



# Insights of Nutritional and Anti-nutritional Retention in Traditionally Processed Millets

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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).

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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, milletrich 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 1,2,3,4,5,6hexakis 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.



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  $-20^{\circ}$ 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  $-20^{\circ}$ 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<sup>TM</sup> 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<sub>3</sub>, and 0.5 ml H<sub>2</sub>SO<sub>4</sub> 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 ( $\sim 1$  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<sub>2</sub>, B<sub>3</sub>, B<sub>5</sub>, B<sub>6</sub>, B<sub>9</sub>, 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<sub>2</sub> and B<sub>3</sub>, 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  $\mu$ m). 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  $\lambda$  at 445 nm and emission  $\lambda$  at 522 nm for the quantification of vitamin B<sub>2</sub>, 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<sub>5</sub> 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  $250 \times 4.6 \,\mathrm{mm}$ , 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  $\times$  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  $\lambda$  at 290 nm and emission  $\lambda$  at 395 nm (Valls et al., 2001). Vitamin B<sub>9</sub> was quantified by U-HPLC after extracting with the tri-enzyme technique.

Briefly, the sample was extracted with phosphate (K<sub>2</sub>HPO<sub>4</sub>) buffer containing ascorbic acid, sodium azide, and 2-mercapto ethanol. The sample was subsequently treated with enzymes ( $\alpha$ 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 ( $150 \times 2 \text{ mm}$ ;  $3 \mu \text{m}$ ) column at a flow rate of 0.5 ml/min, and the folate vitamers were detected using FLD with excitation and emission  $\lambda$  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  $\times$  4.6 mm; particle size, 5  $\mu$ m) 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.

(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  $9.45 \pm 0.37$ ,  $7.18 \pm 0.42$ , and  $10.60 \pm 0.26$  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  $\pm$  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  $\pm$ 0.06, PMFWC-2.61  $\pm$  0.14, and FMFWC-3.81  $\pm$  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

$$Nutrient retention factor = \frac{Nutrient content per 100 g of dish, edible part \times yield factor (YF)}{Nutrient content per 100 g of ingredients (ready - to - cook)}$$
$$YF = \frac{Prepared dish, including waste, in grams}{Total quantity of ingredients (ready - to - cook) in grams}$$

#### **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  $\pm$  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

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  $8.6 \pm 0.15 \text{ mg/g}$  (SMR),  $5.69 \pm 0.19 \text{ mg/g}$  (FMR), and  $4.77 \pm 0.07 \text{ mg/g}$  (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	$10.60 \pm 0.26^{\circ}$	$1.35\pm0.05^{\rm c}$	$1.40\pm0.04^{\text{a}}$	$8.33\pm0.02^{\text{a}}$	$1.09\pm0.03^{\rm a}$	$9.42\pm0.1^{a}$	$77.23\pm0.21^{\text{a}}$	$7.87\pm0.07^{\rm d}$
SMC	$9.91\pm0.18^{\rm d}$	$1.25\pm0.04^{\rm c}$	$1.24\pm0.05^{\rm b}$	$8.05\pm0.14^{\text{a}}$	$1.28\pm0.01^{\text{a}}$	$9.33\pm0.14^{\rm a}$	$78.28\pm0.43^{\text{a}}$	$75.38\pm0.5^{\circ}$
SMFC	$15.60\pm0.07^{\rm b}$	$2.36\pm0.03^{\rm b}$	$0.90\pm0.09^{\rm c}$	$7.96\pm0.01^{\text{a}}$	$1.46\pm0.02^{\text{a}}$	$9.42\pm0.02^{\text{a}}$	$71.71\pm0.90^{\rm c}$	$83.61 \pm 0.56^{\rm b}$
SMFW	$10.13\pm0.09^{\rm cd}$	$1.22\pm0.00^{\rm c}$	$0.62\pm0.02^{\rm e}$	$8.00\pm0.01^{\text{a}}$	$1.96\pm0.01^{\rm a}$	$9.97\pm0.01^{\rm a}$	$78.05\pm0.49^{\rm b}$	$87.86 \pm 1.56^{a}$
SMFWC	$20.57\pm0.37^{\text{a}}$	$2.56\pm0.06^{\text{a}}$	$0.78\pm0.01^{\rm d}$	$8.01\pm0.04^{\text{a}}$	$1.58\pm0.41^{\rm a}$	$9.60\pm0.46^{\text{a}}$	$66.44\pm0.81^{\rm d}$	$88.80\pm0.34^{\text{a}}$
Pearl millet								
PMR	$9.45\pm0.37^{\rm d}$	$1.24\pm0.2^{\rm b}$	$4.30\pm0.02^{\rm a}$	$9.02\pm0.01^{\rm a}$	$1.09\pm0.02^{\circ}$	$10.11 \pm 0.02^{\circ}$	$74.88\pm0.43^{\text{a}}$	$7.87\pm0.09^{\rm d}$
PMC	$9.30\pm0.06^{\rm d}$	$1.35\pm0.04^{\rm b}$	$2.04\pm0.12^{\rm b}$	$8.34\pm0.01^{\circ}$	$1.79\pm0.03^{\rm b}$	$10.13\pm0.05^{\rm c}$	$77.14\pm0.25^{a}$	$72.97\pm0.93^{\rm c}$
PMFC	$16.46\pm0.18^{\rm b}$	$2.56\pm0.04^{\text{a}}$	$1.43\pm0.00^{\rm c}$	$8.42\pm0.03^{\rm c}$	$1.85\pm0.04^{\text{ba}}$	$10.28\pm0.02^{\text{b}}$	$69.26\pm0.68^{\rm c}$	$81.80\pm0.44^{\rm b}$
PMFW	$10.49 \pm 0.11^{\circ}$	$1.41\pm0.48^{\rm b}$	$0.91\pm0.03^{\rm d}$	$8.70\pm0.03^{\rm b}$	$1.93\pm0.01^{\mathrm{ba}}$	$10.64\pm0.04^{\rm a}$	$76.52 \pm 0.79^{\rm b}$	$87.81 \pm 0.20^{a}$
PMFWC	$20.27\pm0.27^{\text{a}}$	$2.61\pm0.14^{a}$	$0.64\pm0.02^{\rm e}$	$8.11\pm0.02^{\rm d}$	$2.02\pm0.02^{\text{a}}$	$10.13\pm0.04^{\rm c}$	$66.32\pm0.38^{\rm d}$	$88.24\pm0.34^{\text{a}}$
Finger millet								
FMR	$7.18\pm0.42^{\rm d}$	$2.14\pm0.04^{\rm b}$	$1.36\pm0.03^{\text{a}}$	$9.42\pm0.13^{\text{a}}$	$2.08\pm0.04^{\text{a}}$	$11.41\pm0.10^{a}$	$77.91 \pm 1.31^{a}$	$10.50\pm0.00^{\rm e}$
FMC	$8.09\pm0.08^{\rm c}$	$2.27\pm0.09^{\rm b}$	$0.58\pm0.06^{\rm d}$	$9.04\pm0.03^{\text{ab}}$	$2.00\pm0.02^{\text{a}}$	$11.13\pm0.07^{\text{ab}}$	$77.95\pm0.48^{\text{a}}$	$68.03 \pm 0.51^{d}$
FMFC	$15.68\pm0.09^{\rm b}$	$3.70\pm0.04^{\text{a}}$	$0.98\pm0.01^{\rm b}$	$8.66\pm0.04^{\rm bc}$	$1.79\pm0.14^{\rm a}$	$10.45\pm0.10^{\rm cb}$	$69.10\pm1.03^{\rm b}$	$77.88 \pm 0.47^{\circ}$
FMFW	$7.88\pm0.12^{\rm c}$	$2.41\pm0.07^{\rm b}$	$0.64\pm0.02^{\rm d}$	$8.42\pm0.02^{\rm c}$	$1.85\pm0.01^{\text{a}}$	$10.27\pm0.03^{\rm c}$	$78.79\pm0.72^{a}$	$82.55\pm0.79^{\rm b}$
FMFWC	$16.92\pm0.07^{\rm a}$	$3.81\pm0.05^{\text{a}}$	$0.83\pm0.01^{\circ}$	$9.05\pm0.01^{\text{ab}}$	$1.81\pm0.02^{a}$	$10.86\pm0.03^{\rm b}$	$67.68\pm0.72^{\rm b}$	$84.12\pm0.38^{\text{a}}$

Values represent mean  $\pm$  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:  $3.75 \pm 0.06$ ; PMFWC:  $3.37 \pm 0.17$ ; SMFWC:  $3.28 \pm 0.09$  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  $(3.32 \pm 0.15 \text{ mg}/100 \text{ g})$ , followed by finger millet (2.09  $\pm$  0.09 mg/100 g) and sorghum (1.95  $\pm$  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  $\pm$  0.75 mg/100 g), followed by sorghum (3.32  $\pm$ 0.12 mg/100 g) and pearl millet  $(3.29 \pm 0.19 \text{ mg}/100 \text{ g})$ . Among traditionally processed millet foods, elevated Fe content was distinguished in the millets fermented overnight after traditional cooking (SMFW, 7.24  $\pm$  0.52 mg/100 g; PMFW, 8.40  $\pm$  0.334 mg/100 g; FMFW, 8.95  $\pm$  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;







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<sub>2</sub> (0.223  $\pm$  0.018 mg/100 g) compared to the other millets. Among traditionally processed millets, vitamin B<sub>2</sub> was significantly (p < 0.05) higher in PMFC (0.173  $\pm$  0.002 mg/100 g), followed by SMFW (0.136  $\pm$  0.039 mg/100 g) and FMFWC (0.115  $\pm$  0.012 mg/100 g). However, maximum B<sub>2</sub> retention was seen in SMFW (64.4%). Vitamin B<sub>3</sub> content was higher in sorghum (2.588  $\pm$  0.112 mg/100 g) and lowest in finger millet (1.723  $\pm$  0.108 mg/100 g). Vitamin B<sub>3</sub> content was reduced to 1.806  $\pm$  0.132 mg/100 g in sorghum (SMFWC),

with 59% retention, whereas  $B_3$  was reduced to 1.349  $\pm$  0.067 mg/100 g in pearl millet (PMFW), with 46% retention, and 0.629  $\pm$  0.021 mg/100 g in finger millet (FMC), with 23% retention.

Vitamin B<sub>5</sub> was highest in the pearl millet (0.633  $\pm$  0.021 mg/100 g) among the three raw millets analyzed. However, the processing reduced the B<sub>5</sub> content in all the millets. The cooked millet fermented overnight and then added with curd was found to have more vitamin B5 compared to those under the other processes (SMFWC,  $0.204 \pm 0.008$  mg/100 g; PMFWC,  $0.411 \pm 0.011 \text{ mg}/100 \text{ g}$ ; FMFWC,  $0.253 \pm 0.015 \text{ mg}/100 \text{ g}$ ). A maximum of 68% retention was observed in FMFWC, followed by 54% in SMFWC and 39% in PMFWC. Vitamin B<sub>6</sub> content in the raw and processed millets is illustrated in **Figure 4D**. Among the raw millets, highest B<sub>6</sub> content was seen in pearl millet (0.291  $\pm$  0.015 mg/100 g) and the lowest in finger millet (0.071  $\pm$  0.001 mg/100 g). The traditionally cooked millets have higher levels of B<sub>6</sub> (SMC, 0.041  $\pm$  0.002 mg/100 g; PMC, 0.083  $\pm$  0.004 mg/100 g; FMC, 0.028  $\pm$  0.002 mg/100 g), with retention of 10.4, 19, and 29.7% in SMC, PMC, and FMS, respectively.

Quantification of vitamin B<sub>9</sub> by the U-HPLC technique in the raw and traditionally processed millets is shown in **Figure 4E**.



**TABLE 2** | Mineral content and phytic acid-mineral molar ratio of traditionally processed sorghum, pearl millet, and finger millet samples.

	iron	Phytic acid–mineral molar ratio	
mg/ <sup>-</sup>	100 g	PA/Zn	PA/Fe
$1.95\pm0.01^{\rm b}$	$3.32\pm0.12^{\rm b}$	43.79	21.94
$2.19\pm0.11^{\rm b}$	$3.88\pm0.12^{\rm b}$	19.80	9.50
$2.55\pm0.05^{\text{a}}$	$6.09\pm0.91^{\text{a}}$	15.22	5.81
$2.72\pm0.05^{\text{a}}$	$7.24\pm0.52^{\text{a}}$	18.61	5.97
$2.59\pm0.02^{\text{a}}$	$4.16\pm0.04^{\text{b}}$	13.60	7.24
$3.32\pm0.15^{\text{a}}$	$3.29\pm0.19^{\rm c}$	14.25	12.26
$3.34\pm0.13^{\text{a}}$	$5.77\pm0.34^{\rm b}$	11.86	5.87
$3.51\pm0.10^{\text{a}}$	$6.75\pm0.616^{\text{b}}$	10.56	4.73
$3.54\pm0.04^{\rm a}$	$8.40\pm0.334^{a}$	11.46	4.12
$3.52\pm0.02^{\text{a}}$	$5.71 \pm 0.215^{\rm b}$	9.48	5.00
$2.09\pm0.09^{\text{a}}$	$5.15\pm0.75^{\rm b}$	26.98	9.34
$2.21\pm0.04^{\text{a}}$	$6.87\pm0.46^{\text{ba}}$	21.35	5.85
$3.19\pm0.10^{\text{a}}$	$7.81\pm0.91^{\rm a}$	14.19	4.95
$3.39\pm0.738^{a}$	$8.95\pm0.63^{\text{a}}$	21.53	5.21
$3.54\pm0.231^{a}$	$6.55\pm0.64^{\text{ba}}$	10.48	4.85
	$\begin{tabular}{ c c c c } \hline mg/\\ \hline 1.95 \pm 0.01^b\\ \hline 2.19 \pm 0.11^b\\ \hline 2.55 \pm 0.05^a\\ \hline 2.59 \pm 0.02^a\\ \hline 3.32 \pm 0.15^a\\ \hline 3.34 \pm 0.13^a\\ \hline 3.51 \pm 0.10^a\\ \hline 3.54 \pm 0.04^a\\ \hline 3.52 \pm 0.02^a\\ \hline 2.09 \pm 0.09^a\\ \hline 2.21 \pm 0.04^a\\ \hline 3.19 \pm 0.10^a\\ \hline 3.39 \pm 0.738^a\\ \hline 3.54 \pm 0.231^a\\ \hline \end{tabular}$	mg/100 g $1.95 \pm 0.01^b$ $3.32 \pm 0.12^b$ $2.19 \pm 0.11^b$ $3.88 \pm 0.12^b$ $2.55 \pm 0.05^a$ $6.09 \pm 0.91^a$ $2.72 \pm 0.05^a$ $7.24 \pm 0.52^a$ $2.59 \pm 0.02^a$ $4.16 \pm 0.04^b$ $3.32 \pm 0.15^a$ $3.29 \pm 0.19^c$ $3.34 \pm 0.13^a$ $5.77 \pm 0.34^b$ $3.51 \pm 0.10^a$ $6.75 \pm 0.616^b$ $3.52 \pm 0.02^a$ $5.15 \pm 0.75^b$ $2.09 \pm 0.09^a$ $5.15 \pm 0.75^b$ $2.21 \pm 0.04^a$ $6.87 \pm 0.46^{ba}$ $3.19 \pm 0.10^a$ $7.81 \pm 0.91^a$ $3.39 \pm 0.738^a$ $8.95 \pm 0.63^a$ $3.54 \pm 0.231^a$ $6.55 \pm 0.64^{ba}$	mode           mg/100 g         PA/Zn $1.95 \pm 0.01^b$ $3.32 \pm 0.12^b$ $43.79$ $2.19 \pm 0.11^b$ $3.88 \pm 0.12^b$ $19.80$ $2.55 \pm 0.05^a$ $6.09 \pm 0.91^a$ $15.22$ $2.72 \pm 0.05^a$ $7.24 \pm 0.52^a$ $18.61$ $2.59 \pm 0.02^a$ $4.16 \pm 0.04^b$ $13.60$ $3.32 \pm 0.15^a$ $3.29 \pm 0.19^c$ $14.25$ $3.34 \pm 0.13^a$ $5.77 \pm 0.34^b$ $11.86$ $3.51 \pm 0.10^a$ $6.75 \pm 0.616^b$ $10.56$ $3.54 \pm 0.04^a$ $8.40 \pm 0.334^a$ $11.46$ $3.52 \pm 0.02^a$ $5.15 \pm 0.75^b$ $26.98$ $2.209 \pm 0.09^a$ $5.15 \pm 0.75^b$ $26.98$ $2.21 \pm 0.04^a$ $6.87 \pm 0.46^{ba}$ $21.35$ $3.19 \pm 0.10^a$ $7.81 \pm 0.91^a$ $14.19$ $3.39 \pm 0.738^a$ $8.95 \pm 0.63^a$ $21.53$ $3.54 \pm 0.231^a$ $6.55 \pm 0.64^{ba}$ $10.48$

Values represent mean  $\pm$  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; PMFWC, pearl millet cooked and fermented with water overnight; PMFWC, pearl millet cooked and fermented with water; FMFC, finger millet cooked and fermented with water; FMFC, finger millet cooked and fermented with water; FMFC, finger millet cooked and fermented with water overnight; FMFWC, finger millet cooked and fermented with water overnight; FMFWC, finger millet cooked and fermented with water overnight; AMFWC, finger millet cooked and fermented with water overnight; FMFWC, finger millet cooked and fermented with water overnight; FMFWC, finger millet cooked and fermented with water overnight; FMFWC, finger millet cooked and fermented with water overnight; FMFWC, finger millet cooked and fermented with water overnight; FMFWC, finger millet cooked and fermented with water overnight; FMFWC, finger millet cooked and fermented with water overnight; FMFWC, finger millet cooked and fermented with water overnight; FMFWC, finger millet cooked and fermented with water overnight; FMFWC, finger millet cooked and fermented with water overnight; FMFWC, finger millet cooked and fermented with water overnight; FMFWC, finger millet cooked and fermented with water overnight; FMFWC, finger millet cooked and fermented with water overnight; FMFWC, finger millet cooked and fermented with water overnight; FMFWC, finger millet cooked and fermented with water overnight; FMFWC, finger millet cooked and fermented with water overnight; FMFWC, finger millet cooked and fermented with water overnight; FMFWC, finger millet cooked and fermented with water over

Vitamin B<sub>9</sub> (total folate) was more in sorghum (44.135  $\pm$  1.30  $\mu$ g/100 g), followed by pearl millet (33.309  $\mu$ g/100 g) and finger millet (27.576  $\pm$  1.23  $\mu$ g/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  $\pm$ 1.08  $\mu$ g/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  $\pm$  0.79 and 16.216  $\pm$  0.28  $\mu$ g/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,  $1.96 \pm 0.18$  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  $\pm$  0.18) and PMC (9.30  $\pm$  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 14h. 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  $\pm$  0.34 g/100 g), PMFWC (88.24  $\pm$  0.34 g/100 g), and FMFWC (84.12  $\pm$  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 ( $3.81 \pm 0.05 \text{ g}/100 \text{ g}$ ), PMFWC ( $2.61 \pm 0.14 \text{ g}/100 \text{ g}$ ), and SMFWC ( $2.56 \pm 0.06 \text{ g}/100 \text{ g}$ ), followed by FMFC ( $3.70 \pm 0.04 \text{ g}/100 \text{ g}$ ), PMFC ( $2.56 \pm 0.04 \text{ g}/100 \text{ g}$ ), and SMFC ( $2.36 \pm 0.03 \text{ g}/100 \text{ g}$ ). 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  $\pm$  0.09), PMFC (1.43  $\pm$  0.00), and FMFC (0.98  $\pm$  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  $\pm$  0.02 g/100 g; PMFW, 0.91  $\pm$  0.03 g/100 g; FMFW, 0.64  $\pm$  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  $\pm$  0.21 (SM) to 66.44  $\pm$  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  $\pm$  0.15, 4.77  $\pm$  0.07, and  $5.69 \pm 0.19$  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  $\pm$ 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  $\pm$  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 acidzinc 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<sub>2</sub>, B<sub>3</sub>, B<sub>5</sub>, and B<sub>9</sub> 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<sub>2</sub>, B<sub>3</sub>, B<sub>5</sub>, and B<sub>9</sub> 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 watersoluble 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<sub>2</sub>), 58% (vitamin B<sub>3</sub>), and 68% (vitamin B<sub>5</sub>) 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.

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#### 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.

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