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Muhammad Hazwan Hamzah,
Putra Malaysia University, Malaysia

*CORRESPONDENCE

Angela Parry-Hanson Kunadu,
✉ aparry-hanson@ug.edu.gh

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Characterization of physicochemical and microbial quality, functional properties, and shelf stability of fermented tigernut-based probiotic beverages

Stephen Yeboah¹, Agatha Ohemeng¹, Leticia Donkor²,
F. K. Saalia², George Amponsah Annor³ and
Angela Parry-Hanson Kunadu^{1*}

¹Department of Food Science and Nutrition, University of Ghana, Accra, Ghana, ²Department of Food Process Engineering, University of Ghana, Accra, Ghana, ³Department of Food Science and Nutrition, University of Minnesota, St. Paul, MN, United States

Tigernuts and millet are indigenous underutilized crops in West Africa that have versatile applications in food processing. These crops are rich in fermentable carbohydrates, resistant starch, fiber, and micronutrients, making them ideal candidates for pre- and probiotic (synbiotic) foods. This study utilized whole tigernuts in a dairy–millet-based fermented beverage called *brukina*, turned it to a synbiotic, and assessed the functional and physicochemical profiles, microbial quality, and shelf stability of the beverage. The tigernut–millet agglomerate was prepared by incorporating cellulose-hydrolyzed tigernut fibrous (TNF) cake and non-hydrolyzed TNF (10% and 15%, respectively) into millet and allowing to ferment for 12 and 24 h. *Brükina* produced from composite tigernut milk: dairy in a ratio of 40%:60% was inoculated with the probiotic *Lactocaseibacillus casei* after pasteurization. The beverage was analyzed for physicochemical, proximate, and functional properties and microbiological stability at 5°C and 25°C. The obtained data were subjected to analysis of variance (ANOVA) in Minitab version 17 using a general linear model to determine the variability, interactions, and significance of the measured product characteristics. The agglomerate water absorption capacity (l/g) ranged from 0.70 ± 0.17 to 0.89 ± 0.17, bulk density (g/l) from 0.55 ± 0.04 to 0.63 ± 0.00, and swell index (%) from 1.62 ± 0.08 to 1.80 ± 0.06. The agglomerate prepared from dough and fermented for 12 h had excellent functional characteristics and was selected for synbiotic *brukina* production. Moisture content of the product decreased ($p < 0.001$) with tigernut incorporation ranging from 78.85% to 70.45%, while sodium, phosphorus, protein, total carbohydrate, and crude fiber increased with tigernut incorporation ($p < 0.05$). Synbiotic *brukina* supported the growth of *L. casei* attaining 11 log CFU/mL with a corresponding increase in lactic acid production and was microbiologically safe at 5°C and 25°C for 5 days compared to unpasteurized and uninoculated probiotic control ($p < 0.05$). The addition of whole tigernuts and *L. casei* to *brukina* enhanced its nutritional content with a shelf stability of 3 days.

KEYWORDS

brukina, tigernut, probiotic, prebiotic, fermentation, synbiotic

Introduction

Cyperus esculentus L. (tigernut) is a tropical crop grown in Southern Europe, Asia, and Africa. In Ghana, tigernut remains classified as a minor crop, which is not captured in official records (SRID Agriculture in Ghana and Facts and Figures, 2010). Enhancing the usability of indigenous plant products like tigernut and millet in a developing country aligns with SDG 2 (Zero Hunger) and SDG 12 (Responsible Consumption and Production), and it can play a significant role in curbing the problem of food and nutritional insecurity. Sánchez-Zapata et al. (2012) explored the use of tigernuts as ingredients in existing foods. The authors incorporated tigernut cake (the fibrous remains after juicing tigernut) into sausage and used the tigernut milk as a carbon source for the development of probiotic products. Tigernut has high nutritional value, and its tubers contain pleasantly flavored oil, much like olive and sweet almond oil (Kim et al., 2007; Yu et al., 2022). Tigernut has long been recognized for its nutritional and health-related benefits. It contains approximately 50% digestible carbohydrates, 10% protein, and 9% dietary fiber, which consists mainly cellulose and lignin (Arafat et al., 2009; Munekata et al., 2021). It has a good micronutrient profile including zinc, magnesium, potassium, calcium, and vitamins C and E (Arafat et al., 2009; Munekata et al., 2021). Tigernut is consumed mainly for its perceived aphrodisiac properties and nourishment as a ready-to-eat (RTE) snack (Asante et al., 2014). Tigernut is highly fibrous, making it a suitable composite material for the production of yogurt, and its yogurt quality is comparable to that of dairy yogurt (Sanful, 2009). Fermentation of dietary fibers in the colon generates short-chain fatty acids such as propionate, which has been shown to inhibit the activity of hydroxy-3-methylglutarylCoA reductase, the limiting enzyme for cholesterol synthesis (Xu et al., 2020). When dietary fiber bonds with bile acids, it prevents reabsorption in the liver, therefore inhibiting cholesterol synthesis (Xu et al., 2020).

Millet is one of the underutilized grains in Africa. In Ghana, it is a staple for the Northern locales (Adebiyi et al., 2016). It is rich in protein, vitamins, essential minerals, and fiber content, which makes it a good candidate for products such as infant food, snacks, and dietary enhancements. Moreover, millet cuisines have progressed toward becoming a part of daily meals in homes and restaurants (Xu et al., 2020). Both millet and tigernut fibers are readily fermentable by colonic microorganisms, such as bifidobacteria and lactobacilli, as carbon sources, making them a decent source of prebiotic. Millet is a key ingredient in the popular fermented dairy beverage known as *brukina* in Ghana but consumed in various parts of West Africa (Adebiyi et al., 2016). *Brükina* is a nutritious snack, widely consumed and thus a good candidate for product development with enhanced nutrition and safety.

Different authors have explored the incorporation of tigernuts into existing food products. Jimoh and Kole (2022) developed a tigernut fruit blend and studied its nutritional profile, sensory properties, and shelf life. Sanful (2009) also developed yoghurt from composite dairy and tigernut milk. All these works prove the feasibility of tigernut in developing nutritious affordable food products. However, none of these authors developed a symbiotic product that had incorporated tigernut fiber and a suitable probiotic to further enhance the nutritional and functional properties of developed food products. Although tigernuts have desirable properties to use in food formulations, its fiber is difficult to swallow, thus limiting its use in foods. Furthermore,

the tigernut milk extract is rich in starch, which causes gelation when heated above 60°C (da Costa Neto et al., 2019). This property limits its functional use in foods like dairy products requiring pasteurization as a legal requirement.

The incorporation of tigernut milk in food requires further research to improve the physicochemical and textural properties of such products. The study presents novel ways of making use of underutilized and affordable whole crops to enhance functional properties, nutritional and microbiological profiles, and stability of *brukina* beverage.

Materials and methods

A brown variety of tigernut tubers (*Cyperus esculentus* L.) (50 kg) was obtained from Twifo Praso in the Central Region of Ghana. Pearl millet and dairy milk powder were also obtained from a local market in Accra. Tigernut and millet were manually sorted to remove the broken pieces and any other physical contaminants before storage at ambient temperature. The probiotic bacteria (*Lactocaseibacillus casei*) strain isolated from human saliva was obtained from the microbiology research laboratory of the Department of Nutrition and Food Science, University of Ghana. The cultures were stored at -80°C in de Man Rogosa Sharpe (MRS) broth (Sigma-Aldrich Co. Ltd., Dorset, UK) containing 10% (v/v) of glycerol.

Experimental design

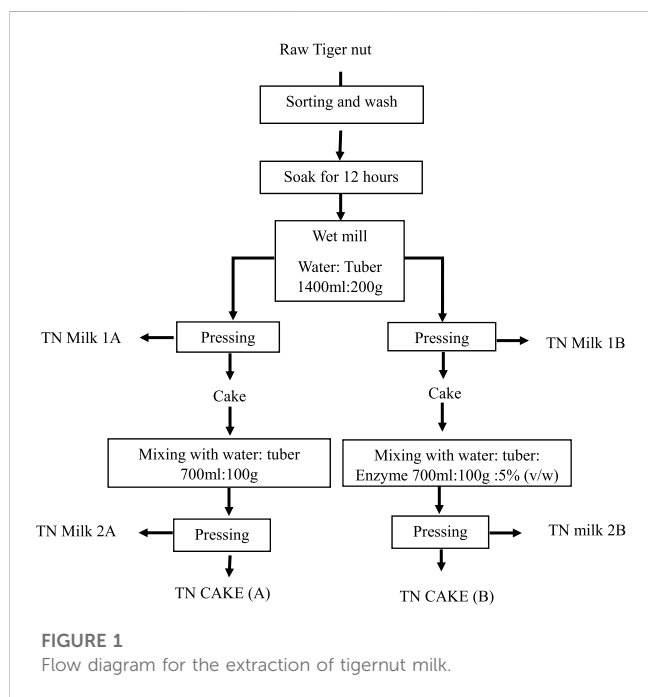
Using Minitab 17, the study employed a mixture design with extreme vertices and two process variables: enzyme treatment of tigernut fibrous cake (TNF) and fermentation time (12 and 24 h). The objective of the study was to investigate the suitability of tigernuts and millet as prebiotic sources in probiotic-based products. The major components of the product formulation were tigernut fibrous cake and wet-milled millet flour. A total of 20 formulations (Ocloo et al., 2014) were generated for the agglomerate formulation process. To ensure the nutritional quality of the product, the fiber content was considered a constraint. The study used the fiber nutritional requirement for processed food products, setting a range of 1.5–3 g of fiber per 100 kcal of processed food (Ioniță-Mîndrican et al., 2022). Based on the highly fibrous nature of the tigernut cake, eight optimized formulations were selected. The rationale behind this selection was to ensure that the fiber content of the tigernut cake component in the chosen formulations fell within the specified range of 1.5–3 g. In addition to the eight optimized selections, a standard control was included requiring no fermentation of the millet (Table 1).

Tigernut milk extraction

The tigernuts were sorted to remove contaminated, defective tubers, and whole tubers were washed with clean sterile water afterward. The tigernuts were then soaked for 12 h and blended with water in a ratio of 1:7 (w/v), and the obtained slurry was divided into two samples labeled A and B. Sample A was enzymatically treated at 55°C with cellulase for 6 h to reduce the starch. The slurries obtained from samples A and B were filtered to obtain the tigernut

TABLE 1 Selected optimized formulations for tigernut-based agglomerate preparations.

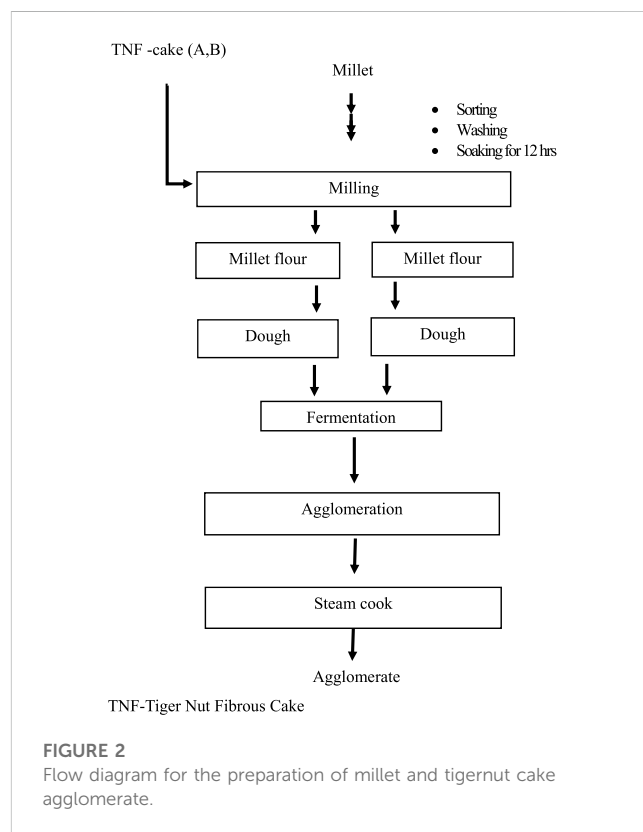
Sample	Fermentation time/ hours	Tigernut cake	Millet	Enzyme treatment	Selected formulations for beverage preparation
A1	0	0	100	No	-
A2	12	0	100	No	A
A3	24	0	100	No	-
B1	24	10	90	No	-
B2	12	10	90	No	B
C1	24	15	85	No	-
C2	12	15	85	No	C
D	12	10	90	Yes	D
E	12	15	85	Yes	E



milk and further homogenized in the colloid mill. The preparation of tigernut milk and cake is presented in [Figure 1](#).

Agglomerate preparation

The millet was sorted, washed, and soaked for 12 h. Using the disc attrition mill (Model SK-30-SS Attrition Mill), the millet was milled to obtain flour. The flour was divided into two equal parts and labeled A and B. Sample A was mixed with TNF in a ratio of 10:90 and 15:85. Both samples were mixed with 1% water and kneaded to form a compact dough. The dough was divided into two parts: one was allowed to spontaneously ferment for 12 h and the other for 24 h. Only the dough fermented for 12 h was used for the preparation of beverage and functional analysis based on the outcome of the functional analysis, which is comparable to the control (data not shown). Further preparation



was carried out on four agglomerate formulations fermented for 12 h only ([Table 1](#), selected formulations for beverage preparation). The fermented dough was then rolled on a sieve with a pore size of 2 mm to form uniform balls (agglomerate) and steam-cooked for 30 min ([Figure 2](#)) ([Baidoo and Parry-Hanson Kunadu, 2018](#)).

Preparation of composite milk

Using Sanful’s method ([Sanful, 2009](#)) with slight modifications, powdered milk was reconstituted by adding 140 g powdered milk to 540 mL potable water. Composite tigernut–dairy milk was prepared in a

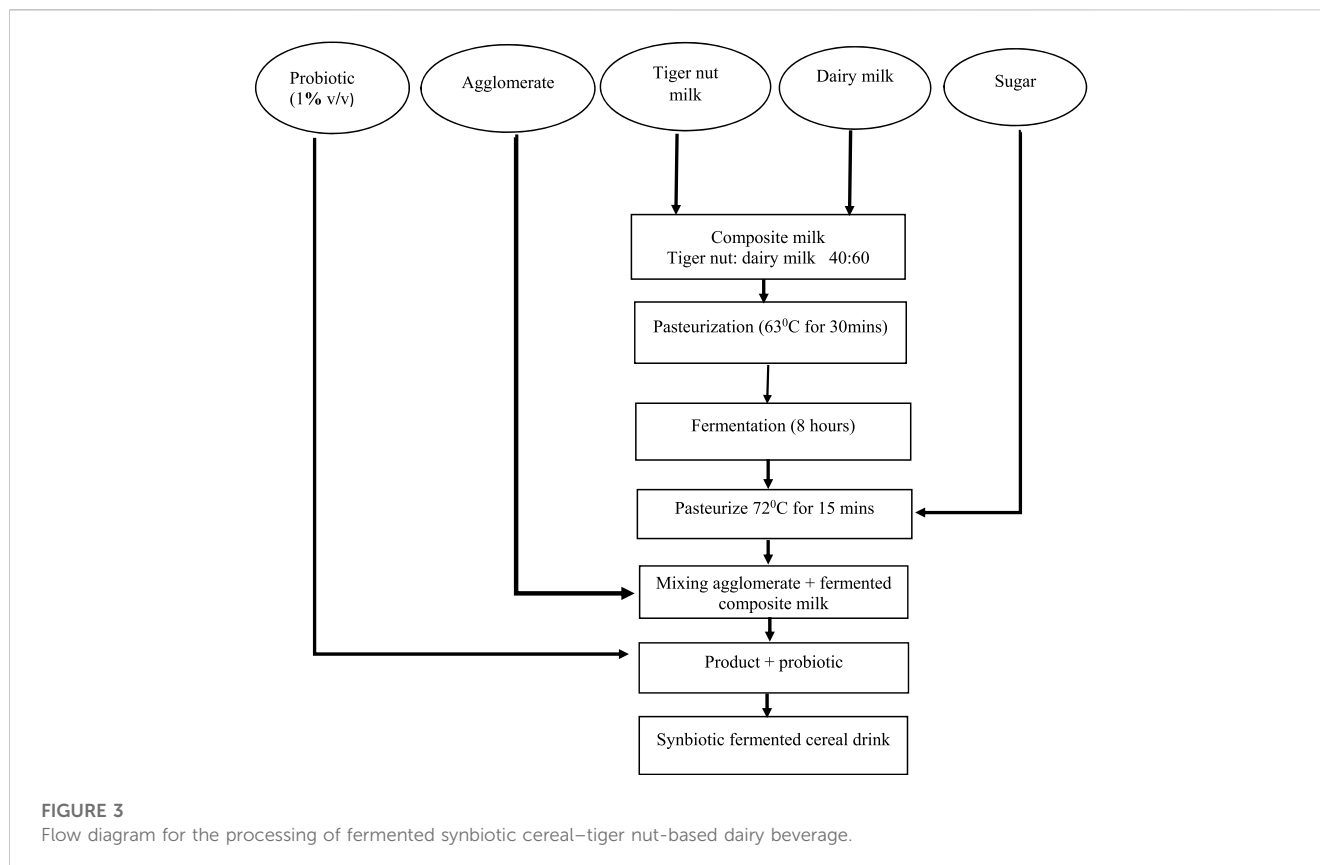


TABLE 2 Functional properties of cellulase- and non-cellulase-hydrolyzed tigernut fibrous cake-based millet agglomerate fermented for 12 h.

Sample ID (M: TNF) %	Swell index	Bulk density (g/l)	Water absorption (l/g)
A (100:0)%	1.81 ± 0.20 ^a	0.54 ± 0.02 ^b	0.86 ± 0.12 ^a
B (85:15)%	1.78 ± 0.16 ^a	0.63 ± 0.04 ^a	0.89 ± 0.17 ^a
C (90:10)%	1.62 ± 0.08 ^a	0.59 ± 0.04 ^b	0.70 ± 0.17 ^a
D (CE) (85:15)%	1.80 ± 0.06 ^a	0.55 ± 0.04 ^b	0.80 ± 0.17 ^a
E (CE) (90:10)%	1.66 ± 0.16 ^a	0.63 ± 0.00 ^a	0.79 ± 0.18 ^a
<i>p</i> -values	0.422	0.009	0.640

The tigernut–millet dough was fermented for 12 h. CE, cellulase enzyme-treated tigernut fibrous cake; M, millet; TNF, tigernut fiber; A–E, sample IDs. For every parameter, values in the same column with different superscripts are statistically different at $\alpha = 0.05$. Sample A (0) (100:0)% is the control sample.

ratio of 60:40. The mixture was pasteurized for 15 min at 62°C. The pasteurized milk (800 mL) was allowed to cool to 43°C and spontaneously fermented in 1,000-mL Erlenmeyer flasks at 37°C for 10 h. The fermented milk was then pasteurized at 72°C for 30 min and allowed to cool.

beverage is depicted in Figure 3. Half of the fermented dairy–tigernut milk composite that did not undergo further pasteurization and probiotic inoculation was used as a control (unpasteurized and uninoculated control).

Probiotic inoculum preparation

The frozen *L. casei* in vials was activated on MRS agar and subsequently cultured into MRS broth anaerobically at 37°C for 12 h. *L. casei* was subsequently cultured in sterile reconstituted dairy milk at 37°C for 20 h. The culture was used as an inoculum at 1% (v/v) in each composite tigernut–dairy milk for the beverages (Rozada-Sánchez et al., 2008). The preparation of the composite *brukina*

Functional properties of the agglomerate

The bulk density was determined in triplicate. The agglomerate sample was put into a 10-ml measuring cylinder and gently agitated continuously until a constant volume was obtained (Ocloo et al., 2014).

$$\text{The bulk density (g/cm}^3\text{)} = \frac{\text{weight of the agglomerate (g)}}{\text{volume of agglomerate (cm}^3\text{)}}. \quad (20a)$$

TABLE 3 Proximate composition of synbiotic cereal-tigernut-based *brukina* with varying compositions of cellulase- and non-cellulase-treated tigernut fibrous cake.

Sample ID (M: TNF)%	Moisture g/100 g	Carbohydrate g/100 g	Fat g/100 g	Crude fiber g/100 g	Ash g/100 g	Protein g/100 g	Energy kcal
A (100:0)%	78.850 ± 0.636 ^a	12.585 ± 0.304 ^c	3.205 ± 0.063 ^d	0.280 ± 0.014 ^b	0.635 ± 0.021 ^b	4.510 ± 0.014 ^c	97.220 ± 1.730 ^d
B (85:15)%	70.685 ± 0.276 ^c	19.240 ± 0.170 ^a	4.895 ± 0.106 ^a	0.565 ± 0.035 ^a	0.665 ± 0.021 ^a	3.940 ± 0.042 ^d	136.780 ± 1.460 ^a
C (90:10)%	70.455 ± 0.361 ^c	18.800 ± 0.311 ^a	4.205 ± 0.063 ^c	0.255 ± 0.007 ^b	0.625 ± 0.049 ^b	5.660 ± 0.056 ^b	135.690 ± 1.590 ^a
D (CE) (85:15)%	75.240 ± 0.070 ^b	15.365 ± 0.191 ^b	4.660 ± 0.084 ^b	0.210 ± 0.014 ^b	0.585 ± 0.007 ^b	3.940 ± 0.042 ^d	119.160 ± 0.170 ^b
E (CE) (90:10)%	78.825 ± 0.276 ^a	9.155 ± 0.247 ^d	4.260 ± 0.042 ^c	0.615 ± 0.092 ^a	0.670 ± 0.028 ^a	6.475 ± 0.049 ^a	100.860 ± 0.410 ^c
<i>p</i> -values	<0.001	<0.001	<0.001	0.001	0.144	<0.001	<0.001

The tigernut–millet dough was fermented for 12 h. CE, cellulase enzyme-treated tigernut fibrous cake; M, millet; TNF, tigernut fibrous cake; A–E, sample IDs. For every parameter, values in the same column with different superscripts are statistically different at $\alpha = 0.05$.

TABLE 4 Mineral composition of synbiotic composite cereal-tigernut-based *brukina* with 10% and 15% of cellulase- and non-cellulase-treated TNF.

Sample ID (M: TNF)%	Na (mg/100 g)	K (Mg/100 g)	Mg (mg/100 g)	Ca (mg/100 g)	Zn (Mg/100 g)	P (mg/100 g)	Fe (mg/100 g)
A (12) (100:0)%	141.82 ± 6.96 ^b	131.33 ± 3.12 ^a	5.26 ± 0.90 ^b	10.38 ± 0.82 ^a	1.18 ± 0.10 ^b	20.60 ± 0.08 ^c	12.71 ± 0.55 ^a
B (12) (85:15)%	187.34 ± 8.18 ^a	147.81 ± 9.99 ^a	8.26 ± 0.98 ^a	10.80 ± 0.40 ^a	2.21 ± 0.42 ^{ab}	36.80 ± 0.80 ^a	10.81 ± 2.33 ^a
D (12E) (85:15)%	110.42 ± 2.65 ^c	137.73 ± 1.07 ^a	6.06 ± 1.22 ^b	7.24 ± 0.00 ^b	1.66 ± 0.09 ^{ab}	16.56 ± 0.32 ^c	12.57 ± 0.06 ^a
E (12E) (90:10)%	163.36 ± 0.85 ^b	149.63 ± 0.73 ^a	3.64 ± 0.24 ^b	10.80 ± 0.40 ^a	2.28 ± 0.29 ^a	30.12 ± 2.92 ^a	10.87 ± 1.49 ^a
<i>p</i> -value	0.003	0.182	0.092	0.018	0.114	0.002	0.667

FT, fermentation time for agglomerate in hours; M, millet; TNF, tigernut fibrous cakes; A–E, sample IDs. For every parameter, values in the same column with different superscripts are statistically different at $\alpha = 0.0$.

TABLE 5 pH and bacteriological quality of pasteurized composite cereal-tigernut-based *brukina* with 15% of cellulase- and non-cellulase-treated TNF before and after probiotic inoculation.

Sample ID (FT) (M: TNF)%	Mean ± SD (log CFU/mL)				
	Total plate count	<i>E. coli</i>	<i>S. aureus</i>	Y&M	pH
B (12) (85:15)%	0.0 ± 0.000 ^b	0.0	0.0	0	4.41
D (12E) (85:15)%	0.0 ± 0.000 ^b	0.0	0.0	0	4.45
B1 (12) (85:15)%	7.98218 ± 0.01280 ^a	0.0	0.0	0	4.35
D1 (12E) (85:15)%	7.9563 ± 0.0238 ^a	0.0	0.0	0	4.36

B and D, before inoculation with probiotic; B1 and D1, after inoculation with probiotics.

The water absorption capacity was determined in triplicate; 5.0 g of the sample was weighed and mixed with 20 ml of distilled water and allowed to stand for 30 min at a room temperature of 28°C. The mixture was then centrifuged (Centurion Scientific centrifuge K3 Series) at 1,512 RCF for 20 min. The excess water was decanted from the centrifuge tubes into a measuring cylinder, and the volume was determined. The water absorption capacity was calculated as volume (water absorbed/g of flour) (Ocloo et al., 2014).

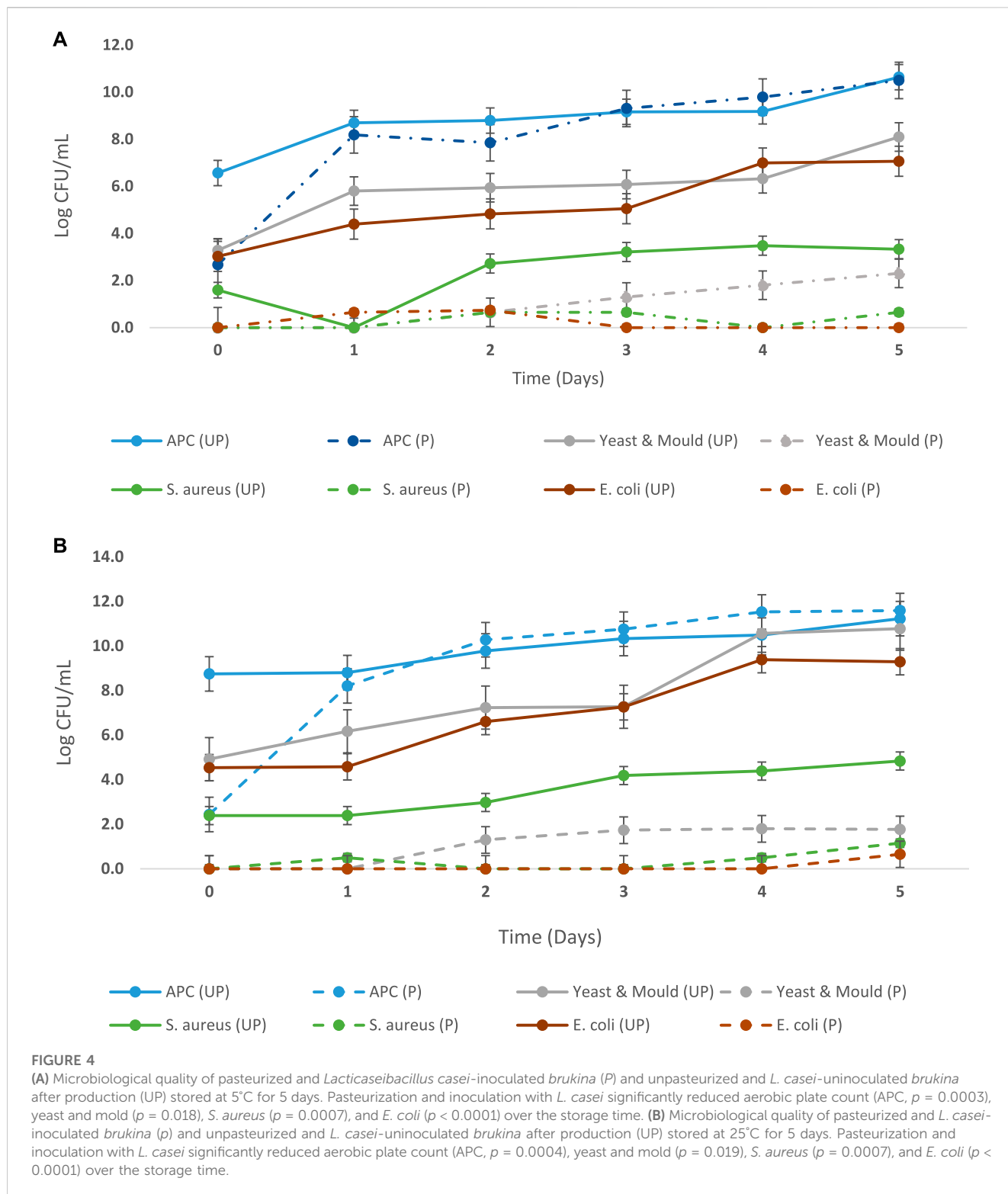
For the swelling index, 5 g of each sample was placed in a graduated measuring cylinder containing 100 ml of distilled water at room temperature. It was tapped gently to eliminate air, and the

initial volume was recorded. It was subsequently swirled around and allowed to stand for 5 h, and the final volume was recorded.

$$\text{Swelling index} = \frac{\text{final volume} - \text{initial volume}}{\text{sample weight}} \quad (20b)$$

Determination of pH and titratable acidity

pH was determined using a digital pH meter (Mettler Toledo SevenCompact). For the titratable acidity, three drops of



phenolphthalein were added to 15 ml of the sample in a beaker. The mixture was titrated against 0.1 N NaOH until the solution turned pink. The volume of NaOH used was recorded, and the titratable acidity was calculated (Feldsine et al., 2002).

$$\text{Titratable acidity (\%)} = \frac{\text{titre value} \times 0.9 \times 100}{\text{volume of sample}} \quad (21)$$

Proximate analysis

Moisture content, crude protein, total ash, crude fiber, and carbohydrate contents were determined using standard methods (AOAC, 1997). The moisture content of the synbiotic beverage was determined using the oven drying method at 105°C for 5 h. The protein content was also determined by employing the micro-

TABLE 6 Probiotic *L. casei* of synbiotic composite cereal–tigernut-based *brukina* with 10% and 15% of cellulase and non-cellulase-treated TNF stored at two varying temperatures 5°C and 25°C for 4 days.

Sample ID (FT) (M: TNF)%	Temp (°C)	Mean ± SD (log CFU/mL)				
		Day 0	Day 1	Day 2	Day 3	Day 4
A (0) (100:0)%	5	7.16 ± 0.06 ^a	7.52 ± 0.09 ^b	8.71 ± 0.39 ^{de}	10.74 ± 0.01 ^a	11.48 ± 0.22 ^a
B (12) (85:15)%		6.89 ± 0.01 ^a	7.47 ± 0.02 ^b	8.84 ± 0.02 ^{cde}	10.6 ± 0.09 ^a	11.52 ± 0.00 ^a
D (12E) (85:15)%		7.07 ± 0.14 ^a	7.18 ± 0.08 ^c	8.48 ± 0.68 ^c	10.09 ± 0.01 ^c	11.35 ± 0.01 ^a
E (12E) (90:10)%		6.52 ± 0.44 ^a	7.38 ± 0.05 ^{bc}	9.10 ± 0.30 ^{bcd}	10.60 ± 0.02 ^{ab}	11.15 ± 0.05 ^a
A (0) (100:0)%		25	7.16 ± 0.06 ^a	8.80 ± 0.05 ^a	10.23 ± 0.01 ^{abcd}	10.78 ± 0.06 ^a
B (12) (85:15)%	6.89 ± 0.01 ^a		8.73 ± 0.10 ^a	10.84 ± 0.85 ^a	10.33 ± 0.09 ^{bc}	11.14 ± 0.03 ^a
D (12E) (85:15)%	7.07 ± 0.14 ^a		8.70 ± 0.00 ^a	10.45 ± 0.19 ^{abc}	10.27 ± 0.14 ^c	11.56 ± 0.26 ^a
E (12E) (90:10)%	6.52 ± 0.4 ^a		8.65 ± 0.03 ^a	10.74 ± 0.03 ^{ab}	10.68 ± 0.10 ^a	11.17 ± 0.02 ^a
<i>p</i> -value			0.161	0.019	0.566	0.000

For every parameter, values in the same column with different superscripts are statistically different at $\alpha = 0.05$.

FT, fermentation time for agglomerate in hours.

M, millet; TNF, tigernut fibrous co-product; A–E, sample IDs.

TABLE 7 Shelf life of synbiotic composite cereal–tigernut-based *brukina* with 10% and 15% of cellulase- and non-cellulase-treated TNF stored for 5 days at 5°C, 25°C, and 35°C.

Sample ID	T	K	R ²	1/T	LN(K)	R	Ea/R	Ea	Shelf life
A (100:0)%	278	0.1753	0.9571	0.003597	-1.74126	-1.986	-1,346.2	2673.553	5.70
	298	0.2507	0.9349	0.003356	-1.3835	-1.986			3.99
	308	0.2783	0.9252	0.003247	-1.27906	-1.986			3.60
B (85:15)%	278	0.1722	0.9558	0.003597	-1.7591	-1.986	-1,393.4	2767.292	5.81
	298	0.2303	0.924	0.003356	-1.46837	-1.986			4.34
	308	0.2843	0.9553	0.003247	-1.25773	-1.986			3.52
C (90:10)%	278	0.1869	0.9428	0.003597	-1.67718	-1.986	-1,014.4	2014.598	5.35
	298	0.2374	0.9067	0.003356	-1.43801	-1.986			4.21
	308	0.2671	0.8996	0.003247	-1.32013	-1.986			3.74
D (CE) (85:15)%	278	0.1856	0.912	0.003597	-1.68416	-1.986			5.38
	298	0.2448	0.8999	0.003356	-1.40731	-1.986	-1,218	2418.948	4.08
	308	0.2858	0.9549	0.003247	-1.25246	-1.986			3.50

Kjeldahl method. Total ash was determined by igniting 2 g product at 550°C for 4 h using a muffle furnace. The crude fiber was determined using the digestion method, and the carbohydrate content was estimated by calculating the difference [100 - (% water + % protein + % fat + % ash + % crude fiber)]. The crude fat was determined using the standard Soxhlet extraction method with diethyl ether as the solvent (AOAC, 2000).

0.3 g of the sample was digested with nitric acid and sulfuric acid in a ratio of 2:1. The digested sample was transferred into a 50-ml volumetric flask; distilled water was added to achieve the final volume of 50 ml. Sodium (Na), potassium (K), magnesium (Mg), calcium (Ca), zinc (Zn), iron (Fe), phosphorus (P), manganese (Mn), and copper (Cu) were all determined in triplicate with the use of the PerkinElmer Atomic Absorption spectrophotometer (Model AA 220FS, Massachusetts, United States) (AOAC, 2005).

Mineral analysis of synbiotic beverage

Based on the results of the proximate composition, sample C was excluded in further mineral analysis. A wet digestion method using a closed-vessel microwave digester (ETHOS-EZ Milestone) was used for the digestion of synbiotic *brukina* for mineral analysis. Approximately

Microbiological assessments

Samples were analyzed for determining the counts of aerobic bacteria, yeast and molds, *Staphylococcus aureus*, *Escherichia coli*, and lactobacilli. A measure of 10 g of each sample was transferred

into 90 mL of 0.1% sterile buffered peptone water (Oxoid, Hampshire) and homogenized in a stomacher for 1 minute. Appropriate serial dilutions were prepared from homogenates. Yeast and molds were enumerated on malt extract agar by spread plating appropriate dilutions on pre-poured plates. Inoculated plates were incubated at 25°C for 3–5 days before enumeration. *S. aureus* was enumerated by pour plating on mannitol salt agar (MSA, Oxoid CM0085). Inoculated plates were incubated at 37°C for 48 h. Yellow colonies with yellow zones surrounding them were counted and recorded as colony-forming units per ml of sample (CFU/ml). *E. coli* was enumerated on MacConkey agar with 24 h incubation at 37°C. Lactobacilli were enumerated on de Man Rogosa Sharpe agar (MRS Oxoid CM036) by using the pour plate overlay method. Plates were incubated at 37°C for 48 h before enumeration (Maselli and Hekmat, 2016).

Determination of accelerated shelf life using the Arrhenius model

Brukina beverages in plastic bottles with a net weight of 350 mL were stored at critical temperature variations of 5°C, 25°C, and 35°C. The analysis was carried out periodically every 24 h from days 0 to 5, to get five points of observation. Two parameters were used in the shelf life study: the log CFU/mL of *L. casei* and pH value of the product. The result data of each parameter were plotted against time (day) to generate linear equations. The three equations obtained for three conditions of product storage temperature are depicted using the following equations (Pulungan et al., 2018):

$$y = mx + c. \quad (1)$$

The choice of reaction order for a parameter is carried out by comparing the regression value (R^2) of each linear equation at the same temperature. The reaction order with a larger R^2 value is the order of reactions used by that parameter.

$$\ln k = \ln k_0 - \frac{E_a R}{1/T}. \quad (2)$$

From Equation 2, k_0 represents the deteriorating constant. It is the factor that determines the quality of a product kept at a constant temperature.

$$k = k_0 \frac{-E_a R}{T}. \quad (3)$$

Based on the Arrhenius equation (Eq. 3) and the calculation of k , the shelf life of fermented cereal–tiger nut-based drink can be determined using the first-order kinetics:

$$\ln(\text{shelf life}) = \frac{E_a R}{T} + \ln(k),$$

$$\text{shelf life} = e - \left(\frac{E_a R}{T} + \ln(k) \right).$$

Statistical analysis

The obtained data were analyzed using analysis of variance (ANOVA). The physicochemical parameters, functional properties,

the *L. casei* growth, lactic acid production, and the developed agglomerate were used for the analysis of variance. The level of significance was set at $p < 0.05$. A general linear model was used to determine the interactions and variability between the groups influenced by the time, temperature, enzymatic treatment, and compositional blend. The statistical analysis was carried out using Minitab version 17.

Results and discussion

Swell index, water absorption capacity, and bulk density of the composite agglomerate

The compositional blend of 15% and 10% TNF agglomerates for the production of synbiotic *brukina* did not have any significant effect on the swell index and water absorption capacity ($p = 0.422$) and ($p = 0.640$), respectively (Table 2). Bulk density (BD) values for 10% and 15% TNF agglomerates, with and without enzyme hydrolysis, were 0.55–0.63 g/mL and 0.59–0.63 g/mL, respectively (Table 2). The observed difference in BD was significantly higher ($p = 0.009$) than that in the control. Similar bulk density values of 0.59 g/mL (Adejuyitan, 2011) and 0.56 g/mL (Chinma et al., 2009) have been reported for TNF-based products. This observed difference in the bulk density is associated with the varying starch composition of the agglomerate from varying sources, which influences structural differences.

Steam cooking at atmospheric pressure leads to the absorption of water by the agglomerate until gel formation (Zarski et al., 2021). The high-water environment and heating are ideal for starch pasting and gelatinization, leading to starch granule swelling and losing crystallinity through the absorption of water. The overall effect is the creation of a gelatinized starch product. The linear amylose and available amylopectin align to form hydrogen bonding which expels water when cooled (Zarski et al., 2021). For this reason, the formed agglomerate does not absorb much moisture and has little capability to swell further. The similarity in the swell index and water absorption among all samples was expected as these functional properties are characteristics of starches found in all the agglomerate ingredients, as shown in Table 2. According to Wang et al. (2003), hydrolysis has been found to cause changes in the swelling power of starch morsels. Amylopectin plays a critical role in starch granule swelling and water-holding ability. Once the amylopectin structure is disrupted, an intact linkage cannot be formed, and the damaged chains tend to dissolve because they no longer can entrap water.

Proximate composition of the synbiotic *brukina*

The proximate composition of the fermented synbiotic *brukina* samples determined included moisture, ash, protein, fat, carbohydrate, and caloric content. The values were reported on a percentage dry-matter basis except for moisture content values. Table 3 shows the proximate composition of the synbiotic *brukina* samples made from millet–TNF dough fermented for 12 h.

The moisture content of synbiotic *brukina* ranged from 78.85% to 70.45%. The values obtained were comparatively lower than

81.71%–86.45% reported in Awonorin and Udeozor (2014) and 92.44% reported in Badau et al. (2015), but higher than 62.8%–73.5% reported in Musa and Hamza (2013) for tigernut milk. Samples containing 15% and 10% TNF without cellulase hydrolysis recorded lower moisture content ($p < 0.001$) than the samples containing 10% and 15% hydrolyzed TNF-incorporated drinks, as shown in Table 3. TNF without cellulase hydrolysis has a high fiber content. Dietary fiber has the capacity to absorb and hold water molecules by binding them through hydrogen bonding and other interactions (Wong et al., 2022). The hygroscopic nature allows it to trap moisture, reducing the amount of free water available in the sample. The hydrolysis of TNF with cellulase and fermentation of the starch component in the TNF–millet samples led to increased tightly packed crystalline structure due to the preferential cellulase hydrolysis of amorphous regions within the biopolymers of the TNF and millet. The hydrogen bonds formed within the crystalline starch molecule in the product prevented water absorption into the formed gel, making enough moisture available, hence the observed high moisture in the product with hydrolyzed TNF incorporation. The observed moisture for a beverage is good since moisture of approximately 70% will provide enough free water. *Lactobacillus casei* is a mesophilic bacterium that grows optimally under high-to-moderate moisture conditions depending on the pH, temperature, and nutrient availability in its environment. Synbiotic samples with tigernuts had higher carbohydrate contents ($p < 0.001$) than control samples without tigernuts. The carbohydrate content of the samples ranged from 9.15% to 18.85%, which was higher than 8.5% reported in Frimpong (2016). This observation as shown in Table 3 was the same for the fat ($p < 0.001$) caloric content ($p < 0.001$) of the product. The observation can be explained from the physical and chemical changes that occur during processing. Heating starch-containing ingredients in the presence of moisture causes swelling and gelatinization of starch. This process increases digestibility and availability of starch, hence higher carbohydrate concentration. High temperatures also aid in hydrolysis of complex carbohydrates making them more easily digestible and available (Eliass and on, 2017). The incorporation of 40% tigernut milk and 10%–15% fiber into the beverage samples increased the fat content of the product. This characteristic can be attributed to the fact that tigernut is rich in fatty acids (Liu et al., 2019). The fat levels were within the range of 3.2%–4.8%. Tigernut fats, most of which are oleic acid, and monounsaturated fats (Eteshola and Oraedu, 1996) are known to help with weight loss, reduce the risk of heart disease, and decrease inflammation (Adeniyi et al., 2013). The crude fiber in all samples, except samples C and D, showed a significant increase in fiber content ($p = 0.001$) compared to control sample A but for samples C and D. However, the value range (0.21%–0.62%) was higher than 0.20% reported in Belewu and Belewu (2007) in tigernut-based products. The fiber in food means carbohydrates not digested or absorbed in the human small intestine (Lupton, 2010). Fiber increases fecal output and reduces the fecal pH by producing short-chain fatty acids in the colon, which reduces the incidence of colon cancer. It binds to bile to prevent its reabsorption in the liver, leading to inhibition of the synthesis of cholesterol, hence preventing diabetes, heart diseases, obesity, and certain degenerative diseases (Chen et al., 1984; Mann and Cummings, 2009).

The increase in protein and carbohydrate, crude fiber, and fat contents, as observed in the product, affirms the fact that the tigernut is an excellent composite material to be used in combination with cereals for the development of probiotic beverage (Eliasson, 2017). Carbohydrates contribute to the taste and flavor of the products (Eliasson, 2017), and it also serves as a carbon source for the probiotic microorganism *L. casei* in the product.

Mineral composition of synbiotic *brukina* samples

Table 4 shows the mineral profile of *brukina* samples. It was observed that the mineral content of the fermented synbiotic *brukina* samples was high in general. They showed no significant differences across all the minerals examined ($p > 0.05$) except for phosphorus ($p = 0.002$), sodium ($p = 0.003$), and calcium ($p = 0.018$). None exceeded the daily recommended mineral intake level despite the observed high mineral content. The differences in the minerals examined for various synbiotic *brukina* samples can be attributed to the factors such as the processing method employed in the product development and incorporation of whole tigernut in the product. Tigernut and millet are high in minerals such as magnesium, potassium, and phosphorous (Nalini et al., 2018), which contributed to the high mineral content observed in both ash and mineral profiles in Table 3 and Table 4, respectively.

Microbial quality, growth of *L. casei* in probiotic *brukina*, and shelf life of synbiotic *brukina*

E. coli, *S. aureus*, and yeast and molds were not detected in the pasteurized products sampled before and after inoculation with *L. casei* as depicted in Table 5 and Figures 4A,B. However, changes in the *L. casei* count in the synbiotic products were observed during storage at 5°C and 25°C, as shown in Table 6. *L. casei* attained 11 log CFU/mL counts in the *brukina* samples (A, B, D, and E) after 4 days for the storage at 5°C and 25°C. *L. casei* growth paralleled aerobic plate count (APC) growth in pasteurized and probiotic inoculated *brukina* at 5°C and 25°C (Figures 4A,B, respectively). The high count of microbes in the unpasteurized samples reflects the microbiological quality of raw materials that are used as spontaneously fermented products. All the samples showed a similar trend of *L. casei* growth with time. After day 4, products A, B, D, and E stored at 5°C recorded the final pH of 3.54, 3.56, 3.39, and 3.53, respectively, and the most considerable reduction in pH were recorded for all the products. The same trend was observed in the sample stored at 25°C. The observed trend of log increase in *L. casei* at refrigeration temperature is consistent with the result obtained in Parra et al. (2013) using a cereal blend where the viability of *L. casei* during 28 days of refrigeration storage survived and attained the highest populations after 7 days of storage in a cereal-based diet. Furthermore, higher survival of *L. casei* than that of *Lactobacillus acidophilus* has been found in varying functional fermented products and non-fermented products that do not contain milk (Kyung et al., 2005). The growth of *L. casei* depended on its ability to utilize the nutrients available in the

growth medium for its survival. Corcoran et al. (2005) observed that the survival of lactobacilli in acidic environments is enhanced in the presence of metabolizable sugars that allow cell membrane proton pumps to operate and prevent the lowering of intracellular pH. *L. casei* is known to be more resistant to acidic conditions in a grain diet than other lactobacilli (Charal et al., 2002). The composite nature of the product, with cellulase hydrolyzed starch, made metabolizable sugars readily available for *L. casei* metabolic activity in samples kept at different storage temperatures. The initial pH range (4.41–4.75) of the products which resulted from the initial fermentation of both the agglomerate and the composite milk used for the product preparation also influenced the growth of the probiotic microorganism in the product. The beverage supported *L. casei* metabolic activity and growth, noting that *L. casei* possesses mechanisms to counteract the detrimental effects of low pH, such as the production of acid-tolerant enzymes, acidic amylase, acidic protease, and acidic phosphatase, and the maintenance of an internal pH homeostasis (Castro-López et al., 2022), hence the observed trends of increasing lactic acid production with corresponding growth in *L. casei*. For a probiotic-based ready-to-eat dairy-based product, the shelf life is defined by either pH changes (because of the live probiotic activity) or the probiotic count in the product with varying environmental temperature conditions (Kortei et al., 2020). The shelf life was estimated by transforming the pH using the regression of log shelf life versus temperature. The linear regression of pH as expressed by the Arrhenius model predicted the product's shelf life as 5 days for the sample kept at 5°C, 4 days for 25°C, and 3 days for 35°C, as shown in Table 7. However, for uncertainty factors (Tano-Debrah et al., 2019), since only pH was considered a chemical parameter in the Arrhenius model, the shelf life was determined as 3 days to cater for possibilities of temperature abuse during storage.

Conclusion

A novel synbiotic *brukina* was developed incorporating tigernut milk, tigernut fibrous cake, enzyme hydrolysis, and inoculation with *L. casei* to improve the functional properties and nutritional quality and stability of *brukina*. Synbiotic *brukina* exhibited promising results in supporting the growth of *L. casei*, with a significant 1 log increase in probiotic count observed over a 4-day period under both refrigerated (5°C) and room temperature (25°C) conditions. While all formulations had comparable functional properties as the control, the agglomerate formulation that incorporated 10% TNF and enzyme hydrolyzed dough had superior macro- and micronutrient profiles and is thus recommended for commercialization or used in nutrition interventions. The high fiber content ranging from 0.21% to 0.62% indicates potential benefits in terms of increased fecal output, reduced fecal pH, and enhanced protective gut microbes. The utility organisms (yeast and molds) and pathogen indicator microorganism (*E. coli*) were reduced to undetectable levels after pasteurization and *L. casei* inoculation, and their numbers remained low throughout the storage period. This buttresses the microbiological safety of the novel beverage, which is one of the major challenges faced with the traditional *brukina* beverage. The shelf life of 3 days was limited by sourness of

the product from high concentration of the probiotic, *L. casei*, at 35°C. At chilled temperatures, the product is stable for at least 4 days.

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

AP-HK conceptualized the study, supervised the work, and wrote the final draft. SY also contributed to the conceptualization of the work, analyzed the samples, collated and analyzed the data, and wrote the original draft. LD analyzed the samples and edited the first draft of the manuscript. FS supervised the work. AO supervised the work and edited the final manuscript. GA supervised the work and supported with the sample analysis. All authors contributed to the article and approved the submitted version.

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Conflict of interest

The author(s) AP-HK declared that they were an editorial board member of Frontiers, at the time of submission. This had no impact on the peer review process and the final decision.

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.

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Supplementary material

The Supplementary Material for this article can be found online at: <https://www.frontiersin.org/articles/10.3389/frfst.2023.1228643/full#supplementary-material>

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