

Research Article

Effect of *Lacticaseibacillus rhamnosus* Yoba Fermentation on Physicochemical Properties, Amino Acids, and Antioxidant Activity of Cowpea-Peanut Milk

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The global renewed interest in plant-based milk and products is increasing amongst health-conscious consumers. There is increased utilisation of generic probiotics in the processing of legume milk as alternatives to dairy milk are scarce in Africa. This study evaluated the probiotic potential, physicochemical, and sensory properties of novel fermented cowpea-peanut milk with *Lacticaseibacillus rhamnosus* Yoba. A 3 × 1 factorial design as ratio of cowpea-peanut milk (1 : 1, 2 : 1, 3 : 1v/v) and the application of 2% w/v *L. rhamnosus* Yoba obtained from Yoba for Life Foundation, Netherlands, was used. The chemical and mineral contents of the fermented cowpea-peanut milk was analysed using Association of Official Analytical Chemists (AOAC) methods. Quality parameters such as 2,2-diphenyl-1-picrylhydrazyl (DPPH) free radical scavenging rate, total antioxidant activity, antinutrient, and amino acids content were determined. The fermented cowpea-peanut milk samples had 7.7–8.1 log CFU/mL viable *L. rhamnosus* Yoba cells after fermentation. Nutrient content range was given in g/100 g: carbohydrate 5.18–6.05, crude fat 3.3–3.5, crude protein 5.6–7.1, ash 1.04–1.26, crude fibre 0.72–1.18, and total reducing sugars 1.80–2.20. Lysine, leucine, and methionine content was 6.30–7.31, 6.60–8.75, and 1.7–1.86 g/100 g, respectively. Phytic acid and trypsin inhibitor content range was 0.3–0.34 mg/100 g and 0.86–1.12 TIU/mg, respectively. Iron and potassium content (mg/100 g) was 0.48–0.58 and 202–243 with pH 4.1–4.2. DPPH free radical scavenging, and total antioxidant rate was 56–59% and 49–54%, respectively. Physicochemical parameters were significantly different ($p < 0.05$). The fermented cowpea-peanut milk had an acceptance rating of 78%. The successful application and consumer acceptability of the fermented cowpea-peanut milk has the potential to increase the utilisation of these legumes and enhance their market value.

1. Introduction

Of late, the utilisation of alternative protein sources in food processing is on an increase due to the global concerns on food security, protein malnutrition, and population growth. The great shift towards the consumption of plant-based foods which consist of legumes, nuts, seeds, cereals, fruits, and vegetables is being promoted [1]. Plant-based milk is becoming an important food in the vegetarian food industry [2]. They play an important role in the diets of consumers

who are allergic to cow's milk or have lactose intolerance. Plant-based milk is an extracted fluid in water obtained from the process of maceration, grinding, and filtration [3].

COVID-19 is likely to have a major impact on global food and nutrition security, especially in Southern Africa [4]. To further promote the application of plant-based milk in the food industry, it is important to understand their potential to support the growth of probiotic bacteria which influence their technofunctional properties such as foaming, solubility, gelation, and emulsification capacity in food

matrices. Probiotics are defined as live microorganisms which when administered in adequate amounts confer a health benefit on the host [5]. A generic probiotic, *Lactobacillus rhamnosus* Yoba, 2012, is being promoted and applied as a practical solution for accessing probiotic foods, especially to many rural folks in Africa [6]. Studies on the probiotics have found the ability of the bacteria to grow and ferment fruit pulp substrate [7]. A recent study on “a taxonomic note on the genus *Lactobacillus*: Description of 23 novel genera, emended description of the genus *Lactobacillus* Beijerinck 1901, and union of *Lactobacillaceae* and *Leuconostocaceae*” resulted in the reclassification of *L. rhamnosus* to *Lacticaseibacillus rhamnosus* [8].

Previously, legumes have been used in the production of many plant-based milk products such as chocolate milk drinks [9] and powdered milk [10]. The functional properties of legumes and oilseeds make it suitable to combine them and produce acceptable food products. In the case of cowpea milk, it has low energy; whereas, peanut milk is high in energy [11]. The blending of the two products effectively reduces the limitations they have individually [12]. Apart from rich nutrients, cowpeas are good sources of dietary fibre, polyphenols, polyunsaturated fatty acids (PUFA), and antioxidants [13]. Studies on the health benefits of plant-based milk include the reduction of the risk of cardiovascular diseases, atherosclerosis, diabetes, and cancer [14]. The use of lactic acid bacteria (LAB) in the fermentation of plant-based milk is a subtrend of innovative plant-based products that improve their technofunctional properties, nutrition, safety, and shelf life [15]. Studies have been carried out on the functional properties of plant-based fermented milk [15, 16]. Soybean milk fermented with *Lactobacillus paracasei* had a good antioxidative capacity and vitamin B groups [17].

Plant-based milk has problems of low acceptability due to unpleasant taste and smell and low bioaccessibility of minerals due to the presence of antinutritive compounds. Information on the utilisation of cowpea-peanut milk in fermented food is scarce. Conversely, whether the physicochemical and technofunctional properties of cowpea-peanut milk can be positively influenced by fermentation using probiotics that remain unknown. Therefore, this study aimed to evaluate the probiotic potential, physicochemical, amino acid content, antioxidant activity, and sensorial quality of cowpea-peanut milk fermented with *Lacticaseibacillus rhamnosus* Yoba. This information has the potential to increase the application of *Lacticaseibacillus rhamnosus* Yoba in new food product development and promote the use of cowpea-peanut milk as an ingredient in the formulation of many food products.

2. Materials and Methods

2.1. Raw Materials. The most common cowpea variety (*Vigna unguiculata*), locally called “nyemba in Shona” and peanuts (*Arachis hypogaea*), was purchased on a local market in Chinhoyi, Zimbabwe. The legume grains were sorted and cleaned by removing rotten grains as well as any extraneous materials from the lot.

2.1.1. Research Design. A 3×1 factorial design was followed: (a) the ratio of cowpea-peanut (1:1, 2:1, 3:1) and (b) the application of *Lacticaseibacillus rhamnosus* Yoba. The production of legume milk is illustrated using a flowchart, as shown in Figure 1.

2.1.2. Sample Pretreatments. The pretreatments were conducted according to a method described by Asiamah [12], with slight modifications. The weighed (1 kg) cowpeas samples were steeped in distilled water for 5 min and dehulled. The dehulled cowpeas were then steeped in 0.75% w/v baking soda (sodium hydrogen carbonate) (NaHCO_3) at pH 8, for 6 hours (h), and then washed in distilled water. Similarly, weighed peanuts were blanched in hot water (80°C) for 1 min and then dehulled. The peanuts were further steeped in 1% w/v NaHCO_3 at pH 8.2, for 9 h, and then, the dehulled peanuts were washed in distilled water.

2.1.3. Preparation of Legume Milk. The dehulled cowpeas and peanuts were combined using 1:1 w/w, 2:1 w/w, and 3:1 w/w ratios according to the experimental design. The mixed grain samples (cowpeas + peanuts) were then mixed with water in a ratio (1:2 w/v) and further slurried in a blender. The slurry was then filtered using a double-cheese cloth to obtain milk. Milk was pasteurized at 85°C for 5 min and then allowed to cool to 30°C. The milk was divided into 1 L portions. Raw cow’s milk, an animal-based product, was used as a control.

2.2. Source of *Lacticaseibacillus rhamnosus* Yoba. The *Lacticaseibacillus rhamnosus* Yoba culture was purchased from Yoba for Life Foundation, Netherlands. The generic probiotic, *Lacticaseibacillus rhamnosus* Yoba, was isolated from a commercial product containing *Lacticaseibacillus rhamnosus* GG bacteria and identified and confirmed using 16S rRNA sequencing. The purchased *Lacticaseibacillus rhamnosus* Yoba strain was stored at room temperature (20–25°C) according to manufacturer’s guidelines.

2.2.1. Preparation of Inoculum. Pure strains of *Lacticaseibacillus rhamnosus* Yoba were reactivated by subculturing anaerobically in De Man, Rogosa, and Sharpe agar (MRS) broth at 37°C for 18 h. A preculture medium of cowpea-peanut milk was boiled and then cooled to room temperature (25°C). Two grams of each reactivated culture were then precultured in the medium and incubated at 37°C for 36 hours. The growth of the bacterium was then monitored until the total viable count was $>6 \log \text{CFU/mL}$.

2.2.2. Inoculation of Probiotic Culture. Sterilized polyethylene terephthalate bottles (1 L) containing cowpea-peanut milk were opened under aseptic conditions, and the milk was inoculated with 2% (v/v) culture. The culture was gently mixed with the milk samples and incubated at a time of 0 h. In the control experiment, cow’s milk was inoculated.

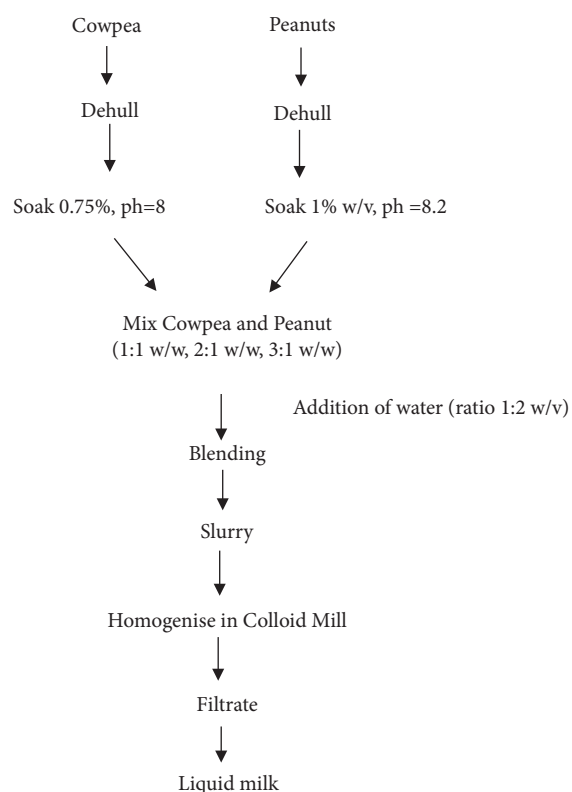


FIGURE 1: Process flowchart of cowpea-peanut milk.

2.3. Determination of Viable Cell Counts. The growth rate of the probiotic bacteria in the cowpea-peanut milk and control milk was monitored for 24 h. Samples were collected every 6 h over the 24 h period. One milliliter (1 mL) of each sample fermented by the probiotic culture was aseptically taken from the sample and suspended in sterile 9 mL of peptone solution (pH 7, 8.5 g/L NaCl, and 1 g/L neutralized bacteriological peptone from Oxoid). De Man, Rogosa, and MRS agar (1.2% agar, bacteriological peptone from Oxoid, added to de Man, Rogosa, and Sharpe broth, Merck) was used for culturing *Lactocaseibacillus rhamnosus* Yoba. Diluents of 100 μ L from each fermented sample were cultured in triplicates. The cultured MRS agar plates were then incubated under anaerobic conditions at 37°C in GasPak anaerobic jars (Becton Dickinson Microbiology Systems, Baltimore, Maryland, USA). The colonies were counted and expressed as colony-forming units per milliliter (CFU/mL) of each probiotic bacteria.

2.4. Chemical Composition. Proximate analysis on ash content using dry ashing (AOAC method 938.08), moisture content using (AOAC method 925.45), crude fibre using the enzymatic gravimetric method (AOAC method 985.29), and crude protein using Kjeldahl (AOAC method 991.20) were determined according to standard methods described by the AOAC [18]. Total carbohydrate was determined by the difference method.

2.5. pH, Titratable Acidity, and Total Sugars. The pH was determined using a digital pH meter (BT-675, BOECO, Hamburg, Germany) which was calibrated with pH 4.0 and 7.0

according to the AOAC method [18]. Total soluble solids ($^{\circ}$ Brix) were determined using a digital refractometer (MA871, North Carolina, Milwaukee Instruments, USA) at 20°C. Titratable acidity (TA) was determined following a standard method by AOAC [18]. Ten milliliters of the sample was titrated against 0.1 M NaOH solution using phenolphthalein as an indicator. The pH and TA measurements were taken at $t = 0, 6, 12, 18,$ and 24 h. The phenol-sulfuric acid method was used to determine the reducing sugars (RS) [19].

2.6. Mineral and Antinutrient Analysis. The mineral content was determined according to the method from AOAC [18] using an inductively coupled plasma optical emission spectrometer (ICP-OES) (Agilent 5100, Agilent Technologies, Santa Clara, California, USA), AOAC [18]. The fermented milk samples were digested using concentrated HNO_3 and H_2SO_4 , followed by the addition of ultrapure H_2O_2 to complete digestion and then analysed. The trypsin inhibitor content was determined according to a method by Kakade et al. [20] with few modifications using bovine trypsin and α -benzoyl-DL arginine 4-nitroanilide hydrochloride (BAPNA) as the substrate and bovine trypsin as the standard enzyme, and the phytic acid content was determined using the method by McKie and McCleary [21] that involved acid extraction of phytic acid and then the dephosphorylation with phytase and alkaline phosphatase.

2.7. Amino Acid Analysis. Essential amino acids were analysed using the HCl hydrolysis process described by Chawafambira et al. [22] with slight modifications. The fermented sample was mixed with 6 N HCl in a vial and then mixed and treated with argon to remove oxygen. The mixture in the vial was heated at 110°C for 18–24 h and cooled. The hydrolysate was then centrifuged, filtrated, dried, and then reconstituted using borate buffer for derivatization. Borate buffer (200 μ L) was then pipetted into a 2 mL glass vial and mixed with pipetted 10 μ L of diluted sample. The mixture was then mixed with 6-aminoquinolyl-N-hydroxysuccinimidyl carbamate chemical and then vortexed. The vortexed mixture was heated at 55°C for 10 min and loaded into an autosampler tray for analysis. The ultra-performance liquid chromatography (UPLC) with a waters photodiode array detector (Waters, Milford, MA, USA) for high-resolution UPLC-UV was used for analysis. The separation process of the sample was performed using an Acquity UPLC BEH C18 (2.1 \times 150 mm; 1.7 μ m particle size) column at 60°C at a flow rate of 0.4 ml/min. Results were determined at a wavelength of 254 nm, and the amino acid content was expressed as mg/100 g.

2.8. DPPH Free Radical Scavenging and Total Antioxidant Activity Assay. The 2,2-diphenyl-1-picrylhydrazyl free radical scavenging activity of the fermented samples was analysed using the method described by AOAC [18] with slight modifications. Each 1 mL sample of 0, 6, 12, 18, and 24 h was mixed with 3 mL of 0.2 mmol/L DPPH methanolic solution. The absorbance of the reaction mixture was

measured at 517 nm using a Spectronic Genesys spectrophotometer (Genesys 5, Thermo Fisher Scientific, Waltham, Massachusetts, USA) after calibration with methanol in darkness at 25°C for 30 min. The DPPH free radical scavenging rate of each sample was determined as the percentage decrease in absorbance with time.

The total antioxidant activity was determined using the ABTS method as described by Yu et al. [23] with modifications. ABTS⁺ (7 mmol/L) and potassium persulfate (K₂S₂O₈) (140 mmol/L) were prepared, and then, 1.76 mL K₂S₂O₈ solution and 100 mL ABTS⁺ were mixed and left to react in the dark. The prepared liquid with 95% ethanol was then diluted, and its absorbance was analysed. 1.0 mL of each sample was then mixed with 3 mL of ABTS radical solution, and the decolorisation of the ABTS + radical cation by the sample was recorded in a Spectronic Genesys spectrophotometer (Genesys 5, Thermo Fisher Scientific, Waltham, Massachusetts, USA) at 734 nm.

2.9. Sensory Analysis. A total of 50 untrained panellists consisting of men and women were randomly selected to participate in the sensory evaluation process. Consent forms were obtained from volunteer participants. The fermented cowpea-peanut milk samples were coded using a three-digit code. Vanilla and sugar were added to the samples. Each panelist was given a pen, a sensory evaluation scorecard, and a bottle of water to rinse their mouth after each analysis and were placed in individual testing booths. A 5-point hedonic scale (1, very bad; 2, bad; 3, average; 4, good; 5, very good) was designed. The panellists were asked to evaluate product attribute: taste, colour, texture, aroma, appearance, and overall acceptance. Panelists were not allowed to discuss their responses during the sensory evaluation process.

2.10. Statistical Analysis. The Kruskal–Wallis nonparametric test will be used to determine any significant differences in the nutritional composition. The least significant difference (LSD) test will be used to compare the means. Multivariate analysis will be performed using analysis of variances (ANOVA) at $p < 0.05$ using Sigma Plot for Windows version 12.0.

3. Results and Discussion

3.1. Chemical Characteristics of the Fermented Peanut-Cowpea Milk. The fermented cowpea-peanut milk had a crude protein content range of 5.6–6.8% (Table 1). The addition of peanut milk was beneficial in improving the protein because of the high protein content of peanuts (25.80 g/100 g) as compared to cowpea (8–13 g/100 g) [24]. The utilisation of proteins during fermentation might have resulted in the lower content than the generally expected value in the fermented cowpea-peanut milk and the control sample.

The carbohydrate content of the control sample (4.25%) was lower as compared to the fermented cowpea-peanut milk samples (5.18–6.05%). The carbohydrate content was high in fermented cowpea-peanut milk sample (3:1) and significantly different ($p < 0.05$) in other fermented samples (1:1, 2:1 and control) after 24 h of fermentation. As the ratio

of cowpeas increased, the carbohydrate content increased in the fermented cowpea-peanut milk samples. This might be attributed to the high carbohydrate content of cowpea (53–66%) which is mostly starch and has a good C-type starch crystallinity and high amylose content [25]. This carbohydrate has a low glycemic index (GI), and this makes the fermented cowpea-peanut milk beneficial in preventing diabetes, obesity, and cardiovascular diseases [26].

The crude fat content was within the expected level in fermented cowpeas-peanut milk samples after the end of fermentation. The use of cowpeas in the ratios and addition of water might have affected the crude fat content of the milk blends. Consequently, during fermentation, some of the lipids might have been utilised by the starter culture to yield energy [10]. Also, Aduol et al. [27] reported a significant difference ($p < 0.05$) in the low crude fat content (0.3–0.5%) in fermented cowpeas milk Yoba Fiti GR-1 culture. The fat content observed in the fermented cowpea-peanut milk is beneficial to human health since it is within the recommended dietary intake level range for fat by the U.S. Department of Health and Human Services and U.S. Department of Agriculture Report of 2015.

The fibre content in fermented cowpeas-peanut milk sample could be attributed to the high dietary fibre ($14.1 \pm 0.3\%$ dry matter (DM) present in processed cowpea of which $1.0 \pm 0.0\%$ DM is soluble fibre and $13 \pm 0.2\%$ DM is insoluble fibre) [28]. Also, the inclusion of peanut milk in the milk blend might have resulted in the observed fibre content.

The control (fermented cow milk) had a higher content of RS (8.40 g/100 g) than the fermented cowpea-peanut milk. This could be attributed to the breakdown of lactose into simple sugars by lactase produced by the bacteria [29]. The observed RS in the fermented cowpea-peanut milk could be explained by the action of *Lacticaseibacillus rhamnosus* Yoba as it produces α -amylase and maltase which degrade the starch into malto-dextrins and simple sugars, respectively [30].

The moisture content of the fermented cowpea-peanut milk ranged between 89.8 and 91.5% and was higher than the fermented cow milk (83.6%) because of the addition of water during the preparation of the plant milk samples.

The fermented cowpea-peanut milk samples had a higher ash content (1.04–1.26%) than the control (0.73%). There was a significant difference ($p < 0.05$) in the total ash content of the fermented cowpea-peanut milk and control samples. As the cowpea milk increased in the peanut: cowpea ratio, the ash content increased in the fermented cowpea-peanut milk because cowpea has an ash content range of 0.21–1.09% [31]. Furthermore, the increase in ash content can be explained by the breakdown of dry matter during fermentation as the probiotic culture degraded carbohydrates and proteins [32].

3.2. pH and Total Acidity. The titratable acidity (TA) of the fermented cowpea-peanut milk samples was low (range 0.70–0.74% v/v lactic acid) as compared with that of the control (0.84% v/v) (Figure 2). This study noted a decrease in the final pH and an increase in the TA of the cowpea-peanut

TABLE 1: Proximate composition of fermented cowpea-peanut milk with *L. rhamnosus* Yoba.

Sample	Mineral content (g/100 g)						
	Crude protein	Crude fibre	Ash	Moisture	Carbohydrates	Crude fat	Reducing sugars
CM:PM (1:1)	6.3 ± 0.01 ^c	1.18 ± 0.01 ^b	1.26 ± 0.03 ^c	89.8 ± 0.01 ^a	5.18 ± 1.80 ^b	3.40 ± 0.01 ^b	2.10 ± 0.01 ^b
CM:PM (2:1)	5.6 ± 0.02 ^b	0.80 ± 0.01 ^a	1.04 ± 0.01 ^b	90.1 ± 0.02 ^b	5.46 ± 1.18 ^b	3.30 ± 0.03 ^b	1.80 ± 0.02 ^a
CM:PM (3:1)	6.8 ± 0.02 ^c	0.72 ± 0.03 ^a	1.10 ± 0.05 ^b	91.5 ± 0.03 ^b	6.05 ± 1.03 ^c	3.55 ± 0.01 ^b	2.20 ± 0.05 ^b
Control	4.1 ± 0.05 ^a	ND	0.73 ± 0.07 ^a	83.6 ± 0.01 ^a	4.25 ± 0.06 ^a	3.04 ± 0.04 ^a	8.40 ± 0.06 ^c
<i>P</i> value	<0.05	<0.05	<0.05	<0.05	<0.05	<0.05	<0.05

CM, cowpea milk; PM, peanut milk; ND, not determined; control, fermented cow milk. Values indicate the means of three replications ± standard deviation. Means values within the same column with different superscript letters (^a, ^b, ^c) are significantly different ($p < 0.05$).

milk samples as the fermentation progressed. This could be ascribed to be the action of *Lacticaseibacillus rhamnosus* Yoba as it breaks down sugars into lactic acid [7]. A similar trend was also observed in the fermented cow milk (control). Mpofo et al. [33] observed similar trends. Adesokan et al. [34] reported the breakdown of sugars by LAB to produce lactic acid as well as other secondary fermentation products such as propionic, acetic, and butyric acids, hence the increase in TA. This is beneficial in limiting the growth of other harmful bacteria that may cause bad fermentations.

At the 6 h fermentation period, the cowpea-peanut milk had a low pH of 4.5 compared to 4.8 observed in the control sample (Figure 3). It can be explained that the pH of the cowpea-peanut milk samples after inoculation (pH 6.3–6.4) was ideal to support the growth of *Lacticaseibacillus rhamnosus* Yoba in all samples at time 0 h. Mäkinen et al. [35] found similar pH trends (6.7–4.34) for cow's milk fermented with related LAB cultures. This is evident by the increase in cell numbers of *Lacticaseibacillus rhamnosus* Yoba in all inoculated samples from time 0 h to 6 h during fermentation as shown in Figure 4. Liew et al. [36] reported the optimum pH range for growth of *Lacticaseibacillus rhamnosus* as 6.4–6.9. As the pH decreased during fermentation, it allowed for the survival of the *Lacticaseibacillus rhamnosus* Yoba in the cowpea-peanut milk but limited its rate of growth.

3.3. DPPH Free Radical Scavenging and Total Antioxidant Activity. The DPPH radical scavenging rate of the fermented cowpea-peanut milk samples ranged from 56 to 59% and was low in the control sample (41.6%) at the end of the fermentation period (Figure 5). The ABTS + free radical scavenging rate (Figure 6) had similar trends as the DPPH radical scavenging rate. This could be attributed to the presence of phenolic acids, flavonoids, anthocyanins, and proanthocyanidins in cowpea [37]. The low antioxidant rate of the fermented cow's milk could be explained by the mode of action of the culture strain during the fermentation of milk [38]. This was in agreement with Lim et al. [39]. More so, the proteolysis of milk proteins such as α -casein, α -lactalbumin, and β -lactoglobulin by the action of starter culture and the production of bioactive peptides has been found to induce in vitro antioxidant capacity [40]. Also, the formation of conjugated linoleic acid (CLA) in fermented milk and the presence of coenzyme Q10 and vitamins A and E contribute to the antioxidant activity [41].

3.4. Amino Acids Composition. The amino acid composition of the fermented cowpea-peanut milk and control (cow milk) is given in Table 2. The fermented cowpea-peanut milk sample (3:1) with *Lacticaseibacillus rhamnosus* Yoba had the highest lysine content (7.31 mg/100 g) and was significantly different to all other fermented milk samples. This could be ascribed to the high concentration of cowpea used in the formulation and the high lysine content in cowpea [42]. The fermented cow milk samples had a methionine content (1.95 mg/100 g) that was not significantly different in fermented cowpea-peanut milk (3:1) and higher when compared to fermented cowpea-peanut milk samples (1:1 and 2:1) because cowpea has low methionine and cysteine content when compared to animal proteins [42]. During fermentation, *Lacticaseibacillus rhamnosus* Yoba was able to break down the proteins into peptides and free amino acids in all the fermented milk samples. Ghosh et al. [43] observed the presence of free histidine, cystine, histidine, and asparagine in fermented cow and soybean milk curd.

3.5. Mineral and Antinutritional Composition. Table 3 provides that fermented cow milk (control) had higher phosphorus, calcium, zinc, and sodium content because raw milk from cows is naturally an excellent source of many minerals. The process of filtering and wash water treatment using cheesecloth might have affected the mineral content of the fermented cowpea-peanut milk blends [10]. Difo et al. [44] observed a decrease of over 90% and 50% in calcium and iron after fermentation of cowpea flours, and this suggests the observed results. The blending of cowpeas and peanuts milk might have had an additive effect on some of the minerals, particularly iron and phosphorus. Cowpea is rich in potassium (957–1251 mg/100 g) with good amount of calcium (29–44 mg/100 g), magnesium (116–130 mg/100 g), and phosphorus (105–276 mg/100 g) [45].

The trypsin inhibitor and phytic acid content in fermented cowpea-peanut milk were very low after fermentation (Table 3). This could be due to the beneficial action of the probiotic microorganism in removing the antinutritive compounds that promote protein cross-linking (phenolic and tannin compounds) and inhibit digestive enzymes (trypsin and chymotrypsin inhibitors) from food material and production of microbial proteases, which could degrade and release some of the proteins from

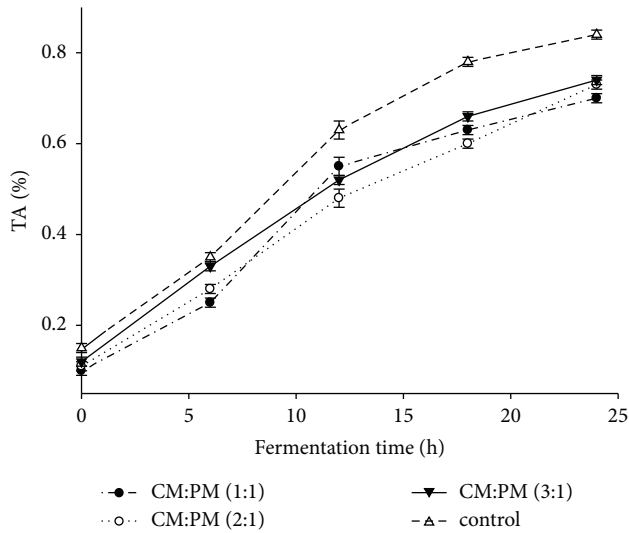


FIGURE 2: Titratable acidity values of four samples at different fermentation times. All the values are expressed as mean \pm SD ($n=3$). CM, cowpea milk; PM, peanut milk.

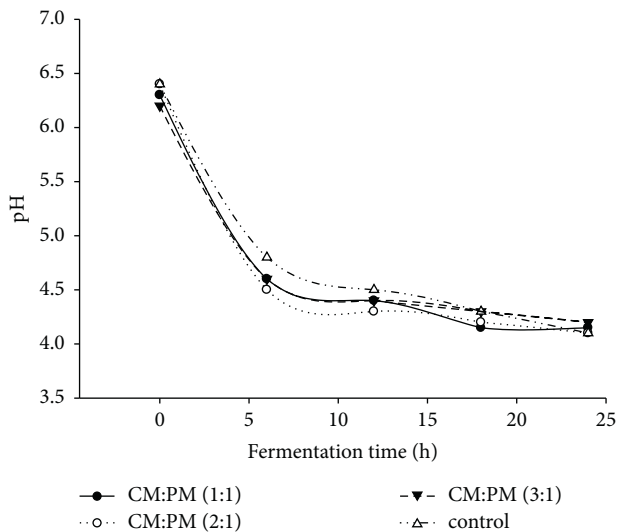


FIGURE 3: The pH values of four samples at different fermentation times. All the values are expressed as mean \pm SD ($n=3$). CM, cowpea milk; PM, peanut milk.

the matrix during fermentation [7, 46]. Also, the use of NaHCO_3 during soaking might have reduced the trypsin inhibitor and phytic acid content. Vadivel and Pugalenti [47] reported a significant reduction in phytic acid (75–78%), trypsin inhibitor activity (81–82%), α -amylase inhibitor activity (82–84%), total free phenolics (82–83%), and tannins (74–84%) in velvet beans soaked in NaHCO_3 .

3.6. Cell Viability. The viable plate count of *Lactocaseibacillus rhamnosus* Yoba increased from 6.1–6.2 log CFU/mL to 7.7–8.1 log CFU/mL ($t=0$ hr) after fermentation (Figure 4). This suggests that the cowpea-peanut milk matrix was an ideal environment to support the growth of *Lactocaseibacillus rhamnosus* Yoba. Cowpea is rich in

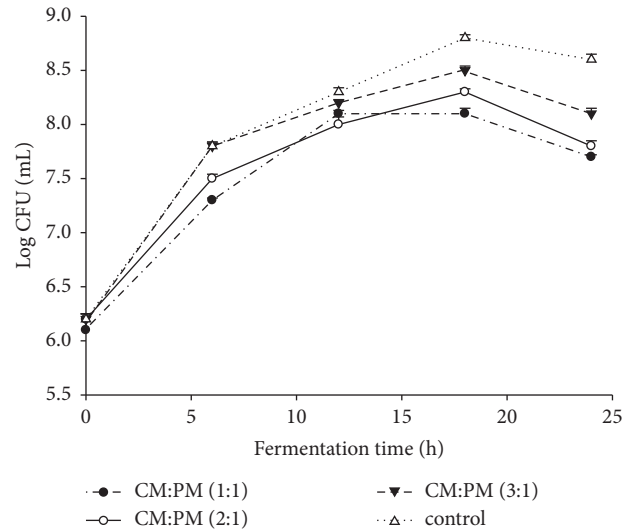


FIGURE 4: *Lactocaseibacillus rhamnosus* Yoba viable cell counts of four samples at different fermentation times. All the values are expressed as mean \pm SD ($n=3$). CM, cowpea milk; PM, peanut milk.

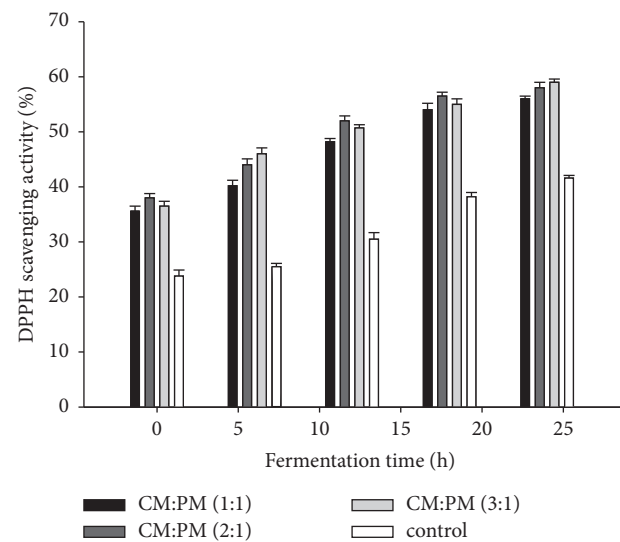


FIGURE 5: DPPH free radical scavenging rate of four samples at different fermentation times. All the values are expressed as mean \pm SD ($n=3$). CM, cowpea milk; PM, peanut milk.

α -galactosides (raffinose, verbascose, and stachyose), also referred to as the raffinose family oligosaccharides (RFOs) which act as prebiotics [48]. The prebiotics might have supported the growth of *Lactocaseibacillus rhamnosus* Yoba in fermented cowpea-peanut milk to some extent although the use of NaHCO_3 during soaking might have lowered the oligosaccharides. This was supported by Vadivel and Pugalenti [47]. The results of this study showed the potential to attain cell viability of over 6 log CFU/mL in fermented cowpea-peanut milk that is comparable to fermented cow's milk (control). This is similar to results obtained on the survival of *Lactocaseibacillus rhamnosus* GG in various leguminous porridges [49].

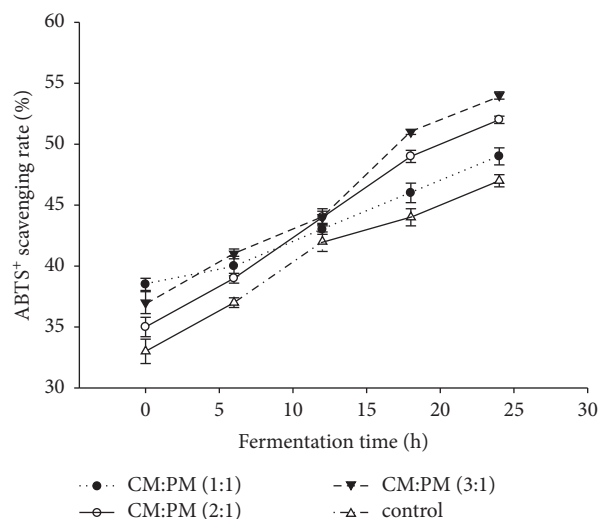


FIGURE 6: ABTS + free radical scavenging rate of four samples at different fermentation times. All the values are expressed as mean \pm SD ($n=3$). CM, cowpea milk; PM, peanut milk.

TABLE 2: Amino acid content of fermented cowpea-peanut milk with *L. rhamnosus* Yoba.

Amino acid (g/100 g)	Fermented milk samples			Control	P value
	CM:PM (1:1)	CM:PM (2:1)	CM:PM (3:1)		
Essential					
Valine	4.40 \pm 0.01 ^a	4.86 \pm 0.02 ^a	5.01 \pm 0.01 ^b	4.78 \pm 0.05 ^a	<0.05
Lysine	6.30 \pm 0.02 ^a	7.31 \pm 0.02 ^b	6.70 \pm 0.01 ^a	6.60 \pm 0.06 ^a	<0.05
Threonine	3.26 \pm 0.01 ^b	2.65 \pm 0.05 ^a	3.80 \pm 0.01 ^c	3.85 \pm 0.04 ^c	<0.05
Tryptophan	1.50 \pm 0.03 ^a	2.10 \pm 0.06 ^b	2.65 \pm 0.07 ^c	1.15 \pm 0.05 ^a	<0.05
Leucine	6.60 \pm 0.01 ^a	8.02 \pm 0.01 ^c	8.75 \pm 0.05 ^c	7.08 \pm 0.05 ^b	<0.05
Phenylalanine	5.23 \pm 0.01 ^b	5.80 \pm 0.03 ^b	5.65 \pm 0.07 ^b	4.10 \pm 0.02 ^a	<0.05
Isoleucine	3.05 \pm 0.05 ^a	2.96 \pm 0.06 ^a	3.40 \pm 0.01 ^b	4.25 \pm 0.08 ^c	<0.05
Tyrosine	1.08 \pm 0.02 ^a	1.23 \pm 0.08 ^a	1.80 \pm 0.04 ^b	4.60 \pm 0.06 ^c	<0.05
Nonessential					
Serine	2.15 \pm 0.02 ^a	1.90 \pm 0.07 ^a	2.25 \pm 0.08 ^b	4.74 \pm 0.04 ^c	<0.05
Proline	2.60 \pm 0.06 ^a	2.78 \pm 0.05 ^b	2.46 \pm 0.07 ^a	6.85 \pm 0.09 ^c	<0.05
Glycine	2.22 \pm 0.06 ^b	2.70 \pm 0.05 ^c	2.90 \pm 0.07 ^c	1.05 \pm 0.05 ^a	<0.05
Alanine	3.08 \pm 0.04 ^b	3.65 \pm 0.08 ^b	4.10 \pm 0.01 ^c	2.30 \pm 0.02 ^a	<0.05
Glutamic	16.6 \pm 0.05 ^c	15.4 \pm 0.06 ^b	17.3 \pm 0.07 ^c	12.4 \pm 0.06 ^a	<0.05
Methionine	1.70 \pm 0.06 ^a	1.50 \pm 0.05 ^a	1.86 \pm 0.08 ^b	1.95 \pm 0.07 ^b	<0.05
Histidine	2.01 \pm 0.08 ^b	1.87 \pm 0.02 ^a	1.90 \pm 0.03 ^a	1.88 \pm 0.08 ^a	<0.05

CM, cowpea milk; PM, peanut milk; control, fermented cow milk. Values indicate the means of three replications \pm standard deviation. Means values within the same row with different superscript letters (^a, ^b, ^c) are significantly different ($p < 0.05$).

TABLE 3: Mineral and antinutritional composition of fermented cowpea-peanut milk with *L. rhamnosus* Yoba.

Sample	Mineral content (mg/100 g)						Phytic acid (%)	Trypsin inhibitor (TIU/mg)	
	Phosphorus	Calcium	Iron	Magnesium	Zinc	Sodium			Potassium
CM:PM (1:1)	60.2 \pm 0.06 ^a	45.3 \pm 0.08 ^a	0.48 \pm 0.05 ^b	20.8 \pm 0.06 ^b	0.18 \pm 0.06 ^a	13.8 \pm 0.08 ^a	222.5 \pm 0.05 ^c	0.34 \pm 0.02 ^a	1.05 \pm 0.03 ^b
CM:PM (2:1)	66.7 \pm 0.07 ^b	51.2 \pm 0.02 ^b	0.50 \pm 0.06 ^b	18.6 \pm 0.02 ^b	0.21 \pm 0.05 ^a	13.4 \pm 0.05 ^a	202.3 \pm 0.01 ^b	0.30 \pm 0.05 ^a	1.12 \pm 0.04 ^b
CM:PM (3:1)	69.2 \pm 0.04 ^b	60.4 \pm 0.07 ^b	0.58 \pm 0.03 ^b	21.4 \pm 0.05 ^c	0.27 \pm 0.06 ^a	14.2 \pm 0.03 ^a	243.1 \pm 0.06 ^c	0.32 \pm 0.06 ^a	0.86 \pm 0.08 ^a
Control	123.2 \pm 0.05 ^c	145.5 \pm 0.06 ^c	0.06 \pm 0.05 ^a	8.1 \pm 0.04 ^a	0.48 \pm 0.02 ^b	48.4 \pm 0.06 ^a	115.7 \pm 0.05 ^a	ND	ND
P value	<0.05	<0.05	<0.05	<0.05	<0.05	<0.05	<0.05	<0.05	<0.05

CM, cowpea milk; PM, peanut milk; ND, not determined; control, fermented cow milk. Values indicate the means of three replications \pm standard deviation. Means values within the same column with different superscript letters (^a, ^b, ^c) are significantly different ($p < 0.05$).

TABLE 4: Mean sensory scores of fermented cowpea-peanut milk with *Lactocaseibacillus rhamnosus* Yoba.

Sample	Taste	Colour	Texture	Aroma	Appearance	Overall acceptability
AAB	3.9 ± 0.1 ^a	3.7 ± 0.1 ^a	3.9 ± 0.2 ^b	4.0 ± 0.1 ^a	3.8 ± 0.1 ^b	4.0 ± 0.1 ^a
ABA	4.0 ± 0.1 ^a	3.5 ± 0.2 ^a	3.7 ± 0.1 ^a	3.9 ± 0.1 ^a	3.7 ± 0.1 ^s	3.9 ± 0.1 ^a
ABB	3.8 ± 0.2 ^a	3.6 ± 0.1 ^a	3.5 ± 0.1 ^a	3.8 ± 0.2 ^a	3.6 ± 0.1 ^s	3.8 ± 0.2 ^a
BAA	4.0 ± 0.1 ^a	4.0 ± 0.1 ^b	4.0 ± 0.2 ^c	4.0 ± 0.1 ^a	4.1 ± 0.1 ^c	4.1 ± 0.0 ^a
<i>P</i> value ^a	ns	<0.05	<0.05	ns	<0.05	ns

AAB, CM: PM (1:1) + sugar + vanilla; ABA, CM: PM (2:1) + sugar + vanilla; ABB, CM: PM (3:1) + sugar + vanilla; BAA, control + sugar + vanilla; ns = not significant. Mean ± standard deviations are reported. ^aFriedman's test. Means values within the same column with different superscript letters (^a, ^b, ^c) are significantly different ($p < 0.05$).

3.7. Sensory Evaluation. The mean scores for the sensory attributes of the fermented cowpea-milks and control samples are given in Table 4. The observed results indicated a significant difference ($p < 0.05$) in colour, texture, and appearance. However, no significant differences ($p > 0.05$) were observed in taste, aroma, and overall acceptability. The ratings of colour in fermented cow's milk could be attributed to the reflection of fat in cow's milk. The taste might have been influenced by the low residual sugar content and titratable acidity although the addition of sugar greatly improved the rating for taste. The flavour ratings could be attributed to the presence of acetaldehyde, (E)-2-pentenal, hexanal, 3-methyl-butanal, 2, 3-butanedione, (E)-2-octenal, nonanal, acetoin, 2-non-anone, ethenyl ester formic acid, and 2-heptanone [50], especially in fermented cow's milk. Also, the microbial catabolism of lactose, proteins, and fatty acids by *Lactocaseibacillus rhamnosus* Yoba could have produced acetaldehyde, acetoin, and diacetyl [51]. Fermented milk produces small amounts (0.001–1,000 µg/L) of volatiles that influence the aroma and flavour thresholds [52]. They were no significant differences in the overall acceptability of the fermented cowpea-peanut milk and cow milk.

4. Conclusion

The application of *Lactocaseibacillus rhamnosus* Yoba as a method of fermenting cowpea-peanut milk proved an effective food processing technique for enhancing customer acceptability and improving the nutritional content. The cowpea-peanut milk matrices were able to support the growth of *Lactocaseibacillus rhamnosus* Yoba, and this was shown by the viable cell counts of 7.7–8.1 log CFU/mL after fermentation. The TA had a significant effect on the amount of pH obtained. The decrease in pH from 6.3 to 4.3 resulted in the significant growth of *Lactocaseibacillus rhamnosus* Yoba during fermentation. However, pH 4.1 decreased the growth of the probiotic bacteria although it allowed for its survival. There was an increase in antioxidant activity and reduction in antinutritive compounds as the *Lactocaseibacillus rhamnosus* Yoba degrade the proteins to produce peptides. The increase in iron and potassium content may be attributed to the possible breakdown of the phytates during fermentation and the release of bound minerals. The results confirm the potential economic benefits of using the underutilised legume crops in the development of novel fermented cowpea-peanut milk with a generic probiotic.

This technique is beneficial in the removal of antinutrients and improving customer acceptability of fermented legume-based milk. Further studies will focus on the bioaccessibility and bioavailability of minerals in the fermented cowpea-peanut milk using the generic probiotic. The possibility of inactivation of pathogenic microorganisms in the media of cowpea-peanut milk fermented with *Lactocaseibacillus rhamnosus* Yoba will also be explored in our further studies.

Data Availability

The data used to support the findings of this study are available from the corresponding author upon request.

Conflicts of Interest

The authors declare that there are no conflicts of interest.

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