection and disease (28).

Mushrooms as heterotrophic organisms are unable to perform photosynthesis. They reproduce sexually or asexually, producing special cells called spores, with the potential to produce one billion offspring in a single day. They are saprophytes as they, through their enzymes, break down and absorb complex organic compounds from various environmental matrices, as they are unable to synthesize their own organic matter (29).

Mushrooms contain various bioactive flavonoids and over 30 polyphenolic compounds which act as successful antioxidants rooted in their outstanding capacity to scavenge free radicals and act as reducing agents (30). The abundance of these fungi's small organic molecules exhibits an effective repercussion on the immune system of the human host consumer (31).

Lignocellulose biomass waste is the most significant natural complex biopolymer on earth, and its structure of cellulose, hemicellulose, and lignin, being so intricate, affects its biodegradation and rate-limiting steps in the global carbon cycle, atmosphere, oceans, fossil fuels, land, and forests (32). Most species of mushrooms synthesize numerous fungal enzymes that play important functions in various applications for human, plant, and environment systems (33, 34).

The endless list of mushroom enzymes comprises ligninolytic enzymes (e.g., laccase and lignin peroxidase), hemicellulases (e.g., degrading galactans, xylans, mannans, and arabans), acid protease, β-glucanases, β-glucosidase, esterases, ribonucleases, and many others (35). These mushroom

FIGURE 1
Multiple roles of mushrooms in several dysfunctions and health concerns.

exoenzymes are major decomposing agents of utmost organic matter, biodegrading larger complex molecules, and releasing smaller fragment elements useful as nutrients or energy (36).

Woodland native *Hericium erinaceus* fruiting bodies were reported to have cytoprotective effects and consumed to prevent gastric and duodenal ulcers, in traditional medicine in North and West Africa (37). The *Hericium* fruiting body accommodates copper metalloenzymes (e.g., polyphenol oxidases, SOD, tyrosinase, and laccases), which are multifunctional and have strong antioxidant properties (38).

Proteolysis, the enzymatic hydrolysis of proteins into smaller peptides or amino acids, is vital in many physiological and metabolic mechanisms and pathways in all ecosystems. Basidiomycete mushrooms harbor rich reservoirs of proteases used in vital mechanisms of living organisms, in bioconversion of agro-wastes toward useful substrates, and agricultural and biotechnological processes (39).

In addition to the presence of enzymes, mushroom secondary metabolites can be used in unconventional applications such as processes associated with the development of cancer and as an alternative anti-nematode agent (17, 40, 41).

The large-scale research on proteomes' vanguard technique has been utilized as a sound means to investigate the biophysical properties of the protein fraction of edible mushrooms, namely Basidiomycota phyla, focussing on the molecular interactions involved in developmental milestones (42).

This includes bioactive peptides, released and active after the fractionation of proteins, as a promising strategy with potential or already involved in the synthesis of elements with anti-cancer, anti-inflammatory, anti-hypertensive, anti-microbial, anti-diabetic, antioxidant, anti-biotic properties, and in other responses to environmental changes (43).

Nucleosides and nucleotides take part in the onset and maintenance of energy for anabolism, the synthesis of polymers, and in interspecies interaction between cells, interacting with cell surface protein binding receptors. This enables the transmission of communication and exchanges within the cell and, therefore, the regulation of biological phenomena in the human body through the wide spectrum of purinergic and pyrimidine surface receptors (44).

The distribution of purine nucleobases (adenine and guanine), pyrimidine nucleobases (cytosine, uracil, and thymine), nucleosides (uridine, guanosine, adenosine, and cytidine), and novel monomeric units for nucleic acids (nucleosides and nucleotides) in edible and non-edible mushrooms needs further studies (45).

Redox imbalance generated from by-products of normal cellular metabolism spawns a vast number of modified nucleotides, created by multi-enzymatic cascade reactions and by spontaneous, without energy input, chemical reactions (46).

Elevated levels of blood nucleic acids have been related to several disorders and aging. Possible neurotoxic effects of nucleic acids from foods were investigated and the determination of levels of nucleic acid in several mushrooms showed that it is safe to consume mushrooms as daily food (47). Nevertheless, when studying *Agaricus bisporus* microRNAs (miRNAs), it was considered that they may interfere with important biological processes related to cancer, infection, and neurodegenerative diseases (48).

The few available viricidal and antiviral therapies can be complemented with nutraceuticals from mushrooms epigenetically active. MicroRNAs post-transcriptionally regulate viral and host gene expression by controlling the expression of their target messenger RNAs.

Mushroom species have been identified by cell-free nucleic acids also used as molecular signatures for prospective cancer biomarkers (49). Recently, nucleic acid-based biosensors gained importance due to their broad usefulness in monitoring parameters, which is very important in the fields of clinical diagnosis, drug development, and the food industry, among others (50).

Various therapeutic platforms delivering oligonucleotide drugs have been established and licensed, although limited by the challenges of safety and efficient consignment (51). The pyrimidine ring structure (along with purines) serves as the informational monomers of RNA and DNA, features of the gene pool, and has been disclosed to have curative prospects and valuable biomedical claims (Figure 2).

Nucleic acids are present in most foods, and meat, fish, seafood, legumes, and mushrooms contain the highest levels of these compounds varying from 2 to 6 g nucleic acid per 100 g crude protein. Targeting nucleotide (purines and pyrimidines) metabolism may stimulate the immune system and combined with immunotherapeutic treatment may control various malignancies (52).

Dietary purines produce dose-proportional increases in plasma uric acid concentrations and renal excretion, responsible for gout and renal calculi, and may influence the biosynthesis of pyrimidines, although the understanding of the inter-relationships of purine and pyrimidine metabolism requires both a global and targeted study of unprecedented scale, limited by the technology available (53).

Cordyceps sinensis, a mushroom that contains cordycepin, a derivative of the nucleoside adenosine and activator of its receptors, stimulates the production of interleukin 10, an anti-inflammatory cytokine (54). It is claimed that cordycepin products could be used as a potential medicinal adenosine receptor agonist, which could play a favorable part in the relief of COVID-19 pneumonia and the protection of the brain (55).

Mushroom secondary metabolites triterpenes, abundant in reishi mushroom (*Ganoderma lucidum*), overpower various pro-inflammation markers such as TNF-α, interleukin-6, nitrogen monoxide free radical, prostaglandin E, nuclear factor kappa B (NF-κB), and cyclooxygenase-2 (COX-2) (56, 57).

Poria cocos mushroom, besides their high content in oligosaccharides and glycoproteins, incorporates lanostane triterpenoids, showed no immunotoxicity, and was observed to improve inflammation and treat tumors (58).

Other mushrooms deploy anti-inflammatory effects indirectly, by smothering harmful free radicals and preventing oxidative damage. Chaga mushroom (*Inonotus obliquus*), for instance, has antioxidant activity, protecting cells against oxidative damage *in vitro* (59, 60). Oyster mushroom (*Pleurotus ostreatus*) has an antioxidant effect and showed no cytotoxic activity (61).

The positive health effects of mushroom consumption during inflammation have been demonstrated by inhibiting the production of pro-inflammatory mediators (62). However, there were modest recorded effects of *in vivo* consumption of edible mushrooms on induced inflammatory responses. The result is foreseeable since it would inevitably be detrimental if strongly instigated or hampering immune function after intake of a commonly ingested food (63).

Mushrooms are rich sources of dietary fiber with two main components: soluble fiber and insoluble fiber, richer in stems rather than in caps. The insoluble fraction includes chitin (some 5 g/100 gDM) and oligosaccharides β-glucans, being the most representative. Others, such as heteropolysaccharides (e.g., pectineus substances, hemicellulose, and polyuronides), make overall as much as 10–50% of DM. The interference of these fibers on nutrient bioaccessibility has been the subject of many studies taking into consideration their diversity, types, and quality (64).

Much of the diverse array of bioactive molecules includes active polysaccharide peptides found in the mycelium, while the fruiting body mainly contains oligo- and polysaccharide β-glucans (65). The interest in these bioactive compounds is extended to their potential in the prevention and treatment of COVID-19 (66).

These dietary healthy fibers cannot be metabolized by enzymes encoded in the human genome but can be degraded and fermented by enzymes of some resident species of gut microbiota. Although not all β-glucans are capable to modulate immune functions (67), mushroom β-glucans contain β-1, 3-glucan linkages and occasionally β-1, 6 linkages, a structure which is recognized by specific receptors located on the surface of immune cells and conferring immunomodulating effects (68).

Mushroom non-cellulosic β-glucans act as "biological response modifiers" or "biotherapy," a type of treatment that uses substances contrived from living organisms to treat disease, enhancing the body's own use of macrophages and T-lymphocytes, rather than directly attacking any tumors (69).

Despite their high-molecular-weight, oligosaccharide β-(1,3)-glucans and similar compounds are absorbed *via* M cells of the intestinal cell wall into the lymph, where their fragments are captured by macrophages, subsequently released and conveyed by other granulocytes, monocytes, and dendritic immune cells, leading to various immune responses and functions (70, 71).

Mushrooms were reported to stimulate cell surface receptor activity, enhancing the activity of NK (natural killer) cells, neutrophils, and macrophages, which are the backbone of the innate immune system, thus responsible for antiviral and anti-tumor responses (72). Mushrooms also have the aptitude to support, enhance, or activate the humoral and cellular adaptive

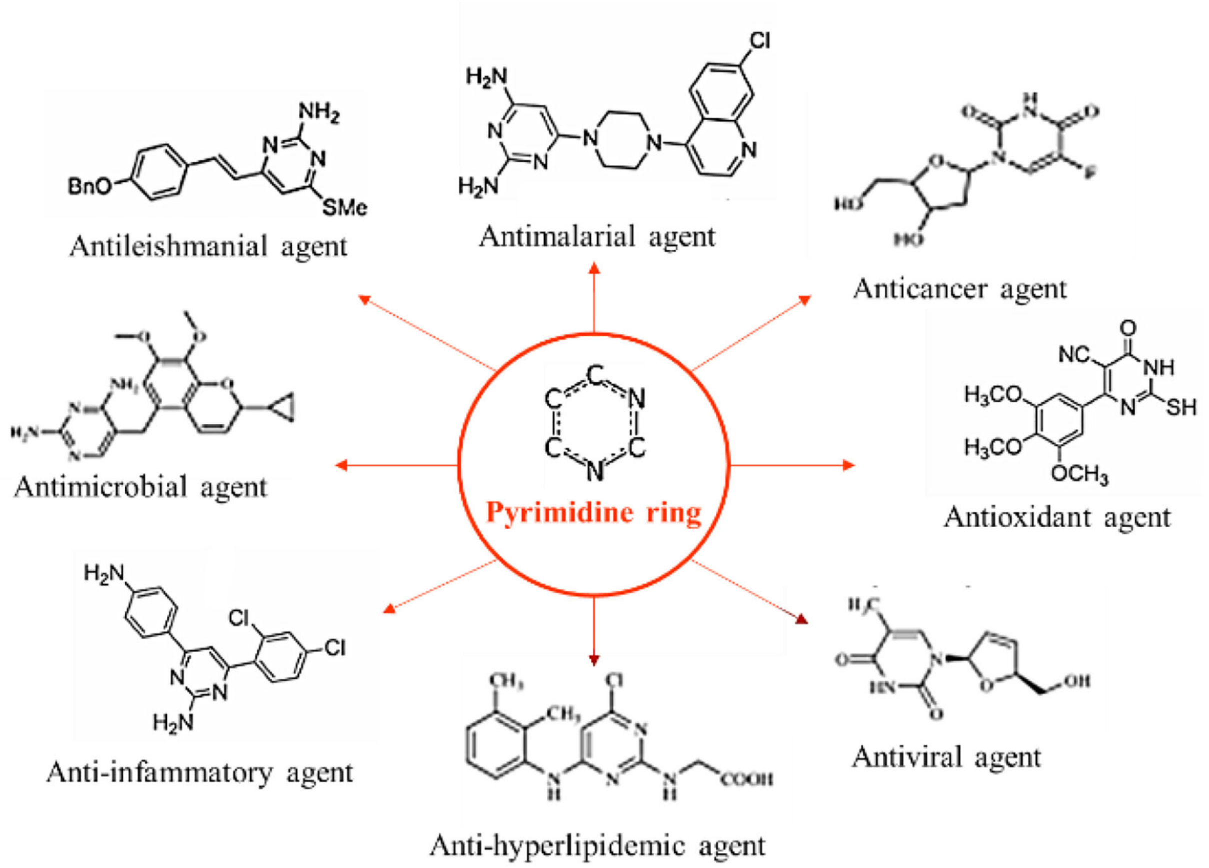

FIGURE 2
Wide range of potential functions of pyrimidine derivatives in several health conditions.

immunity system, much slower than an innate response, after initial risk subjection to an antigen or pathogen (73).

Coriolus versicolor extract, containing polysaccharide K (PSK) and polysaccharide peptide (PSP), is able to immunomodulate and induce the production of lymphocytes and cytokines, such as interferons and interleukins, exhibiting antioxidant activities (74) and used in anti-cancer mycotherapy (75).

Neuroinflammation is a specialized immune response that occurs in the central nervous system (CNS), connected to chronic neurodegenerative disorders (e.g., ALS—amyotrophic lateral sclerosis, MS—multiple sclerosis, Parkinson's disease, and particularly Alzheimer's disease) that negatively affect mental and physical functioning and are characterized by synaptic dysfunction and a gradual loss of neurons from specific regions (76).

The mitochondria participate in critical central metabolic energy-yielding pathways with vital functions in cellular senescence and the pathological mechanisms underlying cancer, neurodegenerative, and other diseases. Mitochondrial function emerges at the interface of determining health and disease, controls intestinal epithelial stem cell niche, crucial for tissue homeostasis, self-renewal, and differentiation, which can be affected by diet as nutritional changes can alter mitochondrial morphology, energy metabolism, and dynamics (77).

Mushrooms and cyanobacteria (known as blue-green algae) *Spirulina* contain a unique naturally occurring specific molecule, which humans are unable to synthesize, the amino acid ergothioneine (range between 0.06 and 5.54 mg/g DM), with powerful metabolic properties, and considered the last undiscovered vitamin (78). Ergothioneine from diet accumulates in many other tissues, and there has been a myriad of alternative applications, playing a critical role in human development and health (72, 79).

For many years PSP, pleuran, lentinan, grifolan, krestin-PSK, schizophyllan, and scleroglucan have been well-described bioactive polysaccharides from the mushroom origin, used as biopharmaceuticals with widespread clinical significance (75).

Mushrooms' omission in dietary guidelines

Globally, protein requirements have been organized under the concept of being either animal- or plant-based disregarding other substantial protein sources such as mycoprotein has been widely neglected in all dietary guidelines (80).

The daily intake of mushrooms worldwide, namely in western countries, has been rather low and considered an extravagant food delicacy; however, their incorporation in diets grants the crucial provision of micronutrients and medicinal bioactive components (81).

Mushrooms have a single and vital nutrient portrait reinforcing the general suggestion of consuming lower energy and sodium, and they are uniquely high in bioavailable and stable vitamin D2. The nutritional impact of adding mushrooms to current dietary intakes has been studied (82).

Fungi products are commercialized as fresh or dried, as biomass dietary supplements, or as extracts. This has an impact on marketing since mushroom extracts are legally considered medicinal nutraceuticals or drugs, while biomass is deemed as foodstuffs or dietary supplements (83).

However, the world general legislation governing dietary supplements rests imprecise because there is no general agreement and they can either be considered as foodstuffs and/or medicinal products as determined by various factors (19, 84, 85).

A harmonious and broad-ranging "Food Guideline" should be eclectic and comprise advice concerning various food categories, food safety elements, provision of meals, volume of (un)refined foodstuffs, processing phases, traditional food items, fermented foods and beverages, salt and sugar maximum levels of intake, sociocultural habits, and even creed and religious faiths (86).

Even considering developed countries, only in 2021, there has been a pioneer recommendation on food guidelines of adding a serving (84 g/day) of mushroom mixtures to USDA Food Patterns, the equivalent of five medium white mushrooms (87).

Anti-inflammatory activity of mushrooms

Inflammation is an immune reaction that shares the complex biological response to detrimental injury, infection, or disease. It is a physiological protective mechanism and a necessary part of healing (88). Evidence supporting the impact of specific foods on chronic persistent inflammation in the body is limited (89). Inflammation is useful because it is the result of the body fighting the infection; however, it is adverse because it damages a lot of the healthy cells in the process.

Inflammation, the fundamental principle of pathology, indicative key of injury and disease, is a critical component of metabolic syndrome, consisting of a carefully controlled flow stage-managed by immune signaling responses termed pro-inflammatory cytokines (IL-1, IL-6, and TNF-α.) and chemokines (90).

Inflammation can be suppressed by ingestion of some foods; however, diet is not the sole factor, and it is intangible the frequency and the abundance required for this advantage. Though there is promising research on the impact of particular foods, with no attention given to long-term eating habits and an anti-inflammatory lifestyle, there is no anti-inflammatory miracle food and although the diet is important, it is not the single cause.

To escape chronic inflammation, it is important to follow healthy dietary patterns, avoiding pro-inflammation diets based on processed red meat, carbohydrates, fried chips, fizzy drinks, and processed sausages. Although there is epidemiological evidence on the benefit of several phytonutrients and other factors in health, only limited clinical data share this outlook (91).

The vast list on news media of alleged anti-inflammatory foods is sometimes super-misleading and not evidence-based. The inventory includes mushroom metabolites, berries rich in anthocyanins and polyphenols, fatty fish and olive oil sources of omega-3s, broccoli rich in sulforaphane, avocados containing carotenoids, peppers providing quercetin, green tea with epigallocatechin-3-gallate (EGCG), grapes supplying anthocyanins, turmeric providing curcumin, tomatoes rich in lycopene, and dark chocolate assigning flavanols.

Mushroom extracts and bioactive components were proven to display anti-inflammatory activity serving as a clinical tool for the natural, safe, and controlled reduction of inflammation and as alternatives to conventional therapeutics (92).

Agaricus, *Pleurotus*, *Cordyceps*, and *Coriolus* are the most researched species, having the ability to reduce inflammation, but it is unclear how often and how much is needed to ingest for this benefit. Through diet, inflammation may be reduced or restrained, but it must be noted that there is no anti-inflammatory marvel food, since it is not the sole factor involved and lifestyle is also crucial (93).

Mushrooms also have an effect on immune function, but that effect is evident only when the immune system is challenged (86). Mushrooms have anti-inflammatory control by suppressing the NF-κB signaling pathways, which control the expression of cytokines, chemokines, adhesion molecules, and cell survival. Bioactive compounds of mushrooms target this major transcription factor highly implicated in both the onset and resolution of acute inflammation and activation of the immune system (Figure 3).

After plant cellulose, chitin is the most abundant biodegradable polymer in nature. Several mushrooms (e.g., *Pleurotus*, *Termitomyces*, *Lactarius*, and *Agaricus*) are rich in chitin $(C_8H_{13}O_5N)_n$, a long-chain polymer of N-acetylglucosamine, and the main component of the cell walls of

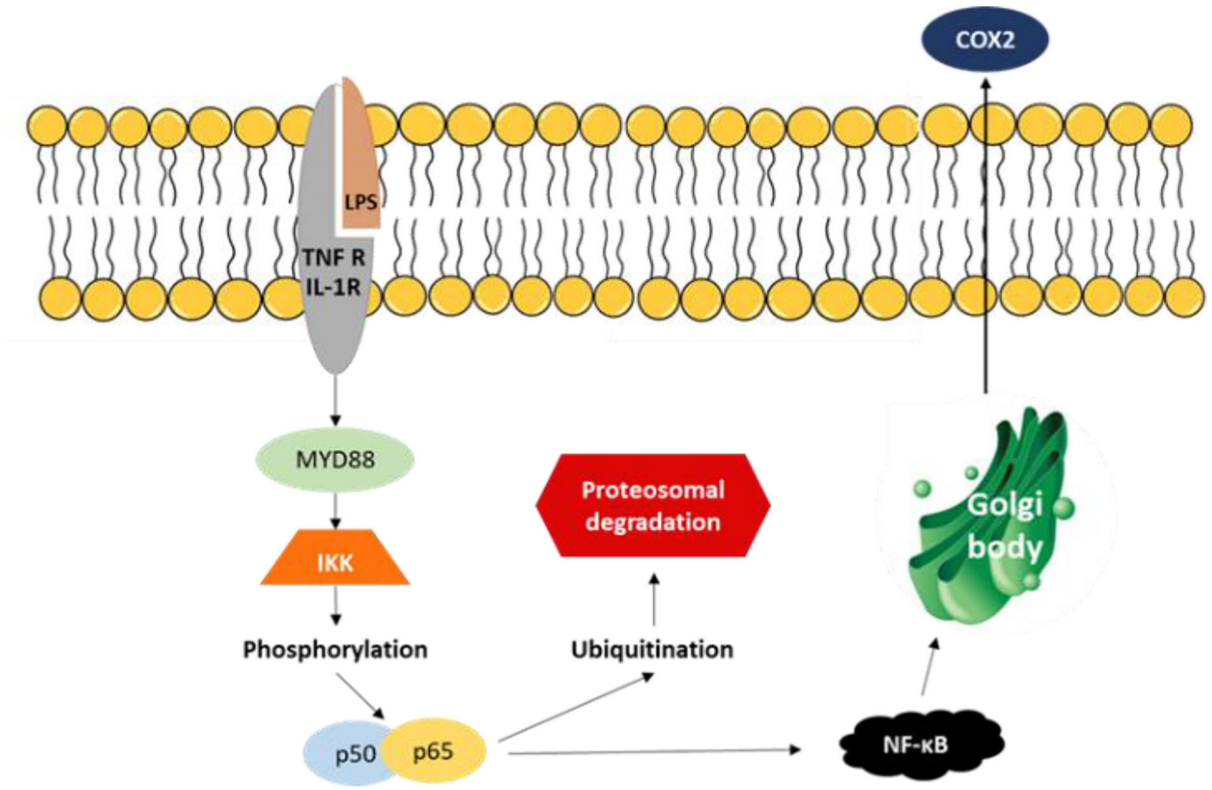

FIGURE 3
TNF R, tumor necrosis factor receptors; TLR ligands (class of proteins that play a key role in the innate immune system); LPS, lipopolysaccharides; IL-1R, interleukin-1 receptor; MYD88—innate immune signal transduction adaptor—provides instructions for making a protein involved in signaling within immune cells; NF-κB is a major transcription factor, essential for inflammatory responses, that regulates genes responsible for both the innate and adaptive immune response; IKK (classical Kinases responsible for the activation of NF-κB); p50 and p65, subunits of NF-kappa B; COX2, enzyme inhibitor that suppresses inflammatory pathways; proteasome degradation: protein complexes that degrade unneeded or damaged proteins by proteolysis; ubiquitination: a protein is inactivated by attaching a single-chain polypeptide (ubiquitin) to it.

fungi, insects, shrimp, and crustaceans shells (94). Chitin can be decomposed into glucosamine, which is involved in the creation of molecules that protect joints from inflammation and shields from other impacts on health (Figure 4).

Other mushrooms act directly on inflammation, and *Agaricus blaz*ei, *Hericium erinaceus*, and *Grifola frondosa* have been shown to exert this activity even in severe lung inflammation that often follows COVID-19 infection (95).

Cordyceps spp, entomopathogenic fungi, enclosing more than 700 species of the same genus, originated from the Tibet region, is widely used in Chinese medicine, and *C. sinensis* and *C. militaris* being the most frequently used in a wide range of biotherapeutic activities (54).

This mushroom is plentiful and assorted in highlands in humid temperate and tropical forests of the high Tibetan Plateau and contains a low-molecular-weight nucleoside compound, cordycepin, which stimulates the production of interleukin 10, an anti-inflammatory cytokine (96).

Poria cocos mushrooms incorporate hydrocarbon terpenes, described to improve inflammation and treat cancers (97). Other macrofungi deploy an indirect anti-inflammatory effect by curbing harmful free radicals and hindering oxidation. Chaga mushrooms (*Inonotus obliquus*) bestow antioxidant activity, shielding cells against redox imbalance (98). Oyster mushrooms (*Pleurotus ostreatus*) also show an antioxidant effect, preventing damage to DNA and cell membranes (99).

It is important to understand the difference in the chemical nature of extracted β-glucan from various sources. Cereals contain β-glucans at different levels, some 2.5 % (rye and wheat) up to 4.5% (oats and barley), but these are not capable of controlling immune functions, as they are usually processed prior to intake (100).

They are nevertheless considered beneficial for lowering the postprandial glucose response and the improvement of blood cholesterol levels (101). However, mushroom β-glucans, consisting mostly of 1,3-β linked glycopyranosyl residues with small numbers of (1,6)-β-linked side chains, operate as bioactive molecules with immunomodulating properties (102).

These BRD-biological response modifiers (1,3)-β-glucans interlink with the brush border enterocyte villi cells and are

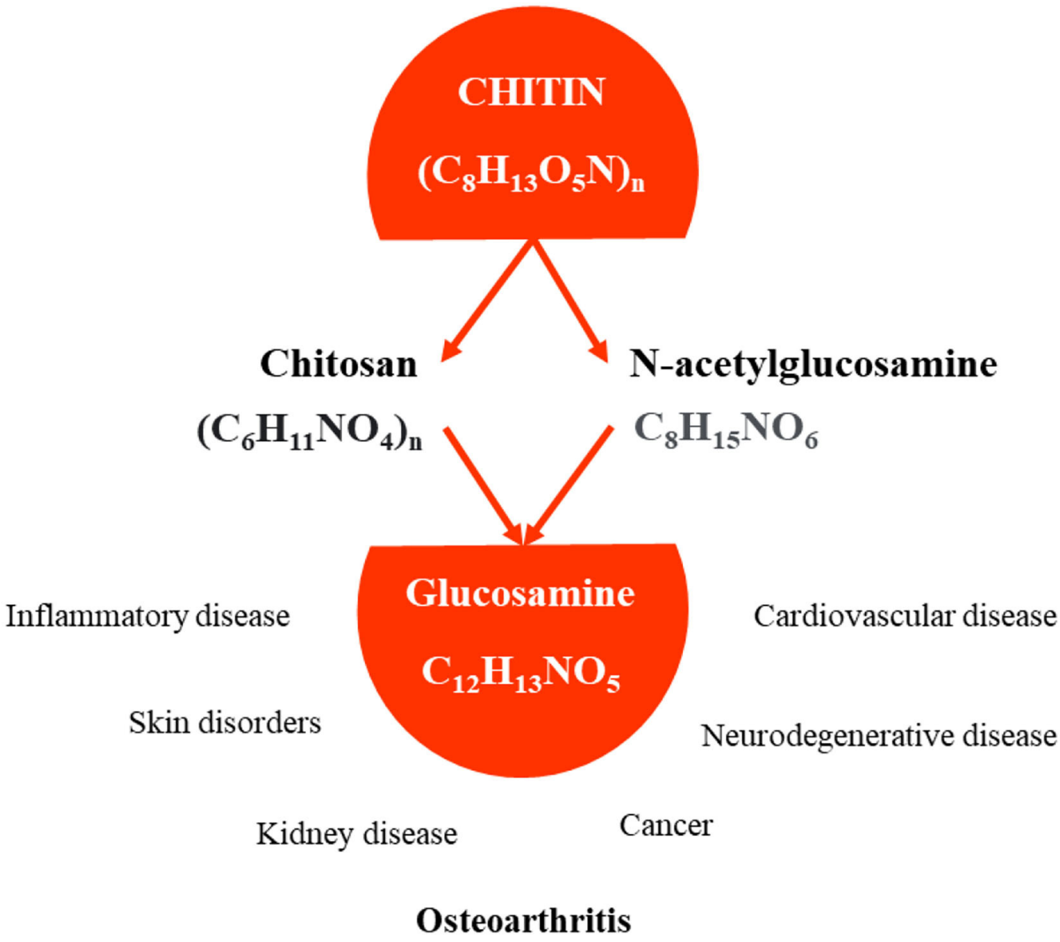

FIGURE 4
Although the effects of glucosamine are still unclear and controversial, it has been widely used as a dietary supplement for relieving complaints of osteoarthritis.

absorbed into the lymph capillaries (103). Here, they enroll the immune sentinel cells, neutrophils, and macrophages, and activate the production of cytokines crucial in controlling and stimulating immune function (Figure 5).

Neuroinflammation is a specialized immune response that occurs in the central nervous system, and it is linked to chronic neurodegenerative disorders (e.g., amyotrophic lateral sclerosis-ALS, multiple sclerosis, Huntington's disease, Parkinson's disease, and particularly Alzheimer's), negatively affecting mental and physical functioning being characterized by synaptic dysfunction and a gradual loss of neurons from specific regions (104).

Mushrooms integrate and are excellent suppliers of ergothioneine, compound humans cannot produce, a distinctive antioxidant, cytoprotective, and anti-inflammatory stem from food histidine. The unique sulfur-containing antioxidant on a deliberate cytoprotective mechanism can accumulate to high levels in red blood cells and many other tissues, functioning both as a therapeutic and possibly as a preventative agent for several diseases (Figure 6).

Antiviral properties of mushrooms

New viruses surface all the time, in a process called zoonotic spill over, jumping from wildlife and animals to humans, purely by transmutation of an ongoing native virus, and can be major hazards to public health. A virus is a disease-producing pathogen metabolically dormant and made up of a core of genetic material, either DNA or RNA, and an external lipoprotein shell, unable to replicate unless inside the host cell proteins and mechanisms.

The viral replication cycle differs by the viral type, and according to the host species which it infects, yielding new viral genomes and proteins. It involves attachment, penetration, uncoating, replication, assembly, maturation, and release steps (105).

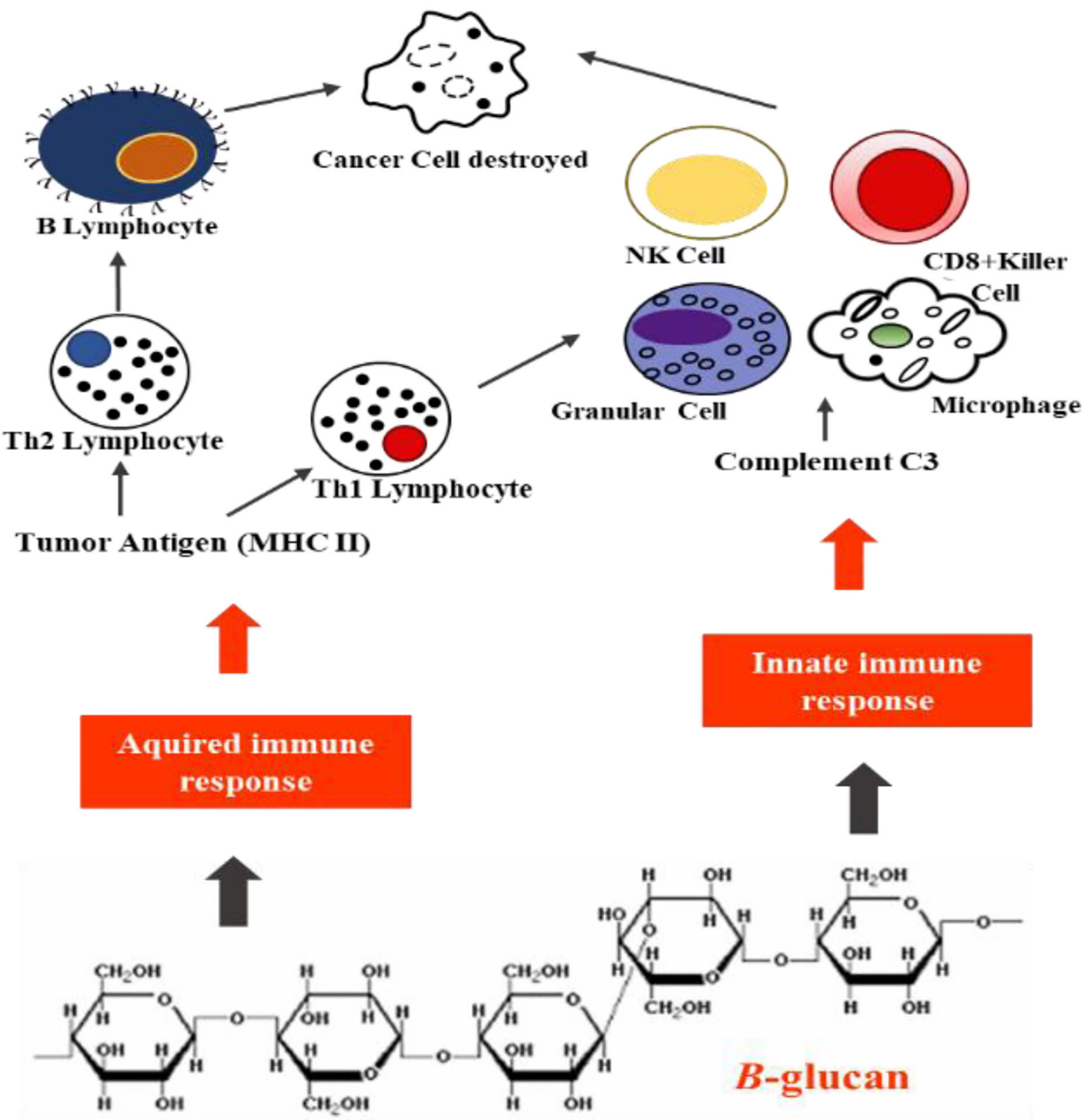

FIGURE 5
System of antineoplastic activity of bioactive β-glucan. The complexity and multi-step cancer cell recognition by the immune system, and the possible therapeutic applications of tumor-specific major histocompatibility complex (MHC-II). Normally, tumor cells do not express MHC-II genes.

Mushroom biomass and extracts downgrade viral disease by primarily addressing viral attachment and entrance to the cell, affecting genome reproduction, reducing the receptor binding of viral envelope glycoproteins by mushroom polysaccharides and triterpenes, and by influencing immune modulation (106).

Numerous previous studies have demonstrated mushrooms as exhibitors of potential antiviral efficacy, including human exposure to the SARS-CoV-2 virus (24, 107).

The natural bioactive compounds from mushrooms and dietary health supplements are responsible for the safeguard and therapy of viral infections (108) mainly through the improvement of the activation or suppression of the immune system, where immune responses are induced, amplified, attenuated, or prevented (Figure 7).

The biological active compounds function chiefly by obstructing the entrance of the virus into the cell, by causing the

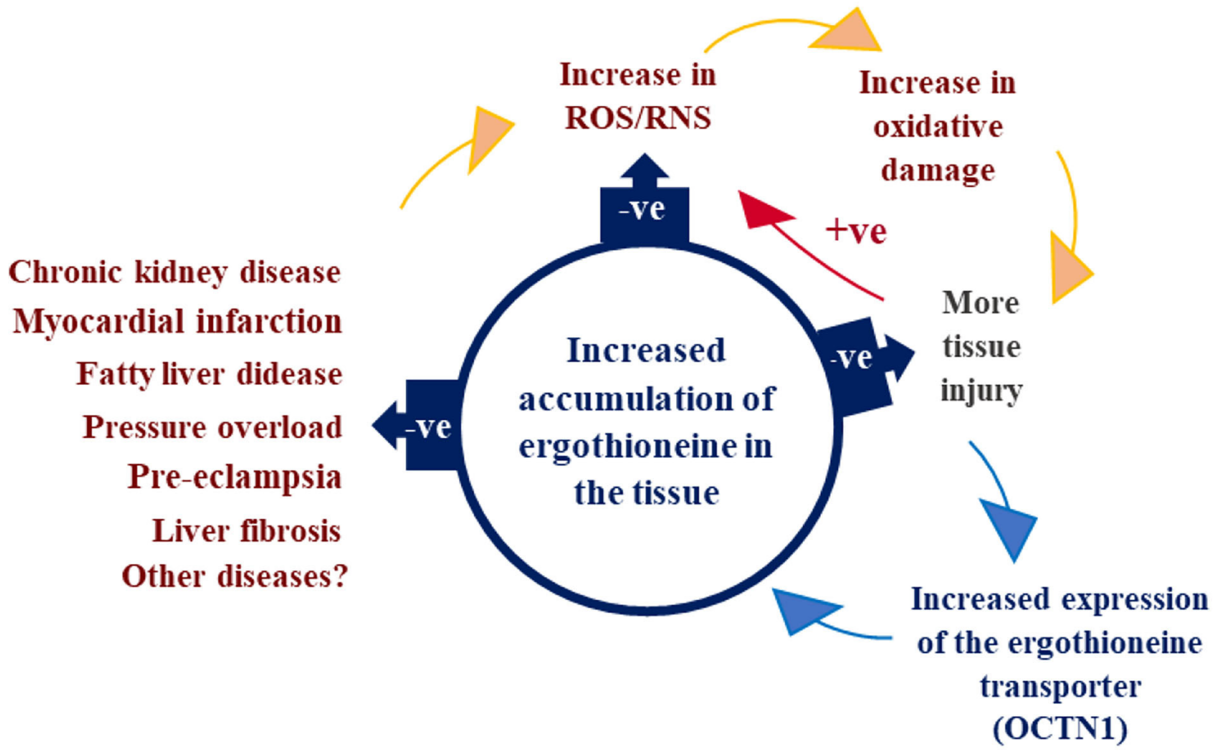

FIGURE 6
Ergothioneine, a potent intra-mitochondrial antioxidant. A diet-derived antioxidant with therapeutic potential, approved by EFSA and FDA, referred to as a "longevity vitamin".

lysis of infected cells by triggering the boosting of NK, CD8+, and T cells, by anti-neuraminidase increased inhibitory activity, and by promotion of cell-mediated immune response (109).

Several clinical studies, specifically for fighting viruses with mushroom products, have enhanced the role of *Cordyceps* fighting the flu virus by boosting the body NK cell activity and other virus-killing cytokines. Herpes simplex and hepatitis C virus were destroyed using *Ganoderma* (reishi). Similar data were obtained with *Grifola* (maitake) and *Lentinula* (shiitake).

Human immunodeficiency virus (HIV)

The pathogenesis of this disease is considered of multifactorial nature and occurs in communities where malnutrition is endemic and WHO has long established the nutrient requirements for people living with HIV/AIDS (110).

Extensive comprehensive protection for people living with HIV/AIDS requires nutritional care mediation, bioactive molecules of mushrooms may play crucial assistance to patients, and the nutritional guidelines previously established for diet management need updating, especially in limited-resource settings (105).

The evaluation of the complex interplay between HIV/AIDS and under-nutrition and the degree of severity is crucial to forecast the evolution of the disease and the probability of morbidity and death toll, namely in children (111).

Mushrooms may play a decisive role since β-glucans activate the complement system, increase CD4 cell production, boost NK cell production, and stimulate the immune system macrophages (112). Even when infected with HIV, the macrophages fight effectively, reducing HIV replication and mediators of chronic inflammation.

Many studies reveal that several triterpenes from *Ganoderma lucidum* act as antiviral agents against HIV type 1 protease (113) or by boosting the immune system indirectly blocking its multiplication (114).

Direct antiviral consequences involve blockage of viral enzymes and synthesis of viral nucleic acids and adsorption or uptake of viruses. Indirect antiviral effects are completed by vitalizing the immune response or developing biochemical factors, such as pH increase, which interdicts viral replication (115). Mushroom components, namely β-glucans, flavonoids,

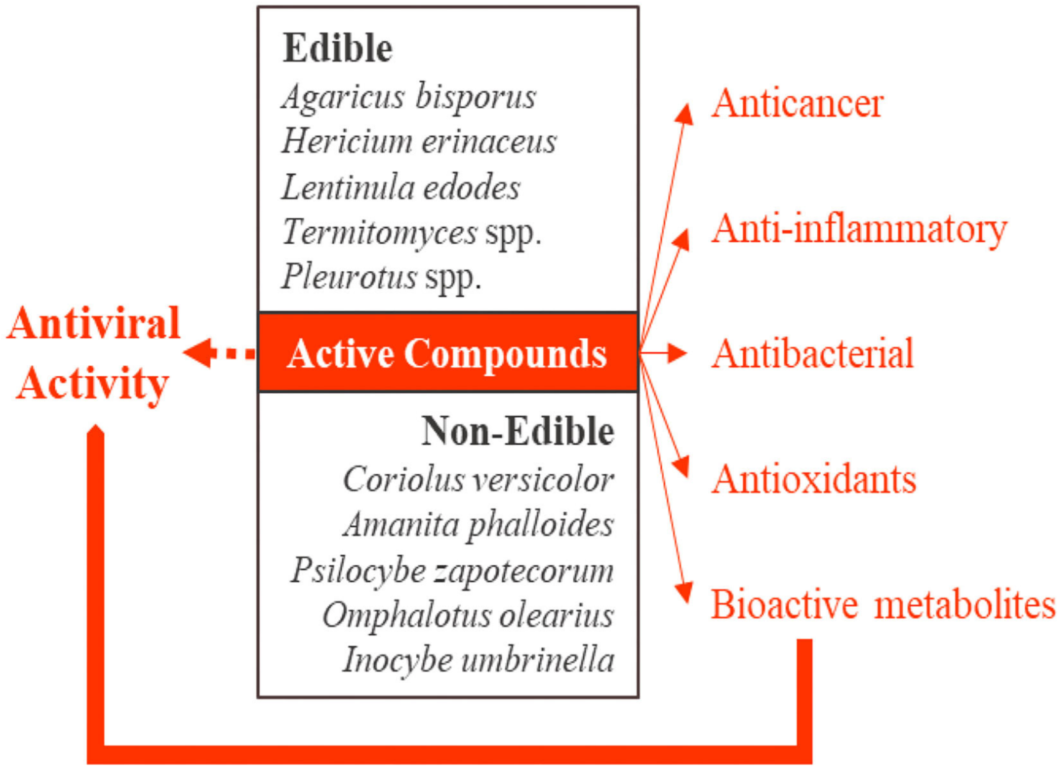

FIGURE 7
With several 100 bioactive compounds, mushrooms demonstrate antiviral outcomes and are used as dietary supplements, functional food, or medicinal products.

glycoproteins, melanin pigments, triterpenoids, and nucleosides, have all shown powerful antiviral activity (116).

Several studies revealed that *Ganoderma lucidum, Pleurotus eryngii,* and *Lentinus tigrinus* can yield laccases, cellulase, xylanases, and ligninolytic peroxidases, enzymes that can provide HIV-1 reverse transcriptase inhibitory activity against HIV (117).

The HIV-1 has a lymphotropic nature, meaning that they infect human T cells, causing relentless disruption of the lymphatic system and attached structures like the nodes, spleen, and thymus, rising numbers of dendritic cells, and antigen functions.

HIV-1 infection is currently manageable but resistance to anti-retroviral (ARV) agents is emerging, and many people infected with HIV have serious adverse reactions. Antiviral drugs do not cure HIV infection but suppress viral replication. Antiviral bioactive compounds from mushrooms and other herbal remedies are potent bio remedies, namely in the pre-exposure prevention phase, acting directly on the metabolic pathways of the human host, and regulating the interactions between viral RNAs and host cell proteins (118).

Herpes Simplex Virus (HSV)

The Herpes Simplex Virus (HSV-1) is developed in humans for millennia in a sustained, active, and perpetual shuffle where the pathogen is present at a high incidence, affecting globally half of the human population, some 3.8 billion people (119).

As over 130 known herpes viruses have been disclosed, eight infect humans and two strains occur in most β-amyloid plaques of Alzheimer's disease (AD). Some two-thirds of their proteins are indistinguishable, hinting that this frequent virus is a potential risk factor for AD, emphasizing that a particular viral species may bluntly constitute a risk of developing AD (120, 121).

The neurotropic virus either persists in an inert attitude, intercalated with reactivation events, or even engenders serious acute nervous syndromes, based on neuroinflammation and secondary effects, able to induce unsafe neural diseases (122).

Once inside the cell, HSVs dramatically modify human metabolism, inducing antiviral immune responses and assigning cell apoptosis in non-immune cells, immune cells, and T cells, while viral reproduction occurs in somatic cells prior to outbreaking into nerve centers, producing a symptomless infection (123).

The anti-herpetic activity of fruit bodies, cultivated mycelia, as supplements or teas and infusions, from *Coriolus versicolor* and several other mushrooms (e.g., *Inonotus, Pleurotus*, *Fomes*, *Auriporia*, and *Polyporus*) has been demonstrated for many years, including the antiviral activity against herpes simplex virus type 2 (HSV-2) (124, 125). As with many supplements and medications, the use of these products as therapeutics may also interact with other pharmaceuticals and carry some risks.

Influenza viruses

Global ecological fluctuations in climate and land may be responsible for zoonotic spill over transmission where a virus circulating quietly may jump from wildlife to humans, causing a disease emergence (126). This is serious in view of the great degree of transmissibility and the ability of these viruses to generate successful escape mutations.

Fresh mushrooms or dietary supplements are successful in intercepting transmission and handling several viruses such as viral rhinitis and the influenza respiratory viruses. *Grifola frondosa*, *Ganoderma lucidum*, and *Inonotus obliquus* were proven to be effective against the flu-causing influenza viruses (127).

Although a mineral deficiency is rare, some mushrooms are a rich source of specific minerals (e.g., selenium, magnesium, and zinc), which may play a direct or indirect role in their anti-influenza properties, performing a crucial role in boosting the immune system and preventing the viral infections, and preserving the homeostasis process in the human body (128). Dried mushrooms might be a source of mineral components indispensable for human health (129).

Human papillomaviruses (HPVs)

The administration of *Coriolus versicolor* biomass dietary supplement in women for a period of 1 year showed considerable success in the relapse of the cervical abnormal condition in the low-grade squamous intraepithelial lesion (LSIL) or in the removal of the high-risk HPV that can cause cancer (130).

Also, the efficacy of a *Coriolus versicolor*-based vaginal gel in women with human papillomavirus-dependent cervical lesions was positively evaluated in repairing mucosa lesions with low-grade Pap smear alterations (131).

This was replicated with a proprietary compound, active hexose correlated compound (AHCC), a fermented extract of *Lentinula edodes* mycelia, following administration for at least 6 months with a 60% successful elimination of human papillomavirus (HPV) infections in women with positive PAP (132).

Mushroom biomass forms, like other natural regimens, appear a safe treatment and are usually administered as a dietary supplement in combination with other treatments and safely boosting radiation and chemotherapeutic efficacy, as well as surgery, with a significant impact on cytotoxic activity of natural killer (NK) and T cells which play important roles in host immune defense.

The novel coronavirus (SARS-CoV-2)

The research has been intensive during the past 2 years, and there is a vast abundance of publications on COVID-19, while currently only one medication has been approved for this disease. The curious fact about the SARS-CoV-2 virus is the disparity of symptoms, ranging from no tenable manifestations to extensive signs of severe disease, namely pneumonia with fever, dry cough, and dyspnoea, spreading the virus and transmitting the disease.

Data on the role of mushrooms in this pandemic are still scarce. Indeed, clinical trials are presently underway, sanctioned by the FDA, with two polypore mushrooms, *Fomitopsis officinalis* and *Coriolus versicolor* known to strongly induce an array of differential cytokine responses (133).

The immunomodulation influential properties of *Cordyceps sinensis* (only grow in nature) and *Cordyceps militaris* were evaluated and considered fine for the prevention and treatment of COVID-19, as they are traditionally used mushrooms to improve lung functions. As severe disease indicates inflammatory response and cytokine storm plays a crucial role in the disease's pathophysiology, it is imperative to reduce the pro-inflammatory cytokines, avoid chronic interstitial pneumonitis, normalize tolerance to low levels of oxygen in the blood, and obstruct viral enzymes (134).

Several edible mushroom species including shiitake (*Lentinus edodes*), Chaga (*Inonotus obliquus*), and maitake (*Grifola* frondosa) are established as natural antivirals but not yet used for treatment against SARS-CoV-2, a present great threat to public health and global economies (66).

As the possibilities of natural substances as effective treatments against COVID-19 seemed promising, the popular mushrooms, white button (*Agaricus blazei*), reishi (*Ganoderma lucidum*), lion's mane (*Hericium erinaceus*), and maitake (*Grifola frondosa*) were tested in Norway and regarded to have prophylactic or healing outcomes upon the critical respiratory diseases frequently occurring in combination with COVID-19 infection (95).

Ganoderma lucidum consumption showed in Iraq to have a prominent role in health assessment through the diagnostic and prognostic value of the hematological and immunological parameters in 150 patients with COVID-19, reducing the

FIGURE 8
Blue chanterelle mushroom *Polyozellus multiplex* from the family *Thelephoraceae*.

number of infections and assisting in the treatment of the pandemic (135).

Encouraging impact counteracting the main protease (Mpro) of SARS-CoV-2 from edible mushrooms has recently been disclosed in January 2022 in India and concluded that edible mushroom blue chanterelle *Polyozellus multiplex* (Figure 8) has the aptitude for controlling SARS-CoV-2 infection and determined the elements that could be further studied as medicinal barriers against SARS-CoV-2 (136).

Other unpublished investigation strongly supports that the natural bioactive compounds from edible mushrooms and marine fungi have promising therapeutic potential which can be further exploited for the rapid development of nutraceutical against different virus including the SARS-CoV-2 virus (137).

New data indicate that polysaccharides from the mushroom Chaga (*Inonotus obliquus*) are potent natural resources for antiviral therapy as an adjuvant to anti-SARS-CoV-2 vaccination and medicaments. Indeed, the envelope S1 spike glycoprotein, the main antigen component, of coronavirus assists the entrance of the virus into the host cell. Some Chaga components specifically interact and attach with the terminal domains of the SARS-CoV-2 S-proteins, regulating its receptors and inhibiting virus entry into the host shelter cell (138).

From the aforementioned, even mushrooms having high safety margins cannot be deduced or recommended mushroom use as functional foods, for SARS-CoV-2 infection, since the focus is to mobilize the non-specific immunity to precipitate prompt antiviral immune natural resistance (139).

Moreover, there have not been adequate long-term risk/benefit assessments of specific mushrooms commonly suggested for pulmonary illness within the frame of the COVID-19 pandemic as an adjuvant treatment.

Anti-tumor activity of mushrooms

Most cancers or tumors are chronic multifactorial, involving single or combined genetic, environmental, lifestyle, medicinal, and other articles, including a third being clinical studies, revealed the significant impact of administration of *Ganoderma lucidum* and *Coriolus versicolor* mushrooms on the elimination of detrimental health consequences, improving lifestyle quality in oncological patients (140).

Lectins, non-immunoglobulin sugar-binding, and cell-agglutinating proteins are universally distributed in viruses, microorganisms, algae, animals, and plants and are reported as modulators *in vivo* and *in vitro*. These molecules also play a role in the induction of mitosis and immune responses, contributing to the resolution of infections and inflammations (141).

There are several types of lectins (Figure 9) that perform different functions. For example, plants use lectins to defend themselves from harmful microorganisms and insects. Some lectins are good and harmless, others are not, depending on food processing. Lectins are used not only as molecular glue for protein immobilization but also serve as a recognition element

FIGURE 9
Food sources of anti-nutrient lectins may negatively impact gut permeability and alter the population of good microbiota. Mushrooms' lectins, however, show potential for treating several ailments.

of biosensors and have high potential in the diagnosis and therapeutic of diseases (142).

More than 100 mushroom lectins have been identified (143) which exhibit a diversity of chemical characteristics possessing immune modulating and/or direct cytotoxic activity toward tumor cell lines (144).

The lectins found in some mushrooms have higher specificity for N-acetyl galactosamine and are known to possess antiviral and immune-stimulating properties to fight certain illnesses (145). Numerous lectins from mushrooms have shown potent inhibitory activity toward cancer cells through fungal extracellular ribonucleases acting as immunotoxins and are characterized by exerting their cellular cytotoxicity by inactivating ribosomes, leading to protein biosynthesis inhibition and death of neoplastic cells (146, 147).

The anti-tumor activity of mushroom compounds is not by way of direct lysis of the neoplastic cell but by means of mobilization and activation of the body's first line of defense, the non-specific innate immune system born with the host. The operating principle is related to the presence of toll-like receptors agonists that identify mushroom-specific macromolecular polysaccharides as patterns associated with pathogens (148).

As a result, in a cascade of reactions, activated macrophages generate signaling molecules, chemokines, and cytokines (TNF, IL-1β, IL-6, IL-12, and IL-23), which control the balance between immune tolerance and immunogenicity, and onset the processes disputing infections when detecting intruder and neoplastic cells (149, 150).

Fungal cell walls contain chitin, α-glucans, and namely β-glucans polysaccharides with diverse structures and functions. Mushrooms are characterized by the structure of β-1,3-glucans with short β-1,6-side chains which are adapted and recognized by specific receptors. Selected β-glucans from mushrooms (e.g., krestin, grifolan, lentinan, schizophyllan, and pleuran) showed immunomodulatory activity by binding to particular sensory receptors, suggesting a role even in the prevention and treatment of COVID-19 (66, 151).

Anti-tumor activity has been demonstrated from several mushroom components including the macromolecular polysaccharides and the highly oxygenated terpenoids, carotenoids, and steroids. The latter are secondary metabolites from mushroom metabolism, which can regulate cell death through multiple pathways including apoptosis, causing tumor cells to self-destruct, a key part of the immune response (152).

Cancer, globally the leading cause of death, has been the subject of many prophylaxes. Prevention with mushroom products has been used for many years in Asia. Natural components, polysaccharide extracts, from *Hericium erinaceus* are active against hepatocellular carcinoma (153), in gastrointestinal cancers and other types (154).

On an extensive study with almost 100 different mushroom species, it was proven the reduction of the viability of various cancers, including breast tumors (155). High intake of dietary mushrooms, the common *Agaricus bisporus* and *Lentinula edodes*, reduced the risk of breast carcinoma in pre- and postmenopausal women (156).

Oncological patients administered maitake mushroom (*Grifola frondosa*) showed significant control of cancerous growth (157). This was considered due to increased release from the spleen, organ of the lymphatic system, of pro-inflammatory

FIGURE 10
Chaga mushroom (*Inonotus obliquus*) is a parasitic fungus from the family Hymenochaetaceae with potential in oncology.

cytokines tumor necrosis factor (TNF)-α and interferon (IFN)-γ, which synergistically enhance NK cell cytotoxicity (158).

Chaga mushroom (*Inonotus obliquus*), common in cold climates, is used as tea or extracts from a mixture of wood from the substrate tree and the mycelium of the invasive fungus (Figure 10). It has been evaluated for its potential on most ailments including cancer, demonstrating its antioxidant and immunostimulatory effects both *in vitro* and *in vivo* (159).

Chaga mushroom, used traditionally for beauty and longevity, is a traditional edible mushroom with proven therapeutic value and contains biologically active substances like long-chain homopolysaccharide β-glucan, galactomannan, and the unique terpenoid betulinic acid. However, very few or no clinical trials have assessed Chagas safety or efficacy for disease prevention or treatment of cancer, cardiovascular disease, or diabetes (160).

Prebiotic activity of mushrooms

Prebiotics, non-digestible food ingredients, act as nourishment for probiotics (e.g., good live microorganisms such as bacteria and yeasts). Prebiotics [e.g., β-glucan, fructo-oligosaccharides (FOS), galacto-oligosaccharides (GOS), trans-galacto-oligosaccharides (TOS), and inulin, a type of oligosaccharide] manifest some direct health benefits such as oral anti-hyperglycaemic and improvement of the gastrointestinal function.

Although they have been medically authenticated and certified by food and health officials, the exact mechanisms behind the prebiotic–gut microbe–host interactions are still under investigation. Edible fungi interact with many beneficial gut microbiota and their non-digestible fiber serves as the substrate for microbial fermentation. Endogenous β-glucans show better prebiotic properties on the modulation of gut microbiota than exogenous β-glucans (148, 161).

β-glucan-producing probiotics may have the prebiotic potential to enhance the growth of other beneficial microbiota in the bowel. In the last decade, the number of patents and scientific articles on biotechnological, nutritional, and therapeutic uses regarding the genus *Pleurotus* has exponentially increased. *Pleurotus ostreatus* and *Pleurotus eryngii* have demonstrated potential catalytic aftermath on the proliferation of probiotic bacteria (162).

The inclusion of mushroom *Coriolus versicolor* in the human diet beneficially affected gut physiological processes and behavioral actions performance and, when the substrate was fermented, influenced modifications in the gut microbiota profile (163).

We have advanced a possible new concept where an ultra-trace metalloid element (e.g., germanium) is said to play an eventual prebiotic complementary role in the mode of action of mushrooms (164). Garlic, ginseng, ginger, and above all mushrooms have significant levels of germanium with significant potential for treating various human illnesses and promoting lifestyle.

Diabetes

Diabetes mellitus is a non-communicable global disease and a major cause of morbidity and mortality. Increased and chronic inflammation in the body may originate insulin resistance and alter its action which can lead to a cluster of several metabolic conditions, including diabetes and cardiovascular diseases. Mushrooms are rich in soluble and insoluble fiber, which is known to maintain and reduce the body's blood sugar level being effective in controlling glycaemic levels (165).

Prolonged hyperglycaemia, combined with insulin resistance, dyslipidaemia, hypertension, and chronic inflammation, promotes an increase in oxidative stress and vascular damage. This may cause capillary vascular diseases (retinopathy, nephropathy, and neuropathy) or major vascular diseases (coronary heart disease, stroke, or peripheral artery disease), related to type 2 diabetes (166).

Mushrooms and other varieties of fiber-containing foods provide the fiber responsible for decreasing the glycaemic index of diets (167). An adequate intake value of dietary fiber consumption from all diets is some 25–38 g/day (14 g/1,000 kcal/day) (168).

The mechanism of action may be achieved through a reduction in both postprandial plasma glucose and insulin concentrations, but also an increase of magnesium which plays a key role in regulating insulin action, insulin-mediated-glucose-uptake, and vascular tone. Magnesium electrolyte is a co-factor for enzymes regulating insulin and glucose metabolism, such as tyrosine kinase, and occurs in high levels (9%) in raw mushrooms (129).

Neuroprotective aptitude of mushrooms

Worldwide, there is a preponderance of neurological morbidity and disorders (e.g., mental health, stroke, epilepsy, and dementia), which have been intensifying and are among the main causes of incapacity and even death.

Mitochondria are cytoplasmic organelle authentic cell power plants where energy is produced, and when mutation dysfunctions they are responsible for oxidative stress and the development of neurodegenerative disorders and aging (169). The human body has several strategies to minimize radically induced damages and to control the imbalance between the production of free radicals and the antioxidant system, by either yielding endogenous antioxidant enzymes (e.g., SOD, CAT, and GSH-Px) or through foodstuffs or dietary supplements (170).

Progressive neurodegeneration has a complex etiology and multifactorial pathogenesis is age-related and may derive from disruptions of cellular proteostasis and multiple interactions between circulation and the brain. The bidirectional communication between the brain and gut microbiota may suffer dysregulation, and there is vast evidence that the imbalance of various microbial-derived metabolites and neuroactive compounds may initiate various neurodevelopmental and neurodegenerative diseases (159, 171).

Neuroinflammation is the process whereby the brain's innate immune system is triggered following an inflammatory challenge caused by a virus infection, injury, or toxin exposure, stimulating the host immune system, with various physiological, biochemical, and behavioral consequences. It may result in neuronal disorder and degeneration with irreversible loss of axons and neurons (172).

Mushroom incorporation in diets, at least twice a week, reduced the risk of the early stage of memory loss, usually anticipating neurological diseases (173). Mushrooms enclose countless elements presently being under investigation in relation to brain health, particularly endogenous factors that control cell proliferation and differentiation in the nervous system (162).

Stimulators of nerve growth have been found in *Hericium erinaceus*. From this mushroom, several natural aromatic compounds, hericenones from the fruiting bodies and erinacines from mycelia, were isolated and studied as having potential beneficial effects in ameliorating cognitive functioning, and behavioral deficits in Alzheimer's and Parkinson's diseases (163, 174).

We have investigated the immune modulation role of mushroom biomass of *Coriolus versicolor* in neuroinflammation and neurohormesis and the emerging role of lipoxinA4 and inflammasome in progressive neurodegenerative disorders and distinct configurations of chronic mental illness, proposing a potentially innovative treatment (69).

Coriolus versicolor (Figure 11) and *Hericium erinaceus* have been the subject of neurobiological research on the control of redox-dependent genes, so-called the vitagene family, measuring heat shock proteins, superoxide dismutase, glutathione and thioredoxin systems, lipoxin A4, and sirtuins, as potential neuro-therapeutic targets (165, 175).

Anxiety with overwhelming feelings of worry, nervousness, and fear, and depression with lingering low, sad, or hopeless mood are in developed countries among the first six most common primary care challenges in medical practice. Treatment with psilocybin, the psychedelic compound found in "magic mushrooms," has shown promise in research settings for treating a range of mental health disorders and addictions (176).

Mushrooms (e.g., *Boletus badius*) and tea leaves contain the unique L-theanine amino acid, an analog of amino acids L-glutamate and L-glutamine, which may modulate aspects of brain function in humans. It showed multiple beneficial effects on depressive symptoms, anxiety, sleep disturbance, and cognitive impairments. Although the European Union does not support L-theanine as a supplement on health claims, it is endorsed by the FDA granted GRAS (generally recognized as safe) status by the Food and Drug Administration (167, 177).

Autism spectrum disorder and mushrooms

Autism is also referred to as autism spectrum disorder (ASD) and constitutes a lifelong diverse group of conditions related to the development of the brain. It is characterized by some degree of difficulty with social and communication interaction affecting some 70 million people globally.

According to the World Health Organization, in 2022, one child in 100 worldwide suffers from an autism spectrum disorder (178). Through epidemiological estimates, these numbers have been increasing worldwide, especially in previously under-represented regions such as Africa and the Middle Eastern region; it is shown to be more common in Africa than initially believed, being a growing global public health concern.

Populations in developing countries are largely unaware of autism, diagnosed only around age 8, some 4 years later than worldwide, despite its prevalence universally similar, that is some 1% of the children. This neurological disorder is often hidden due to cultural, religious, and traditional barriers families and confounded with occult bewitching or profanity, ghosts, and devils.

Many children with this lifelong neurodevelopmental disability, more often boys than girls, in low-income neighborhoods are usually concealed indoors; therefore,

FIGURE 11
Edible, not eatable, *Coriolus versicolor* mushroom.

the rate of occurrence is unrevealed, while scarcely any clinician has the know-how or experience to recognize the condition. Indeed, child mental health assistance has been minor, since child hunger, malnutrition, and mortality are considered the paramount concerns in developing countries.

Autism spectrum disorder is a congenital or environmental disorder caused by apoptosis, more than decreased neurogenesis, inducing significant hippocampal-dependent learning and memory impairment. Children with autism have a surplus of synapses in the brain, a slowdown of synaptic pruning, and novel and much-needed treatment may be based on reducing synapsis density (179).

The first FDA-approved psychedelic treatment, controlled substance esketamine, from hallucinogenic mushrooms is considered a major "breakthrough for depression." Edible mushrooms (e.g., Chaga—*Inonotus obliquus*, Reishi—*Ganoderma lucidum*, Turkey Tail—*Coriolus versicolor*, and Shiitake—*Lentinula edodes)* are rich in ketamine and ergothioneine and have been successfully used on mental health highlighting the potential clinical and public health importance of mushroom consumption as a means of reducing depression and preventing other diseases (180, 181).

In this group of developmental disabilities, many, but not all patients with ASD suffer from gastrointestinal comorbidities, including acute and chronic constipation and diarrhea as well as persistent neuroinflammation (Figure 12). Managing an array of comorbidities greatly increases the complexity of managing disease, and it is important to address neuroinflammation by dietary restriction (182).

Preliminary results in the mouse colitis model suggest that *Coriolus versicolor* biomass supplements hold great promise as a useful way to manage inflammatory bowel diseases (183).

Hericium erinaceus various bioactive compounds are soluble, hence absorbed and metabolized, crossing the blood–brain barrier and clinically effective against depression. They condition several functions, promoting oligodendrocyte maturation with an increase in myelin basic protein, triggering the production of nerve growth factor (NGF) produced by local fibroblasts, a powerful neural survival factor after nerve injury or prolonged inflammation (174).

Mushroom nutrition in Meniere's disease

Meniere's disease (MD) is a clinical syndrome affecting ∼12 in every 1,000 people worldwide, and the underlying etiology of MD remains largely unknown. It is characterized by episodes of spontaneous dizzy spells (vertigo) associated with fluctuating, low-to-medium frequencies sensorineural hearing loss, tinnitus, and aural fullness in one or both ears. Meniere's disease can affect social life, productivity, and overall quality of life (184).

Increasing evidence suggests that, as an oxidant disorder, oxidative stress, immunomodulation, and neuroinflammation

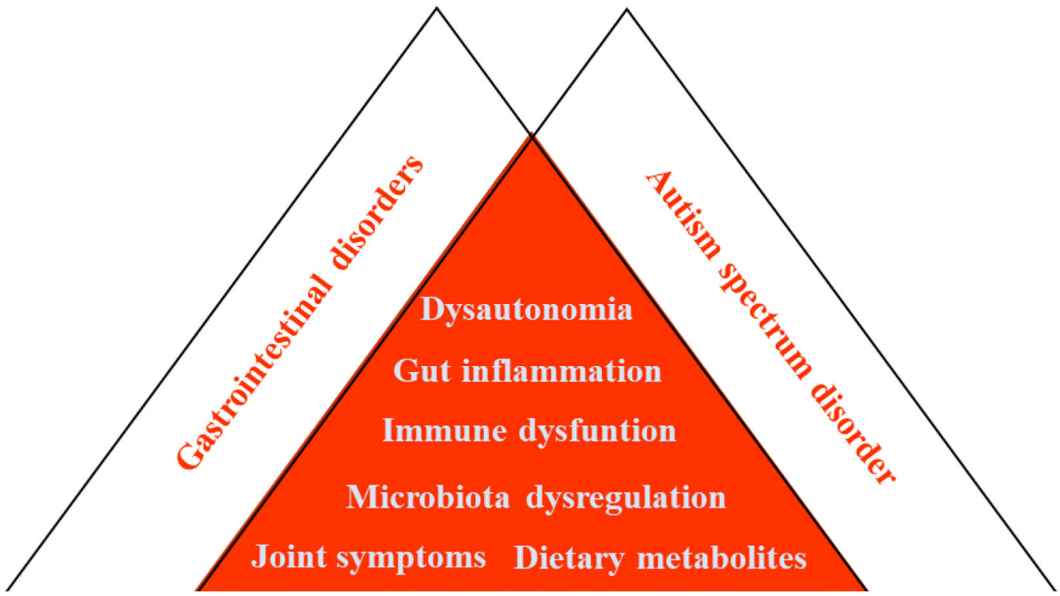

FIGURE 12
Concept of the overlap syndrome of GI disorders and ASD.

may be central to its pathogenesis. At present, there is no cure for this distressing neurodegenerative condition, but surgery and stem cell-based therapy have procedures with some success (185).

An open-label clinical trial in 40 patients with MD suffering from the cochlear sensorineural hearing loss was conducted where 22 of the patients were treated with *Coriolus versicolor* biomass (3 g/day for 2 months), and the remaining 18 were not treated. It was demonstrated that *Coriolus versicolor* supplementation may provide a useful means to modulate and amplify the body's response to oxidative challenge and cellular stress in Meniere's disease. This improved stress response appears to translate into measurable symptom relief, reduction in tinnitus, and improved mood (186).

Concluding remarks

Natural products and structural analogs have historically made a major contribution to pharmacotherapy and are increasing the trust of western people for the treatment and management of several chronic diseases. Mushroom, as functional foods, nutraceuticals, or pharmanutrients are indeed regarded as next-generation functional foods, supporting health and wellness. Nevertheless, despite their very long history of use, safety profile, and clinical use, the differences among fresh mushrooms, their extracts, or biomass dietary supplements' effects on human health are still unidentified.

To aggravate the issue, the specificity of each type of mushroom with its high-molecular diversity and biological attributes, toward a particular health concern, still requires further evaluation namely at the identification of the responsible biomolecule and nanoparticle components as well as the dose response.

Furthermore, mushrooms may be safe as a vaccine adjuvant, but there is mild concern about using them to treat people with for example active SARS-CoV-2 infection since an immune-stimulating agent like mushroom might supercharge an individual's immune response, leading to a cytokine storm, posing the greater risk of COVID-19 mortality.

New efforts are needed to elucidate the still unknown bioactive compounds present in different mushrooms and their therapeutic potential. Novel toxicological studies are needed to ensure their safety and promote pre- and clinical studies.

Author contributions

TF and VB conceived and wrote the article based on previous joint work. VB designed the figures. CS conducted part of the literature review. JG supplied African data and critical feedback. All authors contributed to the article and approved the submitted version.

Funding

The present work was supported by CIISA for publication fees on behalf of FCT—Fundação para a Ciência e Tecnologia, Grant: UIDB/00276/2020.

Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

All claims expressed in this article are solely those of the authors and do not necessarily represent those of their affiliated organizations, or those of the publisher, the editors and the reviewers. Any product that may be evaluated in this article, or claim that may be made by its manufacturer, is not guaranteed or endorsed by the publisher.

References

1. Béné C, Oosterveer P, Lamotte L, Brouwer ID, de Haan S, Prager SD, et al. When food systems meet sustainability – current narratives and implications for actions. *World Dev.* (2019) 113:116–30. doi: 10.1016/j.worlddev.2018.08.011

2. El Bilali H, Callenius C, Strassner C, Probst L. Food and nutrition security and sustainability transitions in food systems. *Food Energy Secur.* (2019) 8:e00154. doi: 10.1007/s12571-019-00922-1

3. Wiltgren AR, Booth AO, Kaur G, Cicerale S, Lacy KE, Thorpe MG, et al. Micronutrient supplement use and diet quality in university students. *Nutrients.* (2015) 7:1094. doi: 10.3390/nu7021094

4. Fernandes T, Garrine C, Ferrão J, Bell V, Varzakas T. Mushroom nutrition as preventative healthcare in sub-Saharan Africa. *Appl Sci.* (2021) 11:1–29. doi: 10.3390/app11094221

5. Fokunang ET, Annih MG, Abongwa LE, Bih ME, Vanessa TM, Fomnboh DJ, et al. Medicinal mushroom of potential pharmaceutical toxic importance: contribution in phytotherapy. In: N Shiomi, A Savitskaya, editors. *Funct Food.* IntechOpen (2022). Available online at: undefined/state.item.id (accessed August 4, 2022).

6. Elvers KT, Wilson VJ, Hammond A, Duncan L, Huntley AL, Hay AD, et al. Antibiotic-induced changes in the human gut microbiota for the most commonly prescribed antibiotics in primary care in the UK: a systematic review. *BMJ Open.* (2020) 10:35677. doi: 10.1136/bmjopen-2019-035677

7. Ashraf SA, Elkhalifa AEO, Siddiqui AJ, Patel M, Awadelkareem AM, Snoussi M, et al. Cordycepin for health and wellbeing: a potent bioactive metabolite of an entomopathogenic medicinal fungus cordyceps with its nutraceutical and therapeutic potential. *Molecules.* (2020) 25:122735. doi: 10.3390/molecules25122735

8. Tiwari M, Pati D, Mohapatra R, Sahu BB, Singh P. The impact of microbes in plant immunity and priming induced inheritance: a sustainable approach for crop protection. *Plant Stress.* (2022) 4:100072. doi: 10.1016/j.stress.2022.100072

9. Tan H, Yu Y, Tang J, Liu T, Miao R, Huang Z, et al. Build your own mushroom soil: microbiota succession and nutritional accumulation in semi-synthetic substratum drive the fructification of a soil-saprotrophic morel. *Front Microbiol.* (2021) 12:895. doi: 10.3389/fmicb.2021.656656

10. Moënne-Loccoz Y, Mavingui P, Combes C, Normand P, Steinberg C. Microorganisms and biotic interactions. *Environ Microbiol Fundam Appl.* (2015) 2015:395–444. doi: 10.1007/978-94-017-9118-2_11

11. Huang W, van Bodegom PM, Declerck S, Heinonsalo J, Cosme M, Viskari T, et al. Mycelium chemistry differs markedly between ectomycorrhizal and arbuscular mycorrhizal fungi. *Commun Biol.* (2022) 5:1–8. doi: 10.1038/s42003-022-03341-9

12. Lücking R, Aime MC, Robbertse B, Miller AN, Ariyawansa HA, Aoki T, et al. Unambiguous identification of fungi: where do we stand and how accurate and precise is fungal DNA barcoding? *IMA Fungus.* (2020) 11:1–32. doi: 10.1186/s43008-020-00033-z

13. Morte A, Kagan-Zur V, Navarro-Ródenas A, Sitrit Y. Cultivation of desert truffles—a crop suitable for arid and semi-arid zones. *Agron.* (2021) 11:1462. doi: 10.3390/agronomy11081462

14. Hawksworth DL, Lücking R. Fungal diversity revisited: 2.2 to 3.8 million species. *Microbiol Spectr.* (2017) 5:2016. doi: 10.1128/microbiolspec.FUNK-0052-2016

15. Riaz S, Ahmad A, Farooq R, Ahmed M, Shaheryar M, Hussain M. Edible mushrooms, a sustainable source of nutrition, biochemically active compounds and its effect on human health. *Funct Food.* (2022) 2022:102694. doi: 10.5772/intechopen.102694

16. Rangel-Vargas E, Rodriguez JA, Domínguez R, Lorenzo JM, Sosa ME, Andrés SC, et al. Edible mushrooms as a natural source of food ingredient/additive replacer. *Foods.* (2021) 10:112687. doi: 10.3390/foods10112687

17. Ziaja-Sołtys M, Kołodziej P, Stefaniuk D, Matuszewska A, Jaszek M, Bogucka-Kocka A. Low-molecular-weight secondary metabolites from fungi: cerrena unicolor as a new proposal of an effective preparation against rhabditis nematodes. *Molecules.* (2022) 27:1660. doi: 10.3390/molecules27051660

18. Merényi Z, Virágh M, Gluck-Thaler E, Slot JC, Kiss B, Varga T, et al. Gene age shapes the transcriptional landscape of sexual morphogenesis in mushroom-forming fungi (Agaricomycetes). *Elife.* (2022) 11:e71348. doi: 10.7554/eLife.71348

19. Chugh RM, Mittal P, MP N, Arora T, Bhattacharya T, Chopra H, et al. Fungal mushrooms: a natural compound with therapeutic applications. *Front Pharmacol.* (2022) 2022:2666. doi: 10.3389/fphar.2022.925387

20. Assemie A, Abaya G. The effect of edible mushroom on health and their biochemistry. *Int J Microbiol.* (2022) 2022:8744788. doi: 10.1155/2022/8744788

21. Kumar K, Mehra R, Guiné RPF, Lima MJ, Kumar N, Kaushik R, et al. Edible mushrooms: a comprehensive review on bioactive compounds with health benefits and processing aspects. *Foods.* (2021) 10:2996. doi: 10.3390/foods10122996

22. Ruiz-Almenara C, Gándara E, Gómez-Hernández M. Comparison of diversity and composition of macrofungal species between intensive mushroom harvesting and non-harvesting areas in Oaxaca, Mexico. *PeerJ.* (2019) 2019:8325. doi: 10.7717/peerj.8325

23. Alzand KI. Mansor?, Bofaris SM, Ugis A. Chemical composition and nutritional value of edible wild growing mushrooms: a review. *Kutaiba al World J Pharm Res.* (2019) 8:31–46. doi: 10.20959/wjpr20193-14261

24. Phillips JM, Ooi SL, Pak SC. Health-promoting properties of medicinal mushrooms and their bioactive compounds for the COVID-19 era—an appraisal: do the pro-health claims measure up? *Molecules.* (2022) 27:72302. doi: 10.3390/molecules27072302

25. Matsugo S, Sakamoto T, Wakame K, Nakamura Y, Watanabe K, Konishi T. Mushrooms as a resource for Mibyou-care functional food; the role of basidiomycetes-X (Shirayukidake) and its major components. *Nutraceuticals.* (2022) 2:132–49. doi: 10.3390/nutraceuticals2030010

26. Watanabe F, Yabuta Y, Bito T, Teng F. Vitamin $B1_2$-containing plant food sources for vegetarians. *Nutrients.* (2014) 6:1861–73. doi: 10.3390/nu6051861

27. Wilson DN, Cate JHD. The structure and function of the eukaryotic ribosome. *Cold Spring Harb Perspect Biol.* (2012) 4:a011536. doi: 10.1101/cshperspect.a011536

28. Bains A, Chawla P, Kaur S, Najda A, Fogarasi M, Fogarasi S. Bioactives from mushroom: health attributes and food industry applications. *Materials.* (2021) 14:247640. doi: 10.3390/ma14247640

29. van den Brandhof JG, Wösten HAB. Risk assessment of fungal materials. *Fungal Biol Biotechnol.* (2022) 9:1–20. doi: 10.1186/s40694-022-00134-x

30. Mwangi RW, Macharia JM, Wagara IN, Bence RL. The antioxidant potential of different edible and medicinal mushrooms. *Biomed Pharmacother.* (2022) 147:112621. doi: 10.1016/j.biopha.2022.112621

31. Chun S, Gopal J, Muthu M, Pablos PA, Omaye S. Antioxidant activity of mushroom extracts/polysaccharides—their antiviral properties and plausible anti-COVID-19 properties. *Antioxidants.* (2021) 10:1899. doi: 10.3390/antiox10121899

32. Borrero-López AM, Valencia C, Franco JM. Lignocellulosic materials for the production of biofuels, biochemicals and biomaterials and applications of lignocellulose-based polyurethanes: a review. *Polymers.* (2022) 14:881. doi: 10.3390/polym14050881

33. El-Gendi H, Saleh AK, Badierah R, Redwan EM, El-Maradny YA, El-Fakharany EM. A comprehensive insight into fungal enzymes: structure, classification, and their role in mankind's challenges. *J Fungi.* (2022) 8:10023. doi: 10.3390/jof8010023

34. Shankar A, Sharma KK. Fungal secondary metabolites in food and pharmaceuticals in the era of multi-omics. *Appl Microbiol Biotechnol.* (2022) 106:3465–88. doi: 10.1007/s00253-022-11945-8

35. Elkhateeb WA, EL-Ghwas DE, Daba GM. Mushrooms as efficient enzymatic machinery. *J Biomed Res Env Sci.* (2022) 3:423–8. doi: 10.37871/jbres1460

36. Barros A, Bell V, Ferrão J, Calabrese V, Fernandes T. Mushroom biomass: some clinical implications of β-glucans and enzymes. *Curr Res Nutr Food Sci J.* (2016) 4:37–47. doi: 10.12944/CRNFSJ.4.Special-Issue-October.06

37. Wong JY, Abdulla MA, Raman J, Phan CW, Kuppusamy UR, Golbabapour S, et al. Gastroprotective effects of lion's mane mushroom *Hericium erinaceus* (Bull:Fr) Pers (Aphyllophoromycetideae) extract against ethanol-induced ulcer in rats. *Evid Based Complement Alternat Med.* (2013) 2013:492976. doi: 10.1155/2013/492976

38. Kim S. Antioxidant compounds for the inhibition of enzymatic browning by polyphenol oxidases in the fruiting body extract of the edible mushroom *Hericium erinaceus*. *Foods.* (2020) 9:951. doi: 10.3390/foods9070951

39. Sabotič J, Kos J. Microbial and fungal protease inhibitors–current and potential applications. *Appl Microbiol Biotechnol.* (2012) 93:1351–75. doi: 10.1007/s00253-011-3834-x

40. Litwin A, Nowak M, Rózalska S. Entomopathogenic fungi: unconventional applications. *Rev Environ Sci Bio/Technol.* (2020) 19:23–42. doi: 10.1007/s11157-020-09525-1

41. Julianti E, Abrian IA, Wibowo MS, Azhari M, Tsurayya N, Izzati F, et al. Secondary metabolites from marine-derived fungi and actinobacteria as potential sources of novel colorectal cancer drugs. *Mar Drugs.* (2022) 20:10067. doi: 10.3390/md20010067

42. Al-Obaidi JR. Proteomics of edible mushrooms: a mini-review. *Electrophoresis Electrophoresis.* (2016) 37:1257–63. doi: 10.1002/elps.201600031

43. Akbarian M, Khani A, Eghbalpour S, Uversky VN. Bioactive peptides: synthesis, sources, applications, and proposed mechanisms of action. *Int J Mol Sci.* (2022) 23:1445. doi: 10.3390/ijms23031445

44. Yuan JP, Zhao SY, Wang JH, Kuang HC, Liu X. Distribution of nucleosides and nucleobases in edible fungi. *J Agric Food Chem.* (2008) 56:809–15. doi: 10.1021/jf0719205

45. Phan CW, Wang JK, Cheah SC, Naidu M, David P, Sabaratnam V. A review on the nucleic acid constituents in mushrooms: nucleobases, nucleosides and nucleotides. *Crit Rev Biotechnol.* (2018) 38:762–77. doi: 10.1080/07388551.2017.1399102

46. Tanaka M, Chock PB. Oxidative modifications of RNA and its potential roles in biosystem. *Front Mol Biosci.* (2021) 8:685331. doi: 10.3389/fmolb.2021.685331

47. Li GSF, Chang ST. The nucleic acid content of some edible mushrooms. *Eur J Appl Microbiol Biotechnol.* (1982) 15:237–40. doi: 10.1007/BF00499963

48. Marin FR, Dávalos A, Kiltschewskij D, Crespo MC, Cairns M, Andrés-León E, et al. RNA-seq, bioinformatic identification of potential MicroRNA-like small RNAs in the edible mushroom *Agaricus bisporus* and experimental approach for their validation. *Int J Mol Sci.* (2022) 23:4923. doi: 10.3390/ijms23094923

49. Mandal S, Khanna K, Johnson-Buck A, Walter NG. A guide to accelerated direct digital counting of single nucleic acid molecules by FRET-based intramolecular kinetic fingerprinting. *Methods Methods.* (2022) 197:63–73. doi: 10.1016/j.ymeth.2021.06.014

50. Carvajal Barbosa L, Insuasty Cepeda D, León Torres AF, Arias Cortes MM, Rivera Monroy ZJ, Garcia Castaneda JE, et al. Nucleic acid-based biosensors: analytical devices for prevention, diagnosis and treatment of diseases. *Vitae.* (2021) 28:347259. doi: 10.17533/udea.vitae.v28n3a347259

51. Wu L, Zhou W, Lin L, Chen A, Feng J, Qu X, et al. Delivery of therapeutic oligonucleotides in nanoscale. *Bioact Mater.* (2022) 7:292–323. doi: 10.1016/j.bioactmat.2021.05.038

52. Wu H, Gong Y, Ji P, Xie Y, Jiang YZ, Liu G. Targeting nucleotide metabolism: a promising approach to enhance cancer immunotherapy. *J Hematol Oncol.* (2022) 15:1–21. doi: 10.1186/s13045-022-01263-x

53. Groebner W, Zoellner N. The influence of dietary purines and pyrimidines on purine and pyrimidine biosynthesis in man. *Infusionstherapie und Klin Ernahrung - Forsch und Prax.* (1977) 4:305–7. doi: 10.1159/000220046

54. Das G, Shin HS, Leyva-Gómez G, Prado-Audelo MLD, Cortes H, Singh YD, et al. *Cordyceps* spp.: a review on its immune-stimulatory and other biological potentials. *Front Pharmacol.* (2021) 11:2250. doi: 10.3389/fphar.2020.602364

55. Du J, Kan W, Bao H, Jia Y, Yang J, Jia H. Interactions between adenosine receptors and cordycepin (3'- deoxyadenosine) from cordyceps militaris: possible pharmacological mechanisms for protection of the brain and the amelioration of covid-19 pneumonia. *J Biotechnol Biomed.* (2021) 4:26–32. doi: 10.26502/jbb.2642-91280035

56. Dasgupta A, Acharya K. Mushrooms: an emerging resource for therapeutic terpenoids. *Biotech.* (2019) 9:2. doi: 10.1007/s13205-019-1906-2

57. Darshani P, Sen Sarma S, Srivastava AK, Baishya R, Kumar D. Anti-viral triterpenes: a review. *Phytochem Rev.* (2022) 2022:1–82. doi: 10.1007/s11101-022-09808-1

58. Chao CL, Huang HW, Su MH, Lin HC, Wu WM. The lanostane triterpenoids in *Poria cocos* play beneficial roles in immunoregulatory activity. *Life.* (2021) 11:1–10. doi: 10.3390/life11020111

59. Cui Y, Kim DS, Park KC. Antioxidant effect of *Inonotus obliquus*. *J Ethnopharmacol J Ethnopharmacol.* (2005) 96:79–85. doi: 10.1016/j.jep.2004.08.037

60. Duarte AP, Luís Â, Gallardo E, Alhallaf W, Perkins LB. The anti-inflammatory properties of Chaga extracts obtained by different extraction methods against LPS-induced RAW 2647. *Molecules.* (2022) 27:4207. doi: 10.3390/molecules27134207

61. Egra S, Kussuma IW, Arung E, Kuspradini H. The potential of white-oyster mushroom (*Pleurotus ostreatus*) as antimicrobial and natural antioxidant. *Biofarmasi J Nat Prod Biochem.* (2019) 17:102. doi: 10.13057/biofar/f170102

62. Rowaiye A, Wilfred OI, Onuh OA, Bur D, Oni S, Nwonu EJ, et al. Modulatory effects of mushrooms on the inflammatory signaling pathways and pro-inflammatory mediators. *Clin Complement Med Pharmacol.* (2022) 2:100037. doi: 10.1016/j.ccmp.2022.100037

63. Yu S, Weaver V, Martin K, Cantorna MT. The effects of whole mushrooms during inflammation. *BMC Immunol.* (2009) 10:1–13. doi: 10.1186/1471-2172-10-12

64. Zhao H, Wang L, Brennan M, Brennan C. How does the addition of mushrooms and their dietary fibre affect starchy foods. *J Futur Foods.* (2022) 2:18–24. doi: 10.1016/j.jfutfo.2022.03.013

65. Wu JY, Siu KC, Geng P. Bioactive ingredients and medicinal values of *Grifola frondosa* (Maitake). *Foods.* (2021) 10:95. doi: 10.3390/foods10010095

66. Mirończuk-Chodakowska I, Kujawowicz K, Witkowska AM. Beta-glucans from fungi: biological and health-promoting potential in the COVID-19 pandemic era. *Nutrients.* (2021) 13:3960. doi: 10.3390/nu13113960

67. Stier H, Ebbeskotte V, Gruenwald J. Immune-modulatory effects of dietary Yeast Beta-1,3/1,6-D-glucan. *Nutr J.* (2014) 13:1–9. doi: 10.1186/1475-2891-13-38

68. Vetvicka V, Teplyakova TV, Shintyapina AB, Korolenko TA. Effects of medicinal fungi-derived β-glucan on tumor progression. *J Fungi.* (2021) 7:250. doi: 10.3390/jof7040250

69. Banerjee S, Parasramka M, Paruthy SB. Polysaccharides in cancer prevention: from bench to bedside. *Polysaccharides.* (2015) 2015:1–30. doi: 10.1007/978-3-319-0375 1-6_26-1

70. Rahar S, Swami G, Nagpal N, Nagpal M, Singh G. Preparation, characterization, and biological properties of β-glucans. *J Adv Pharm Technol Res.* (2011) 2:94. doi: 10.4103/2231-4040.8 2953

71. Han B, Baruah K, Cox E, Vanrompay D, Bossier P. Structure-functional activity relationship of β-glucans from the perspective of immunomodulation: a mini-review. *Front Immunol.* (2020) 11:658. doi: 10.3389/fimmu.2020.00658

72. Arunachalam K, Sasidharan SP, Yang X. A concise review of mushrooms antiviral and immunomodulatory properties that may combat against COVID-19. *Food Chem Adv.* (2022) 1:100023. doi: 10.1016/j.focha.2022.100023

73. Zhao Q, Elson CO. Adaptive immune education by gut microbiota antigens. *Immunology.* (2018) 154:28–37. doi: 10.1111/imm.12896

74. Habtemariam S. *Trametes versicolor* (Synn. Coriolus versicolor) Polysaccharides in cancer therapy: targets and efficacy. *Biomedicines.* (2020) 8:135. doi: 10.3390/biomedicines8050135

75. Sivanesan I, Muthu M, Gopal J, Oh J-W. Mushroom polysaccharide-assisted anticarcinogenic mycotherapy: reviewing its clinical trials. *Molecules.* (2022) 27:4090. doi: 10.3390/molecules27134090

76. Trovato A, Pennisi M, Crupi R, Paola R, Alario A, Modafferi S, et al. Neuroinflammation and mitochondrial dysfunction in the pathogenesis of Alzheimer's disease: modulation by coriolus versicolor (Yun-Zhi) nutritional mushroom. *J Neurol Neuromedicine.* (2017) 2:19–28. doi: 10.29245/2572.942X/2017/2.942X/2017/1.1088

77. Urbauer E, Rath E, Haller D. Mitochondrial metabolism in the intestinal stem cell niche—sensing and signaling in health and disease. *Front Cell Dev Biol.* (2021) 8:602814. doi: 10.3389/fcell.2020.602814

78. Fu TT, Shen L. Ergothioneine as a natural antioxidant against oxidative stress-related diseases. *Front Pharmacol.* (2022) 13:850813. doi: 10.3389/fphar.2022.850813

79. Cheah IK, Halliwell B. Ergothioneine, recent developments. *Redox Biol Redox Biol.* (2021) 42:101868. doi: 10.1016/j.redox.2021.101868

80. Derbyshire EJ. Is there scope for a novel mycelium category of proteins alongside animals and plants? *Foods.* (2020) 9:1151. doi: 10.3390/foods9091151

81. Muszy Z, Shashikant M, Bains A, Chawla P, Fogarasi M, Fogarasi S. The current status bioactivity food and pharmaceutical approaches of *calocybe indica*: a review. *Antioxidants.* (2022) 11:1145. doi: 10.3390/antiox11061145

82. Fulgoni VL, Agarwal S. Nutritional impact of adding a serving of mushrooms on usual intakes and nutrient adequacy using National Health and Nutrition Examination Survey 2011-2016 data. *Food Sci Nutr.* (2021) 9:1504–11. doi: 10.1002/fsn3.2120

83. Anusiya G, Gowthama Prabu U, Yamini NV, Sivarajasekar N, Rambabu K, Bharath G, et al. A review of the therapeutic and biological effects of edible and wild mushrooms. *Bioengineered.* (2021) 12:11239–68. doi: 10.1080/21655979.2021.2001183

84. Dwyer JT, Coates PM, Smith MJ. Dietary supplements: regulatory challenges and research resources. *Nutrients.* (2018) 10:41. doi: 10.3390/nu10010041

85. EFSA. *Food Supplements. Topics.* (2018). Available online at: https://www.efsa.europa.eu/en/topics/topic/food-supplements (accessed August 5, 2022).

86. Bell V, Ferrão J, Fernandes T. Nutritional guidelines and fermented food frameworks. *Foods.* (2017) 6:65. doi: 10.3390/foods6080065

87. Agarwal S, Fulgoni VL. Nutritional impact of adding a serving of mushrooms to USDA Food Patterns - a dietary modeling analysis. *Food Nutr Res.* (2021) 65:1–8. doi: 10.29219/fnr.v65.5618

88. Chen L, Deng H, Cui H, Fang J, Zuo Z, Deng J, et al. Inflammatory responses and inflammation-associated diseases in organs. *Oncotarget.* (2017) 9:7204–18. doi: 10.18632/oncotarget.23208

89. Hess JM, Stephensen CB, Kratz M, Bolling BW. Exploring the links between diet and inflammation: dairy foods as case studies. *Adv Nutr Adv Nutr.* (2021) 12:1S—13S. doi: 10.1093/advances/nmab108

90. Patel H, Patel VH. Inflammation and metabolic syndrome: an overview. *Curr Res Nutr Food Sci.* (2015) 3:263–8. doi: 10.12944/CRNFSJ.3.3.10

91. Monjotin N, Amiot MJ, Fleurentin J, Morel JM, Raynal S. Clinical evidence of the benefits of phytonutrients in human healthcare. *Nutrients.* (2022) 14:1712. doi: 10.3390/nu14091712

92. Rangsinth P, Sillapachaiyaporn C, Nilkhet S, Tencomnao T, Ung AT, Chuchawankul S. Mushroom-derived bioactive compounds potentially serve as the inhibitors of SARS-CoV-2 main protease: an *in silico* approach. *J Tradit Complement Med.* (2021) 11:158–72. doi: 10.1016/j.jtcme.2020.12.002

93. Taofiq O, Martins A, Barreiro MF, Ferreira ICFR. Anti-inflammatory potential of mushroom extracts and isolated metabolites. *Trends Food Sci Technol.* (2016) 50:193–210. doi: 10.1016/j.tifs.2016.02.005

94. Liu BQ, Meng X, Li C, Gao YY, Li N, Niu XF, et al. Glucosamine induces cell death via proteasome inhibition in human ALVA41 prostate cancer cell. *Exp Mol Med.* (2011) 43:487. doi: 10.3858/emm.2011.43.9.055

95. Hetland G, Johnson E, Bernardshaw S V, Grinde B. Can medicinal mushrooms have prophylactic or therapeutic effect against COVID-19 and its pneumonic superinfection and complicating inflammation? *Scand J Immunol.* (2021) 93:e12937. doi: 10.1111/sji.12937

96. Lo HC, Hsieh C, Lin FY, Hsu TH. A systematic review of the mysterious caterpillar fungus *Ophiocordyceps sinensis* in Dong-ChongXiaCao and related bioactive ingredients. *J Tradit Complement Med.* (2013) 3:16. doi: 10.1016/S2225-4110(16)30164-X

97. Li X, He Y, Zeng P, Liu Y, Zhang M, Hao C, et al. Molecular basis for *Poria cocos* mushroom polysaccharide used as an antitumor drug in China. *J Cell Mol Med.* (2019) 23:4–20. doi: 10.1111/jcmm.13564

98. Lu Y, Jia Y, Xue Z, Li N, Liu J, Chen H. Recent developments in *Inonotus obliquus* (Chaga mushroom) polysaccharides: isolation, structural characteristics, biological activities and application. *Polymers.* (2021) 13:91441. doi: 10.3390/polym13091441

99. Jaya BPD, Widiastuti EL, Nurcahyani E. Sutyarso. Taurine and oyster mushroom (*Pleurotus ostreatus*) prevents oxidative damage in liver of mice induced by paraquat. *Biomed Pharmacol J.* (2017) 10:1993–2000. doi: 10.13005/bpj/1320

100. Zielke C, Kosik O, Ainalem ML, Lovegrove A, Stradner A, Nilsson L. Characterization of cereal β-glucan extracts from oat and barley and quantification of proteinaceous matter. *PLoS ONE.* (2017) 12:e0172034. doi: 10.1371/journal.pone.0172034

101. Zurbau A, Noronha J, Khan T, Sievenpiper J, Wolever T. The effect of oat β-glucan on postprandial blood glucose and insulin responses: a systematic review and meta-analysis. *Curr Dev Nutr.* (2021) 5:533–533. doi: 10.1093/cdn/nzab041_048

102. Chen O, Mah E, Dioum E, Marwaha A, Shanmugam S, Malleshi N, et al. The role of oat nutrients in the immune system: a narrative review. *Nutr.* (2021) 13:1048. doi: 10.3390/nu13041048

103. Cerletti C, Esposito S, Iacoviello L. Edible mushrooms and beta-glucans: impact on human health. *Nutrients.* (2021) 13:2195. doi: 10.3390/nu13072195

104. Rai SN, Mishra D, Singh P, Vamanu E, Singh MP. Therapeutic applications of mushrooms and their biomolecules along with a glimpse of *in silico* approach in neurodegenerative diseases. *Biomed Pharmacother Biomed Pharmacother.* (2021) 137:111377. doi: 10.1016/j.biopha.2021.111377

105. Burrell CJ, Howard CR, Murphy FA. Virus replication. *Fenner White's Med Virol.* (2017) 7:39–55. doi: 10.1016/B978-0-12-375156-0.00004-7

106. Seo DJ, Choi C. Antiviral bioactive compounds of mushrooms and their antiviral mechanisms: a review. *Viruses.* (2021) 13:20350. doi: 10.3390/v13020350

107. Sevindik M. Mushrooms as natural antiviral sources and supplements foods against coronavirus (COVID-19). *J Bacteriol Mycol Open Access.* (2021) 9:73–6. doi: 10.15406/jbmoa.2021.09.00299

108. Piraino F, Brandt CR. Isolation and partial characterization of an antiviral, RC-183, from the edible mushroom *Rozites caperata*. *Antiviral Res.* (1999) 43:67–78. doi: 10.1016/S0166-3542(99)00035-2

109. Rappuoli R, Giudice G. Inhibiting neuraminidase can make the difference. *J Exp Med.* (2019) 216:251–2. doi: 10.1084/jem.20182245

110. WHO. *Nutrient Requirements for People Living With HIV/AIDS : Report of a Technical Consultation, 13-15 May 2003.* Geneva: WHO (2003). Available online at: https://apps.who.int/iris/handle/10665/42853 (accessed May 13, 2003).

111. Alebel A, Demant D, Petrucka P, Sibbritt D. Effects of undernutrition on mortality and morbidity among adults living with HIV in sub-Saharan Africa: a systematic review and meta-analysis. *BMC Infect Dis.* (2021) 21:1–20. doi: 10.1186/s12879-020-05706-z

112. De Marco Castro E, Calder PC, Roche HM. β-1,3/1,6-glucans and immunity: state of the art and future directions. *Mol Nutr Food Res.* (2021) 65:1901071. doi: 10.1002/mnfr.201901071

113. Choengpanya K, Ratanabunyong S, Seetaha S, Tabtimmai L, Choowongkomon K. Anti-HIV-1 reverse transcriptase property of some edible mushrooms in Asia. *Saudi J Biol Sci.* (2021) 28:2807. doi: 10.1016/j.sjbs.2021.02.012

114. Kálai T, Pongrácz JE, Mátyus P. Recent advances in influenza, HIV and SARS-CoV-2 infection prevention and drug treatment—the need for precision medicine. *Chemistry.* (2022) 4:216–58. doi: 10.3390/chemistry4020019

115. Kausar S, Said Khan F, Ishaq Mujeeb Ur Rehman M, Akram M, Riaz M, Rasool G, et al. A review: mechanism of action of antiviral drugs. *Int J Immunopathol Pharmacol.* (2021) 35:20587384211002621. doi: 10.1177/20587384211002621

116. Claus-Desbonnet H, Nikly E, Nalbantova V, Karcheva-Bahchevanska D, Ivanova S, Pierre G, et al. Polysaccharides and their derivatives as potential antiviral molecules. *Viruses.* (2022) 14:426. doi: 10.3390/v14020426

117. Xu L, Wang H, Ng T. A laccase with HIV-1 reverse transcriptase inhibitory activity from the broth of mycelial culture of the mushroom *Lentinus tigrinus*. *J Biomed Biotechnol.* (2012) 2012:536725. doi: 10.1155/2012/536725

118. Soleymani S, Naghizadeh A, Karimi M, Zarei A, Mardi R, Kordafshari G, et al. COVID-19: general strategies for herbal therapies. *J Evidence-Based Integr Med.* (2022) 27:2515690X211053641. doi: 10.1177/2515690X211053641

119. Mody PH, Pathak S, Hanson LK, Spencer JV. Herpes simplex virus: a versatile tool for insights into evolution, gene delivery, and tumor immunotherapy. *Virology.* (2020) 11:1–12. doi: 10.1177/1178122X20913274

120. Bocharova O, Pandit NP, Molesworth K, Fisher A, Mychko O, Makarava N, et al. Alzheimer's disease-associated β-amyloid does not protect against

herpes simplex virus 1 infection in the mouse brain. *J Biol Chem.* (2021) 297:100845. doi: 10.1016/j.jbc.2021.100845

121. Wainberg M, Luquez T, Koelle DM, Readhead B, Johnston C, Darvas M, et al. The viral hypothesis: how herpesviruses may contribute to Alzheimer's disease. *Mol Psychiatry.* (2021) 26:5476–80. doi: 10.1038/s41380-021-01138-6

122. Vazquez C, Jurado KA. Neurotropic RNA virus modulation of immune responses within the central nervous system. *Int J Mol Sci.* (2022) 23:4018. doi: 10.3390/ijms23074018

123. Moreno-Altamirano MMB, Kolstoe SE, Sánchez-García FJ. Virus control of cell metabolism for replication and evasion of host immune responses. *Front Cell Infect Microbiol.* (2019) 9:95. doi: 10.3389/fcimb.2019.00095

124. Krupodorova T, Rybalko S, Barshteyn V. Antiviral activity of Basidiomycete mycelia against influenza type A (serotype H1N1) and herpes simplex virus type 2 in cell culture. *Virol Sin.* (2014) 29:284–90. doi: 10.1007/s12250-014-3486-y

125. Jing Y, Zhang S, Li M, Ma Y, Zheng Y, Zhang D, et al. Research progress on the extraction structure and bioactivities of polysaccharides from *Coriolus versicolor*. *Foods.* (2022) 11:2126. doi: 10.3390/foods11142126

126. Carlson CJ, Albery GF, Merow C, Trisos CH, Zipfel CM, Eskew EA, et al. Climate change increases cross-species viral transmission risk. *Nature.* (2022) 607:555–62. doi: 10.1038/s41586-022-04788-w

127. Shahzad F, Anderson D, Najafzadeh M. The antiviral, anti-inflammatory effects of natural medicinal herbs and mushrooms and SARS-CoV-2 infection. *Nutrients.* (2020) 12:2573. doi: 10.3390/nu12092573

128. Zhang Y, Zhang G, Ling J. Medicinal fungi with antiviral effect. *Molecules.* (2022) 27:4457. doi: 10.3390/molecules27144457

129. Orywal K, Socha K, Nowakowski P, Zoń W, Mroczko B, Perkowski M. Dried wild-grown mushrooms can be considered a source of selected minerals. *Nutrients.* (2022) 14:2750. doi: 10.3390/nu14132750

130. Couto S. Evaluation of *Coriolus versicolor* supplementation in HPV patients. *Clin J Mycol.* (2007) 2:2–3. Available online at: https://www.aneid.pt/wp-content/uploads/2018/03/Clinical_Journal_of_Mycology_11_uk.pdf (accessed August 9, 2022).

131. Serrano L, López AC, González SP, Palacios S, Dexeus D, Centeno-Mediavilla C, et al. Efficacy of a *Coriolus versicolor*-based vaginal gel in women with human papillomavirus-dependent cervical lesions: the PALOMA study. *J Low Genit Tract Dis.* (2021) 25:130–6. doi: 10.1097/LGT.0000000000000596

132. Smith JA, Gaikwad AA, Mathew L, Rech B, Faro JP, Lucci JAI, et al. AHCC® supplementation to support immune function to clear persistent human papillomavirus infections. *Front Oncol.* (2022) 2022:2921. doi: 10.3389/fonc.2022.881902

133. Slomski A. Trials test mushrooms and herbs as anti–COVID-19 agents. *J Am Med Assoc.* (2021) 326:1997–9. doi: 10.1001/jama.2021.19388

134. Kaymakci MA, Guler EM. Promising potential pharmaceuticals from the genus cordyceps for COVID-19 treatment: a review study. *Bezmialem Sci Bezmialem Vakif University.* (2020) 8:140–4. doi: 10.14235/bas.galenos.2020.4532

135. Obaid AL, Jumaili MM, Al-dulaimi YFK, Ajeel MA. The role of *Ganoderma lucidum* uptake on some hematological and immunological response in patients with coronavirus (COVID-19). *Syst Rev Pharm.* (2020) 11:537–41. doi: 10.24926/iip.v11i2.3249

136. Sen D, Debnath B, Debnath P, Debnath S, Zaki MEA, Masand VH. Identification of potential edible mushroom as SARS-CoV-2 main protease inhibitor using rational drug designing approach. *Sci Rep.* (2022) 12:5349. doi: 10.1038/s41598-022-05349-x

137. Srivastav AK, Jaiswal J, Kumar U. *Tapping the Unexplored Potential of Marine Fungi and Edible Mushrooms for in silico Screening of Anti-Viral Bioactive Compounds Against SARS-CoV-2 for Rapid Development of Nutraceuticals.* (2020). Available online at: https://chemrxiv.org/engage/chemrxiv/article-details/60c7521af96a002d5c288158 (accessed August 11, 2022).

138. Eid JI, Das B, Al-Tuwaijri MM, Basal WT. Targeting SARS-CoV-2 with Chaga mushroom: an *in silico* study toward developing a natural antiviral compound. *Food Sci Nutr.* (2021) 9:6513–23. doi: 10.1002/fsn3.2576

139. Farzana M, Shahriar S, Jeba FR, Tabassum T, Araf Y, Ullah MA, et al. Functional food: complementary to fight against COVID-19. *Beni-Suef Univ J Basic Appl Sci.* (2022) 11:33. doi: 10.1186/s43088-022-00 217-z

140. Jakopovic B, Oršolić N, Kraljević Pavelić S. Antitumor, immunomodulatory and antiangiogenic efficacy of medicinal mushroom extract mixtures in advanced colorectal cancer animal model. *Molecules.* (2020) 25:5005. doi: 10.3390/molecules25215005

141. Coelho LCBB, Silva PMDS, Lima VLDM, Pontual EV, Paiva PMG, Napoleão TH, et al. Lectins, interconnecting proteins with biotechnological/pharmacological and therapeutic applications. *Evid Based Complement Alternat Med.* (2017) 2017:1594074. doi: 10.1155/2017/1594074

142. Mishra A, Behura A, Mawatwal S, Kumar A, Naik L, Mohanty SS, et al. Structure-function and application of plant lectins in disease biology and immunity. *Food Chem Toxicol.* (2019) 134:110827. doi: 10.1016/j.fct.2019.110827

143. Hassan MAA, Rouf R, Tiralongo E, May TW, Tiralongo J. Mushroom lectins: specificity, structure and bioactivity relevant to human disease. *Int J Mol Sci.* (2015) 16:7802–38. doi: 10.3390/ijms16047802

144. Nabi-Afjadi M, Heydari M, Zalpoor H, Arman I, Sadoughi A, Sahami P, et al. Lectins and lectibodies: potential promising antiviral agents. *Cell Mol Biol Lett.* (2022) 27:1–25. doi: 10.1186/s11658-022-00338-4

145. Linnakoski R, Reshamwala D, Veteli P, Cortina-Escribano M, Vanhanen H, Marjomäki V. Antiviral agents from fungi: diversity, mechanisms and potential applications. *Front Microbiol.* (2018) 9:2325. doi: 10.3389/fmicb.2018.02325

146. Sarup Singh R, Preet Kaur H, Rakesh Kanwar J. Mushroom lectins as promising anticancer substances. *Curr Protein Pept Sci.* (2016) 17:797–807. doi: 10.2174/1389203717666160226144741

147. Landi N, Hussain HZF, Pedone P V, Ragucci S, Maro A. Ribotoxic proteins, known as inhibitors of protein synthesis, from mushrooms and other fungi according to endo's fragment detection. *Toxins.* (2022) 14:403. doi: 10.3390/toxins14060403

148. Taban Q, Mumtaz PT, Masoodi KZ, Haq E, Ahmad SM. Scavenger receptors in host defense: from functional aspects to mode of action. *Cell Commun Signal.* (2022) 20:1–17. doi: 10.1186/s12964-021-00812-0

149. Kitaura H, Marahleh A, Ohori F, Noguchi T, Nara Y, Pramusita A, et al. Role of the interaction of tumor necrosis factor-α and tumor necrosis factor receptors 1 and 2 in bone-related cells. *Int J Mol Sci.* (2022) 23:31481. doi: 10.3390/ijms23031481

150. Megha KB, Joseph X, Akhil V, Mohanan P V. Cascade of immune mechanism and consequences of inflammatory disorders. *Phytomedicine.* (2021) 91:153712. doi: 10.1016/j.phymed.2021.153712

151. Caseiro C, Nunes J, Dias R, de Fontes CMGA, Bule P. From cancer therapy to winemaking: the molecular structure and applications of β-glucans and β-1, 3-glucanases. *Int J Mol Sci.* (2022) 23:3156. doi: 10.3390/ijms23063156

152. Siraj MA, Islam MA, Al Fahad MA, Kheya HR, Xiao J, Simal-Gandara J. Cancer chemopreventive role of dietary terpenoids by modulating Keap1-Nrf2-ARE signaling system—a comprehensive update. *Appl Sci.* (2021) 11:10806. doi: 10.3390/app112210806

153. Liu J, Wang W, Hu Q, Wu X, Xu H, Su A, et al. Bioactivities and molecular mechanisms of polysaccharides from *Hericium erinaceus*. *J Futur Foods.* (2022) 2:103–11. doi: 10.1016/j.jfutfo.2022.03.007

154. Younis A. Anticancer potential of *Hericium erinaceus* extracts against particular human cancer cell lines. *Microb Biosyst.* (2017) 2:9–20. doi: 10.21608/mb.2017.5253

155. Nowakowski P, Markiewicz-Zukowska R, Bielecka J, Mielcarek K, Grabia M, Socha K. Treasures from the forest: evaluation of mushroom extracts as anti-cancer agents. *Biomed Pharmacother.* (2021) 143:112106. doi: 10.1016/j.biopha.2021.112106

156. Li J, Zou L, Chen W, Zhu B, Shen N, Ke J, et al. Dietary mushroom intake may reduce the risk of breast cancer: evidence from a meta-analysis of observational studies. *PLoS ONE.* (2014) 9:93437. doi: 10.1371/journal.pone.0093437

157. Kodama N, Komuta K, Nanba H. Effect of maitake (*Grifola frondosa*) D-fraction on the activation of NK cells in cancer patients. *J Med Food.* (2004) 6:371–7. doi: 10.1089/109662003772519949

158. Wang R, Jaw JJ, Stutzman NC, Zou Z, Sun PD. Natural killer cell-produced IFN-γ and TNF-α induce target cell cytolysis through up-regulation of ICAM-1. *J Leukoc Biol.* (2012) 91:299–309. doi: 10.1189/jlb.0611308

159. Ham SS, Kim SH, Moon SY, Chung MJ, Cui CB, Han EK, et al. Antimutagenic effects of subfractions of Chaga mushroom (*Inonotus obliquus*) extract. *Mutat Res Toxicol Environ Mutagen.* (2009) 672:55–9. doi: 10.1016/j.mrgentox.2008.10.002

160. Molina-Morant D, Fernández ML, Bosch-Nicolau P, Sulleiro E, Bangher M, Salvador F, et al. Efficacy and safety assessment of different dosage of benznidazol for the treatment of Chagas disease in chronic phase in adults (MULTIBENZ study): study protocol for a multicenter randomized Phase II non-inferiority clinical trial. *Trials.* (2020) 21:1–10. doi: 10.1186/s13063-020-4226-2

161. Reilly P, Sweeney T, O'Shea C, Pierce KM, Figat S, Smith AG, et al. The effect of cereal-derived beta-glucans and exogenous enzyme supplementation on intestinal microflora, nutrient digestibility, mineral metabolism and volatile fatty acid concentrations in finisher pigs. *Anim Feed Sci Technol.* (2010) 158:165–76. doi: 10.1016/j.anifeedsci.2010.04.008

162. Li S, Shah NP. Effects of *Pleurotus eryngii* polysaccharides on bacterial growth, texture properties, proteolytic capacity, and angiotensin-I-converting enzyme–inhibitory activities of fermented milk. *J Dairy Sci.* (2015) 98:2949–61. doi: 10.3168/jds.2014-9116

163. Benson KF, Stamets P, Davis R, Nally R, Taylor A, Slater S, et al. The mycelium of the Trametes versicolor (Turkey tail) mushroom and its fermented substrate each show potent and complementary immune activating properties *in vitro*. *BMC Complement Altern Med.* (2019) 19:1–14. doi: 10.1186/s12906-019-2681-7

164. Ferrão J, Bell V, Chaquisse E, Garrine C, Fernandes T. The synbiotic role of mushrooms: is germanium a bioactive prebiotic player? A review. *Am J Food Nutr.* (2019) 7:26–35. doi: 10.12691/ajfn-7-1-5

165. Gulati V, Singh MD, Gulati P, Gulati V, Singh MD, Gulati P. Role of mushrooms in gestational diabetes mellitus. *AIMS Med Sci.* (2019) 6:49–66. doi: 10.3934/medsci.2019.1.49

166. Cade WT. Diabetes-related microvascular and macrovascular diseases in the physical therapy setting. *Phys Ther Phys Ther.* (2008) 88:1322–35. doi: 10.2522/ptj.20080008

167. Lunn J, Buttriss JL. Carbohydrates and dietary fibre. *Nutr Bull.* (2007) 32:21–64. doi: 10.1111/j.1467-3010.2007.00616.x

168. King DE, Mainous AG, Lambourne CA. Trends in dietary fiber intake in the United States, 1999-2008. *J Acad Nutr Diet.* (2012) 112:642–8. doi: 10.1016/j.jand.2012.01.019

169. Misrani A, Tabassum S, Yang L. Mitochondrial dysfunction and oxidative stress in Alzheimer's disease. *Front Aging Neurosci.* (2021) 13:57. doi: 10.3389/fnagi.2021.617588

170. Kurutas EB. The importance of antioxidants which play the role in cellular response against oxidative/nitrosative stress: current state. *Nutr J.* (2016) 15:5. doi: 10.1186/s12937-016-0186-5

171. Liu L, Huh JR, Shah K. Microbiota and the gut-brain-axis: implications for new therapeutic design in the CNS. *eBioMedicine.* (2022) 77:103908. doi: 10.1016/j.ebiom.2022.103908

172. Tohidpour A, Morgun AV, Boitsova EB, Malinovskaya NA, Martynova GP, Khilazheva ED, et al. Neuroinflammation and infection: molecular mechanisms associated with dysfunction of neurovascular unit. *Front Cell Infect Microbiol.* (2017) 7:276. doi: 10.3389/fcimb.2017.00276

173. Feng L, Cheah IKM, Ng MMX, Li J, Chan SM, Lim SL, et al. The association between mushroom consumption and mild cognitive impairment: a community-based cross-sectional study in Singapore. *J Alzheimer's Dis.* (2019) 68:197–203. doi: 10.3233/JAD-180959

174. Yu WS, Fung ML, Lee CW, Lim LW, Wong KH. The monkey head mushroom and memory enhancement in Alzheimer's disease. *Cells.* (2022) 11:2284. doi: 10.3390/cells11152284

175. Salinaro AT, Pennisi M, Di Paola R, Scuto M, Crupi R, Cambria MT, et al. Neuroinflammation and neurohormesis in the pathogenesis of Alzheimer's disease and Alzheimer-linked pathologies: modulation by nutritional mushrooms. *Immun Ageing.* (2018) 15:1–8. doi: 10.1186/s12979-017-0108-1

176. Bogadi M, Kaštelan S. A potential effect of psilocybin on anxiety in neurotic personality structures in adolescents. *Croat Med J.* (2021) 62:528. doi: 10.3325/cmj.2021.62.528

177. Saeed M, Khan MS, Kamboh AA, Alagawany M, Khafaga AF, Noreldin AE, et al. L-theanine: an astounding sui generis amino acid in poultry nutrition. *Poult Sci Poult Sci.* (2020) 99:5625–36. doi: 10.1016/j.psj.2020.07.016

178. WHO. *Autism. Fact Sheets.* (2022). Available online at: https://www.who.int/news-room/fact-sheets/detail/autism-spectrum-disorders (accessed August 8, 2022).

179. Hodges H, Fealko C, Soares N. Autism spectrum disorder: definition, epidemiology, causes, and clinical evaluation. *Transl Pediatr.* (2020) 9:S55–65. doi: 10.21037/tp.2019.09.09

180. Bell V, Ferrão J, Chaquisse E, Manuel B, Fernandes T. Role of mushrooms in autism. *Austin J Nutr Food Sci.* (2019) 6:1128. doi: 10.12691/jfnr-6-9-6

181. Gard DE, Pleet MM, Bradley ER, Penn AD, Gallenstein ML, Riley LS, et al. Evaluating the risk of psilocybin for the treatment of bipolar depression: a review of the research literature and published case studies. *J Affect Disord Reports.* (2021) 6:100240. doi: 10.1016/j.jadr.2021.100240

182. Bok E, Jo M, Lee S, Lee BR, Kim J, Kim HJ. Dietary restriction and neuroinflammation: a potential mechanistic link. *Int J Mol Sci.* (2019) 20:464. doi: 10.3390/ijms20030464

183. Impellizzeri D, Fusco R, Genovese T, Cordaro M, D'amico R, Salinaro AT, et al. *Coriolus versicolor* downregulates TLR4/NF-κB signaling cascade in Dinitrobenzenesulfonic acid-treated mice: a possible mechanism for the anti-colitis effect. *Antioxidants.* (2022) 11:406. doi: 10.3390/antiox11020406

184. Basura GJ, Adams ME, Monfared A, Schwartz SR, Antonelli PJ, Burkard R, et al. Clinical practice guideline: Ménière's disease. *Otolaryngology.* (2020) 162:S1–55. doi: 10.1177/0194599820909439

185. De Luca P, Ralli M, Cassandro C, Russo FY, Gioacchini FM, Cavaliere M, et al. Surgical management of intractable Meniere's disease. *Int Tinnitus J.* (2022) 26:50–6. doi: 10.5935/0946-5448.20220007

186. Scuto M, Di Mauro P, Ontario ML, Amato C, Modafferi S, Ciavardelli D, et al. Nutritional mushroom treatment in Meniere's disease with *Coriolus versicolor*: a rationale for therapeutic intervention in neuroinflammation and antineurodegeneration. *Int J Mol Sci.* (2020) 21:284. doi: 10.3390/ijms21010284