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Research Article

The application of blends of bambaranut and millet vegetable milk in the development of plant-based yoghurt analogues: Proximate composition, physiochemical properties, microbial safety and consumer's acceptability

Jasper Okoro Godwin ELECHI, Joseph Oneh ABU, Mike Ojotu EKE

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University of Agriculture, Department of Food Science and Technology, P.M.B. 2373, Makurdi, Nigeria

ORCID IDs of the authors:

J.O.G.E. 0000-0001-5623-4189

J.O.A. Author don't have ORCID

M.O.E. Author don't have ORCID

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Correspondence:

Jasper Okoro Godwin ELECHI

E-mail: helloeljasper@gmail.com

ABSTRACT

This study aimed to produce plant-based yoghurt analogues from the blends of Bambaranut and millet milk. Yoghurt samples were produced from blends of Bambaranut milk and millet milk using *Streptococcus thermophillus* and *Lactobacillus bulgaricus* as starter cultures. Yoghurt samples were subjected to chemical, microbiological and organoleptic assessment. The results of the chemical analysis revealed moisture, protein, ash, fat, fibre, carbohydrates and energy contents ranged from 87.61-78.26%, 6.85-3.68%, 0.76-0.59%, 2.70-1.81%, 0.34-0.26%,12.88- 5.60 and 92.94-57.50% respectively Total solids of between 12.39 and 21.74% were obtained with titratable acidity of 0.21, 0.65, 0.21, 0.23, 0.23, 0.25, 0.30 and 0.90%, respectively. The syneresis of the samples ranged from 40.28 to 18.90% while all the samples showed fairly acidic levels. A viscosity of between 250 and 784cp was obtained. The microbiological examination revealed an acceptable level for all the samples. There were observable significant differences in terms of overall acceptability, taste, and flavour between cow milk yoghurt and the yoghurt analogues.

Keywords: Vegetable milk, Plant-based, Yoghurt analogues, Sustainable diets

Introduction

One of the largest problems facing the world food system in the twenty-first century is meeting the need for protein while maintaining environmental standards. Food systems today are responsible for nearly a quarter of all anthropogenic greenhouse gas (GHG) emissions, as well as deforestation, biodiversity loss, freshwater consumption, and water pollution. They are also ineffective in appropriately feeding people, resulting in a rise in food safety and health issues. Malnutrition affects around a third of the world's population in various ways (hunger, obesity, and micronutrient deficiencies) (Röös *et al.,* 2018; Elechi *et al.,* 2022). Diet-related hazards are now the world's third biggest cause of mortality, and malnutrition is the major cause of lost years of healthy life (Elechi *et al.,* 2022, GBD, 2020). According to Froggatt and Wellesley (2019), meat and dairy production is a major contributor to environmental change and the depletion of natural resources as the livestock industry is responsible for an estimated 40% of the world's arable land, 36% of crop calories produced, 29% of agricultural freshwater (Mottet *et al.,* 2017), and 14.5% of all human greenhouse gas (GHG) emissions. (Gerber *et al.,* 2013). Individuals who consume too much meat or dairy are more likely to be overweight, obese, and develop non-communicable illnesses connected to their diets, such as cardiovascular disease, type 2 diabetes, and certain cancers (Popkin *et al.,* 2012; Rouhani *et al.,* 2014; Bouvard et al., 2015). Additionally, the UN has identified improper animal antibiotic usage as a major contributor to the rise in antimicrobial resistance (Van Boeckel *et al.,* 2017), while the expansion of livestock production raises significant concerns about animal welfare (Mason and Lang, 2017).

Dairy products continue to be important and main agricultural products, with global milk production expected to reach 861 Mt in 2020 and expand at a rate of 1.7% per year to 1020 Mt by 2030 (FAO. 2022). Particularly dairy products have long played a significant and durable role in the diet of the general public, where they are ingested for a variety of reasons, including enjoyment, dietary demands, and special health advantages, including probiotic ingestion. These dairy products range from kefir to yoghurt and other fermented meals to cheese (Tamang *et al.,* 2016; Pua *et al.,* 2022). Consumers are starting to actively look for alternatives to conventional bovine dairy products despite their current popularity because of their possible long-term detrimental effects on human health and the environment as well as other ethical concerns (Mendly-Zambo *et al.,* 2021) which violates sustainable diet principles. A sustainable diet is seen as one that has a minimal environmental effect and contributes to food and nutrition security as well as a healthy life for current and future generations (FAO and Bl 2010). Sustainable diets are culturally acceptable, accessible, economically fair, and inexpensive, as well as nutritionally adequate, safe, and healthy, while also maximising natural and human resources. As a result, greenhouse gas emissions and land usage are the most often employed measures to assess diet sustainability (Jones et al., 2016). Therefore, there is growing agreement in the scientific, environmental, and public health sectors that a fundamental change in eating habits away from excessive meat and dairy consumption is required immediately to address the unsustainable nature of the livestock industry (Froggatt and Wellesley 2019). As a result, there is now more interest in plant-based dairy alternatives, which are thought to have advantages over conventional dairy products.

Due to their additional health advantages, plant-based dairy alternatives are of great interest, and their use and market share have been expanding (Cichonska and Ziarno, 2022). By 2028, the market for plant-based dairy products is projected to grow at a CAGR of 12.5% and total USD 52.58 billion (Grand View, 2022). Vegan-friendly labels (depending on additives) (Mendly-Zambo *et al.,* 2021; Yadav *et al.,* 2017; Roselló-Soto *et al.,* 2019); "free-from" properties (Hartmann et al., 2018) for lactose, cholesterol, and dairy allergens like casein; reductions in consumer concerns about hormone and antibiotic residues; typically high content in vitamins, minerals, other bioactives, phytochemicals, and added functionalities, such as dietary fibre or pre-/probiotic activity (Cichonska and Ziarno, 2022; Roselló-Soto *et al.,* 2019) are some of the attractive features of plant-based dairy alternatives compared to conventional dairy products. Plant-based milk alternatives are liquids made from the breakdown (crushing) of water-extracted plant material (cereals, pseudo-cereals, legumes, oilseeds, nuts), and homogenization of these liquids produces a particle size distribution in the range of 5-20 µm, which resembles cow's milk in appearance and consistency (Sethi *et al.,* 2016). In terms of nutritional content, plant-based milk differs from one another. As a result, mixing two or more types of plant-based milk to produce a product with a high nutritional content equivalent to cow's milk is a crucial processing step (Sethi *et al.,* 2016). Recent research has established the critical role of these plant-based beverages in enhancing or managing the immune system, reducing the risk of cardiovascular and gastrointestinal diseases with improved physiological functions, reducing the risk of low bone mass, and very high levels of antioxidants with free radical scavenging properties (Paul *et al.,* 2019). Furthermore, the addition of some beneficial and nutritionally significant components increases the product's quality, primarily by improving protein quality, mineral bioavailability, and the availability of a few key elements (Akin and Ozcan, 2017).

Yoghurt is arguably the most popular and diverse fermented dairy analogue. Yogurt is manufactured by fermenting milk with lactic acid bacteria - *St. thermophilus and L. bulgaricus*, which are responsible for the characteristic yoghurt flavour. Set, stirred, drinkable, flavoured-the seemingly endless variations of yoghurt products with different fat levels (normal, low-fat, and fat-free) on the market belie the humble makeup of its microbiota (Kayanush *et al.,* 2017). Lactose is converted during the fermentation process into lactic acid, which gives yoghurt its distinct tangy flavour and causes the acid gelation of casein, a significant milk protein (Pua et al., 2022). This creates the cohesive gel that gives dairy yoghurts their distinctive hard, thick texture (Boeck *et al.,* 2021; Aryana and Olson, 2017). The creation of a stable, pleasant, and dairy-like yoghurt alternative has significant challenges due to the absence of casein in plant matrices and dairy-incompatible natural plant flavours (Boeck *et al.,* 2021; Hickisch *et al.,* 2016; Łopusiewicz *et al.,* 2020). The addition of a fermentation process could enhance the organoleptic properties of such plant-based products and create a clean-label fermented dairy analogue with sufficient consumer acceptability, as many plant-based matrices struggle to achieve a natural dairy-like profile without the extensive use of processing aids and flavourings (Pua *et al.,* 2022). The majority of commercially available fermented plant-based yoghurt substitutes are created from nuts, drupes, and seeds (cashew, almond, and coconut). None has been used from the blend of bambaranut and millet.

The Bambaranut (*Vigna subterraenea (L.) verdc*) is a legume with an African origin. It is the third most significant legume, but it is also one of the most neglected (Murevanhema & Jideani, 2013). It has been dubbed a "whole food" because of its high nutritional value. In comparison to fresh whole milk, which contains 88% moisture, 4.8% carbohydrates, 3.2% proteins, 3.4% fat, 0.7% ash, and 0.01% cholesterol, its seeds contain 49-63.5% carbohydrates, 15-25% protein, 4.5-7.4% fat, 5.2-6.4% dietary fibre, 3.2-4.4% ash and 2% minerals. Its chemical makeup is similar to that of soybeans. Furthermore, due to its nutritional content, functional characteristics, antioxidant potential, and drought-resistant cultivation, BGN has been identified as a promising crop. The acceptability of Bambaranut milk was reported to be higher than that of milk derived from other legumes such as soybeans and cowpeas (Murevanhema & Jideani, 2013). Bambaranut milk has a strong enough nutritional profile to support probiotic development. As a result, Bambaranut milk may be fermented with lactic acid bacteria to generate a probiotic drink that not only boosts the nutritional content of the bean but also aids in addressing malnutrition (Murevanhema & Jideani, 2013).

Millions of the poorest people in the places where millet is produced rely on it for energy, protein, vitamins, and minerals. Millet, like sorghum, has 9-13 percent protein on average, however there have been considerable fluctuations in protein content ranging from 6 to 21%. Millet is a popular weaning food since it is one of the least allergic and easily digested grains. It is good for making non-sticky gruel and porridge because it has less water-extractable dietary fibre than wheat and oats.

Diversified dairy products are in short supply in the dairy industries, but demand for them is growing. Although it has been demonstrated that livestock produces around 14.5% of all human-induced greenhouse gas (GHG) emissions, both developing and developed countries continue to consume large amounts of energy-rich meals, which include meat, milk, and other dairy products (Weinrich, 2019). By 2050, it is predicted that 9 billion people will be eating plant-based protein, which has the potential to not only significantly lessen the severely negative environmental effects of meat consumption but also provide a source of good and nutritious meals for those individuals (Chaudhary & Tremorin, 2020; Monnet *et al.,* 2019; Willett et al., 2019). Few legumes and oilseeds have been widely used in this area to produce non-dairy, healthy, affordable plant-based milk alternatives (Sethi *et al.,* 2016). Soy is the most popular plant-based ingredient for yoghurt (Yang *et al.,* 2021) with other plant components like coconut and almonds gaining popularity (Gupta *et al.,* 2022). Novel ingredients, such as lupins (Hickisch *et al.,* 2016), African Yam Bean (Amakoromo *et al.,* 2012); oats (Brückner-Gühmann *et al.,* 2019), sesame seed (Ibrahim, 2013), peas (Ben-Harb *et al.,* 2018), Baobab (Eke *et al.,* 2013), quinoa (Lorusso *et al.,* 2018), Bambara Groundnut (Eke *et al.,* 2012) and flaxseed (Mousavi *et al.,* 2019) are also being assessed. Yoghurt can also be made by making blends from different sources as reported by Olakunle (2012) for soy milk and corn milk and Kolapo and Olubamiwa (2012) for coconut milk and soy milk. Lower protein concentrations and variable gelation characteristics of these proteins compared to casein might be the cause of the various textural qualities of commercial plant-based yoghurts, necessitating the use of gelling agents (Gupta *et al.,* 2022). This study aimed to undertake product development and quality assessment of yoghurt analogues made using Bambaranut and millet milk blends, with implications for nutrient-dense yoghurt product diversification and commercial potential.

Materials and Methods

Source of Material and Preliminary Treatments

About 2.0 kg each of Bambaranut and Millet was purchased from a small market in the Lafia metropolitan region of Nassrawa state, Nigeria. Whole milk powder (Peak), sucrose (Golden Penny brand), and commercially available yoghurt starter cultures (*Streptococcus thermophilillus* and *Lactobacillus bulgaricus*) were acquired at a local shop in Makurdi, Benue state, Nigeria. Tito, a local and prominent dairy yoghurt manufacturer in Nigeria's north-central area, provided the cow's milk yoghurt utilised as a reference sample in this study. The majority of the chemicals and equipment used for sample preparation ($NaHCO_3$) and analysis are analytical grade and were bought from a local Nigerian chemical store and the Department of Food Science and Technology, University of Agriculture, Makurdi-Nigeria. After hand sorting and screening, stones, dirt, and faulty seeds that can affect the beverage's taste and shelf life were removed. Each 5litre plastic bucket was filled with clean Bambaranut and millets. All components are kept in a household refrigerator and employed in product development within seven days.

Preparation of Bambaranut Milk

With minor adjustments, Bambaranut milk was made according to the technique of Eke et al. (2012) as shown in figure 1. One kilogramme of bambaranut was divided into three kilogrammes, i.e. (1:3) warm drinking water with 50 g of sodium bicarbonate, steeped for 18 hours overnight. The Bambaranuts were then drained, washed three times with drinking water, and blanched for 25 minutes in boiling water with baking soda (0.5 percent $NaHCO_3$) to kill the lipoxygenase enzyme. The blanched seeds are rinsed, skinned, and crushed into a paste in the blender (model: Qlink; QBL-15L40) for seven minutes with a little warm water. The paste was prepared up to a 1:3 weight-to-volume ratio. The resultant slurry was filtered and pressed through a double-layered muslin cloth, and the resulting extract (milk) was heated in a pot at 95-100°C for 15 minutes with constant stirring. After heating, the bambaranut milk was then manually homogenised with a glass rod and sieved again. It was then cooled to room temperature of 22°C, kept in a domestic refrigerator at 5°C, and utilised for product formulation within 24 hours.

Preparation of Millet Milk

As described, the Amal *et al.,* (2012) method was adopted and adapted for millet milk manufacturing as shown in figure 1. By soaking and removing the seeds floating on top of one kilogramme of millet, it was cleaned and sorted. The excellent seeds were collected and drained in a plastic basket. After that, the good seeds were steeped for 8 hours. The soaked seeds were drained and crushed in a blender (model: Qlink; QBL-15L40). To extract the milk, the slurry is pressed using cheesecloth. The extract was pasteurised for 5 minutes at 95-100°C. It was homogenised, sieved again, and cooled to room temperature of 22°C before being kept in a household refrigerator at 5°C and utilised within 24 hours for product development.

Cow Milk Yogurt

The plain cow's milk yoghurt utilised in this study came from a manufacturer in Benue State, Nigeria. Ramtito Diary produces Tito yoghurt, which comprises a 2% yoghurt starter made from *S. thermophilus* and *L. delbrueckiisubsp bulgaricus*. The product was chosen as a research reference because it is a popular, affordable, and widely accessible cow's milk-based yoghurt formulation in Benue state and its environs.

Food Products Formulation

Five different food formulations are made by blending the Bambaranut milk (BM) produced with the Millet milk (MM) to obtain various yoghurt analogues as shown in table 1. The resulting blends are homogenized and pasteurized at 72ºC for 5 min in a water bath and cooled immediately to room temperature (28 ±2ºC). These were then packaged in 500mL plastic bottles with air-tight lids as shown in figure 3, and stored in a household refrigerator from where samples are taken for different yoghurt analogues analiysis.

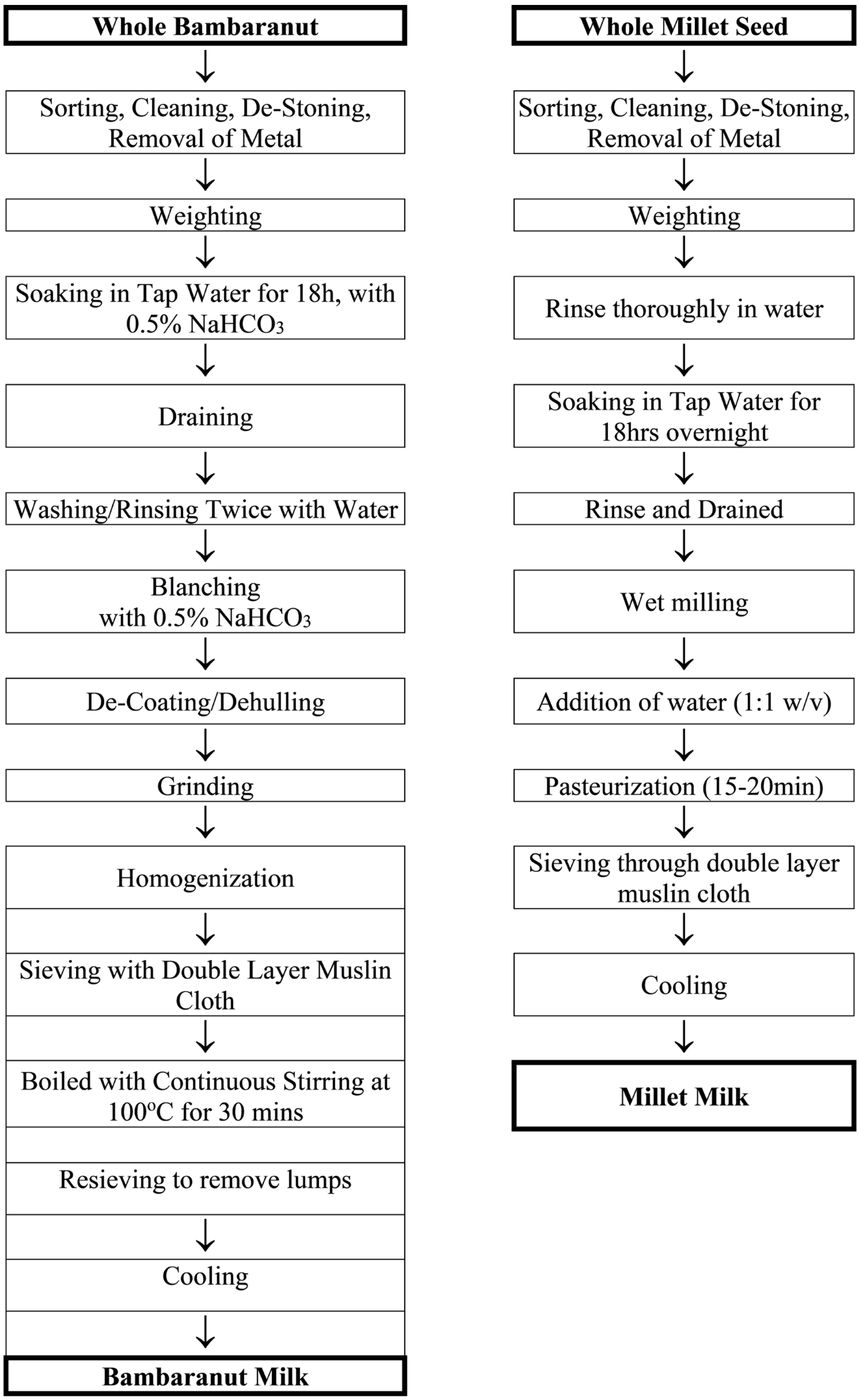

Figure 1. Preparation of bambaranut and millet plant based milk

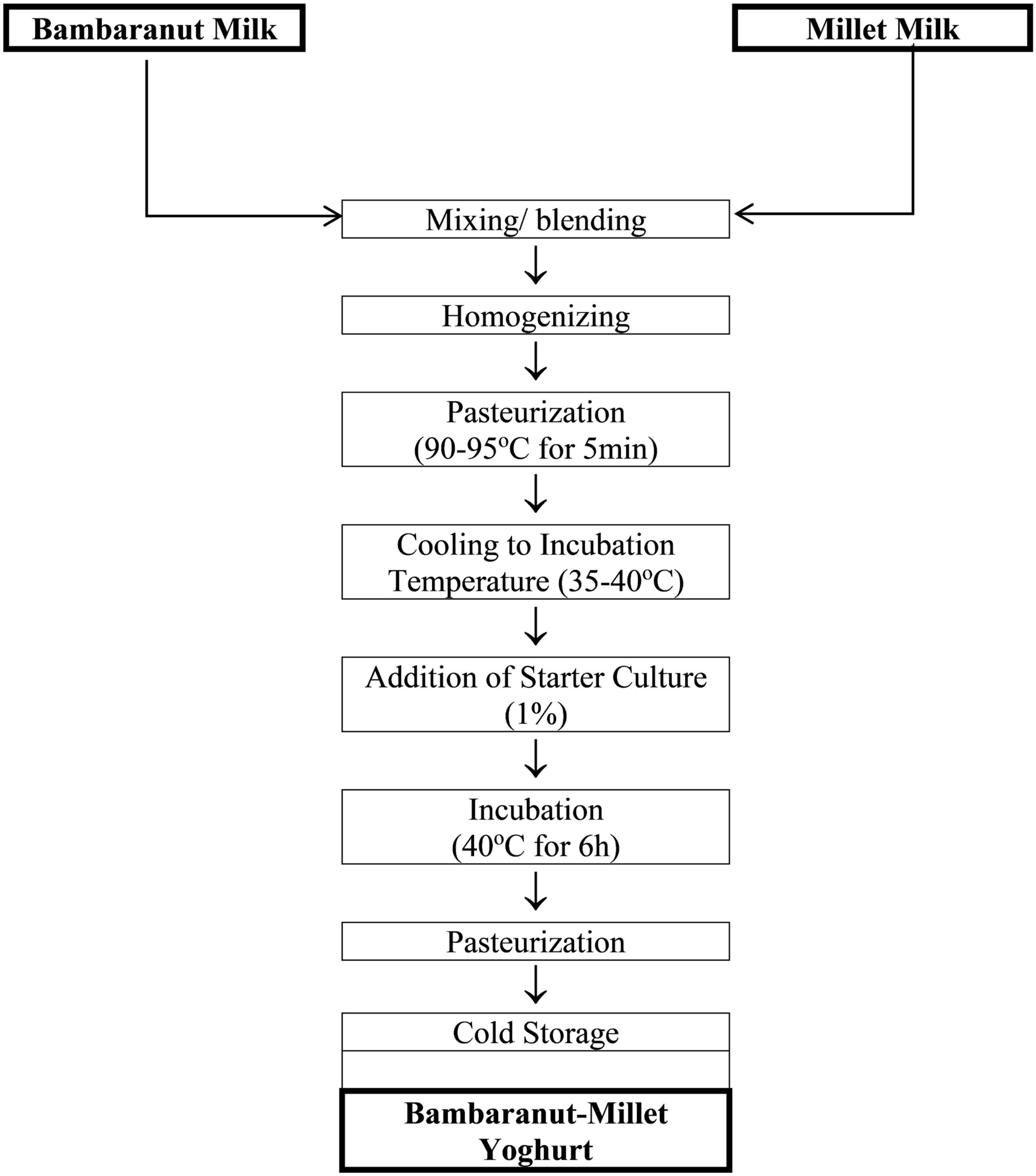

Figure 2. Flow chart for the production of bamaranut – Millet yoghurt analogues

347 = 100% bambara nut without cow milk.
411 = 100% bambara nut with cow milk.
459 = 90% bambara:10% millet.
505 = 80% bambara : 20% millet.
619 = 70% bambara:30% millet
677 = 50% bambara:50%millet.
702= commercial yoghurt cow milk yoghurt

Figure 3. Display of youghurt samples

Preparation of Yoghurt Samples

Yoghurt analogues were made from the food formulations as shown in Figure 2. Five hundred millilitres of prepared milk containing various proportions of Bambaranut and Millet milk were each enriched with 6% sucrose, 5% banana flavouring, and 4% premium whole milk powder and thoroughly blended in a blender. The resulting mixture was sterilised in an autoclave for 15 minutes at 121°C. The sterilised milk was chilled to roughly 40-45°C using a fan. A sterile spoon was used to add 1% of the mixed starter culture (*Streptococcus thermophilus* and *Lactobacillus bulgaricus*), which was then incubated at 40°C for 12 hours. At the completion of the incubation, the mixture was placed in the refrigerator for 3 hours to cease fermentation. The resultant yoghurt analogues products were pasteurized and filled into clean PET bottles, corked and stored from where samples are taken for subsequent analyses.

Analyses

Proximate Composition Analysis of Samples

The ash, crude protein, crude fat, and moisture content of the samples were determined by using standard methods of AOAC (2012). Carbohydrate was determined by difference:

$$Carbohydrate = 100 - (\%\ Moisture, Protein, Fibre, Fat, and\ Ash)$$

The caloric values of the samples will be determined by summing the multiplied values for crude protein, crude fat, and carbohydrate by 4kcal, 9kcal, and 4kcal respectively.

Physio-Chemical Analysis

Syneresis: To determine susceptibility to syneresis (STS), the yoghurt sample (20 mL) was placed on a Whatman No 1 filter paper on top of a funnel and the yogurt was filtered under vacuum for 10 min. The liquid that passed through the filter paper was collected and recorded. The index of syneresis was calculated from the formula:

$$STS\ (\%) = \frac{V1}{V2} \times 100$$

V_1 = Volume of whey collected after drainage

V_2 = Volume of yoghurt sample

Viscosity: A Brookfield viscometer [Brookfield Model RVD E230, USA] with spindle number 6 was used to measure the apparent viscosity at 50 rpm at 25°C.

pH: The pH was determined by the method described by AOAC, (2012) using pH meter. About 2.0 mL of the sample was homogenized in 20 mL of de-ionized water in a beaker. The pH meter (Hanna HI-98128) was standardized using a buffer solution of pH 4.01 and 9.20. The electrode was rinsed with de-ionized water and dipped into the homogenate allowing sufficient time for stabilization before taking the reading.

Total Titratable Acidity (TTA): Ten grams of sample is dissolved in 100mL distilled water contained in a beaker. Then 10mL aliquots are titrated against 0.1M NaOH in the presence of phenolphthalein indicator to a clear endpoint. Titratable acidity is then calculated as given below

$$Lactic\ Acid\ (\%) = \ ml\ NaOH\ \times 0.9$$

Table 1: Recipe Used in Preparing Yoghurt Analogue

Ingredients	Quantity
Bambaranut/Millet Milk	300 mL
Sucrose	18 g
Flavorant (Banana Flavour)	15 mL
Powdered Cow Milk	12 mL
Starter Culture	3g

Table 2: Blend Formulation (%)

Samples	Bambaranut Milk	Millet Milk
A	100	-
B*	100	-
C*	90	10
D*	80	20
E*	70	30
F*	50	50
R	Commercial cow milk	Yoghurt

* Inclusive of 4% (w/v) of powdered cow milk

Total Solids: The yoghurt slurry contains some solid particles that tend to separate on standing based on their density. Three gram of the yogurt was weighed in a round-bottomed metal dish with its weight noted. The dish with the sample was placed in a boiling water bath for 30 min. The dish with the sample was placed in an oven at 100ºC for 2.5 hrs. The dish is cooled in a desiccator for 30 min and weighed. The total solids are calculated as shown:

$$\%TS = 100 - \%\ Moisture\ content$$

Microbiological Analyses

The total viable count of the yoghurt analogues was determined using the pour plate technique. (0.1 mL) of the appropriate dilution was placed on nutrient agar plates. The plates were incubated at 35ºC for 48 h and colony-forming units per g sample (cfu/g) were estimated. For mold and yeast count; the above procedure was repeated using potato dextrose agar and incubation was done at 25ºC for 72 h.

Consumer Acceptability Test

The manufactured yoghurt samples were delivered to 35-member untrained consumers made up of personnel and students from the University of Agriculture, Makurdi, Benue State, who are experienced with yoghurt consumption. On a nine-point hedonic scale, samples were scored for colour/appearance, flavour/aroma, viscosity/consistency, taste/mouthfeel, sourness, and overall acceptability, with 9 indicating extreme acceptance and 1 indicating severe dissatisfaction as shown in the table below. To unify the conditions of the evaluation, all samples were prepared in disposable plastic cups coded with a three-digit number, evaluated by each panelist in a monadic order, following a balanced-incomplete box design (Stone *et al.,* 2020). The samples were served in three sessions consisting of 3–4 samples for each round and served in random order to each panelist. The samples' identities were kept hidden. During the test, the panelists were asked to pause between the sample and cleanse their palates with prepared tap water at room temperature (Samakradhamrongthai *et al.,* 2021). The evaluation was performed in individual booths under white light at the Sensory Evaluation and Consumer Testing Unit (Department of Food Science and Technology, College of Food Technology and Human Ecology, University of Agriculture, Makurdi, Nigeria).

9-Point Hedonic Scale	
Dislike Extremely	1.0
Dislike Very Much	2.0
Dislike Moderately	3.0
Dislike Slightly	4.0
Neither Like nor Dislike	5.0
Like Slightly	6.0
Like Moderately	7.0
Like Very Much	8.0
Like Extremely	9.0

Statistical Analysis

Data were subjected to analysis of variance (ANOVA) by using SPSS Statistics for Windows,

Version 23.0 (IBM SPSS Statistics for Windows, Version 23.0. Armonk, NY: IBM Corp). Where the ANOVA test indicated significant differences and treatment means were separated using Duncan's multiple range test at a 5% probability level.

Results and Discussion

As shown in table 3, the moisture content of 87.61% obtained for sample A (100% BM with no cow milk) is higher than other samples (83.99% for 100% BM with 4% cow milk, 83.19% for 90:10, 82.63% for 80:20, 81.84% for 70:30 and 78.26% for 50:50). This might be because millet starch in form of milk slurry thickened Bambaranut yoghurt (Olakunle, 2012). All of the samples showed a progressive decline in protein content with a significant difference. The augmenting effect of milk powder might explain the greater protein content of the yoghurt samples compared to sample A. The steady loss of protein with increased millet supplementation might be owing to the dilution impact of millet protein on Bambaranut protein, which is richer in protein. However, it is worth noting that the Bambaranut-Millet Yogurt's protein level is of good quality since it includes all of the essential amino acids. It has been demonstrated that by supplementing grain proteins with legume proteins, protein quality equivalent to or greater than that of animal origin may be produced. As a result, yoghurt analogues' protein compares favourably to the control (R) and is high in important amino acids because careful grain and legume selection and combination results in an essential amino acid pattern that is equivalent to or greater than the reference protein. Therefore, the comparative levels of protein in the samples are nutritionally significant in terms of the potential of these beverages to contribute to the increased protein intake of consumers. The protein obtained for sample A agreed with the findings of Kale *et al.* (2011) who reported protein content of date yoghurt was in the range of 3.45% to 3.47% and Gupta *et al.,* (2022) for commercially available dairy and plant-based yogurts.

The values obtained for ash content of the yoghurt samples had a range between 0.59-0.76% which is within the range of market yoghurt sold in Nigeria as reported by Olugbuyiro and Oseh (2011). There was a gradual increase ($p<0.05$) in the ash contents of the samples. The rise in ash content detected in the samples is attributable to the mineral levels generated by the inclusion of millet. The percentage fat content changed Sufficiently ($p<0.05$). The average fat content is 2.70 percent for the highest and 1.81 percent for the lowest. Yoghurt with a fat concentration of less than 0.5 percent is labelled as non-fat yoghurt, while yoghurt with a fat content of 0.5-2.0 percent before adding bulking ingredients is labelled as "low-fat yoghurt" whereas yoghurt with a fat level greater than 3.25 percent is labelled as fat yoghurt according USDA (2001) specifications. As a result, the samples' fat level was within the range of low-fat yoghurt. Gupta *et al.,* (2022) obtained similar results for commercially available dairy and plant-based yogurts. Samples C, D, E, F, and R had low-fat content, which may contribute to a longer shelf life by minimising the risk of rancidity. Higher fat levels found in samples A and B, which might be attributable to the samples being made wholly from Bambaranut-an oilseed, enhanced lipolytic enzyme activity that degraded fat to glycerol and fatty acids, and the release of certain non-lipid ether extractable materials generated by fermenting bacteria.

The fibre content of the yoghurt samples was measured in the range of 0.26-0.34. The role of fibre in human nutrition has been established, fibre containing food are known to stretch the inner walls of the colon, allowing the transit of waste, making them an efficient therapy for constipation; it lowers blood cholesterol levels and reduces the risk of several forms of cancer (Elinge *et al.,* 2012). As a result, the fibre content of Bambaranut-millet yoghurt analogues renders them nutritionally more important than the non-fibre reference sample R. As millet was added to samples C, D, E, and F, the carbohydrate content of these samples increased when compared to sample A. When compared to the content of certain traditional sources, the samples could not be regarded a prospective carbohydrate source (Elinge *et al.,* 2012). However, the yoghurt samples' low carbohydrate and fat content, along with their high fibre content, make them ideal for weight loss and preventing cardiovascular disease. As a result, making yoghurt analogues from grain blends can enhance the nutritional quality of Africa's young population, boost farm gate prices for small farmers, and encourage the cultivation of more grains and legumes in the Global South that can be utilised as yoghurt inputs (Shimelis, 2019).

The result of the physiochemical properties of the various yoghurt samples is presented in Table 4. Milk's nutritional and economic value is directly proportional to its soluble solids concentration. The higher the soluble solids concentration, the higher the nutritious value and the more milk products may be produced. Higher levels of suspended particles caused by particles in bambaranut-millet milk than in bambaranut milk might explain the higher solid values obtained for samples B, C, D, E, and F. The result of this study is in agreement with that of Olakunle (2012), however, the higher value recorded here could be attributed to the effect of powdered milk addition and processing method. All of the designed yoghurt samples had a total solid content that was comparable to commercial yoghurt with samples D, E, and F being higher. These results are in agreement with Eke *et al.* (2013) who observed that the total solid of yoghurt-like product produced from baobab fruit pulp emulsion and powdered milk ranged from 9.24 to 16.58. In another study by [20], the probiotic yogurt from soymilk was shown to be comparable to the standard cow milk yoghurt in terms of physico-chemical attributes.

The pH values in all samples reduced significantly ($P \leq 0.05$), according to statistical analysis. The greatest average pH was 4.64% in Sample A, while the lowest average pH was 3.85% in Sample F. The Bambaranut-millet yoghurts, except for A and R, were more acidic, with a mean pH range of 4.01-3.85%. Because A (4.63) does contain powdered milk, the pH difference between it and other samples might be attributed to the composition and production process and the acidifying action of lactic acid generated during fermentation. This suggests that the rate of acid generation by the starter culture was affected by the addition of millet milk. Despite this, according to FDA standards for yoghurt pH (4.6 or below), the pH findings for yoghurt are within specifications and within the range of yoghurt marketed in Nigeria (Olugbuyiro and Oseh, 2011). The result of this study compared favourably with that of Kolapo and Olubamiwa (2012) Soy-coconut milk-based yoghurt. Due to the predicted effects of poor storage conditions, such as high temperatures in some tropical zones, which might impact the acidity of yoghurt, the pH values of the samples were adequately justified and appropriate for yoghurt marketed in the tropics (Olugbuyiro and Oseh, 2011). The findings regarding the pH of sample A are also in agreement with the findings of Sengupta *et al*. (2013) who found the average pH of whole soy-yoghurt was 4.5. The pH obtained for bambaranut and bambaranut-millet yoghurt is similar to values reported for soy and soy-corn yoghurt (Olakunle, 2012) which range between 4.50 and 4.00. The formation of lactic acid by *Lactobacillus* and other lactic acid bacteria causes the variation in the rate of pH reduction during yoghurt manufacture. Amakoromo *et al,* (2012) reported that the decrease in pH from 5.1 to 4.2 for African Yam Beans yoghurt from whole seeds is a reflection of the souring activity of lactic acid produced during fermentation. In addition to having a fatal and destructive impact on bacteria and inhibiting bacterial multiplication, the formation of lactic acid following fermentation lowers pH, halting any further development of pathogens and other hazardous microbes (Jayeola *et al.,* 2010). Also, each lactic acid starter culture has its own lactose absorption properties and acidification capacities, the pH of yoghurt varies. Furthermore, the addition of 4 percent milk powder to the yoghurt matrix may have buffered the yoghurt matrix, delaying the pH reduction in the samples. Also, Proteins in milk powder are known to act as buffers in food systems because of their ability to release or absorb free hydrogen atoms.

Statistical analysis revealed that during fermentation, titratable acidity increased considerably ($P \leq 0.05$) in all samples (Table 4). The acidity of milk rises with temperature, partially due to changes in the buffering capacity of milk salts and carbon dioxide release during heating. The heat breakdown of lactose into organic acids might be the cause of this rise in acidity. Furthermore, overall acidity increased ($p \leq 0.05$) as the pH decreased ($p \leq 0.05$) as a function of fermentation duration. This may be attributed to the development of lactic acid in the yoghurt (Sengupta *et al.,* 2013). The starter culture utilised and the composition of the premix used for fermentation influenced the development of acidity in the various yoghurt analogues produced. The higher titratable acids obtained for Samples D, E, and F (0.23, 0.25, and 0.34 percent, respectively) compared to Sample A (0.21 percent) might be due to the more acidic composition of the millet protein (Olakunle, 2012). However as titratable acidity increased, the pH decreased as a function of fermentation time. The capacity of the microbes to ferment the available carbohydrates determines how much acid is produced in the medium. Furthermore, the total acidity significantly increased ($p < 0.05$) and pH significantly decreased ($p < 0.05$) which may be attributed to the formation of lactic acid in the Yogurt (Sengupta *et al*., 2013). There was no significant difference ($p \geq 0.05$) in the titratable acidity (TA) for most of the yoghurts prepared. This could be attributed to the Millet and powdered milk being rich protein sources, they exert some buffering action stabilizing the acidity of yoghurt systems.

At 25 °C, the viscosity of experimental yoghurts ranged from 250 to 784 cP (Table 4) which compared favourably with that of Zanhi and Jideani (2012). There was a constant pattern of increased viscosity with increasing millet addition rate except for Sample A, which had no milk powder or millet milk. This related to the finding in Total Soluble Solids that TSS increased as the rate of millet milk addition increased. Sengupta *et al* (2013) reported that Soy Yogurt is non-Newtonian and its viscosity is affected by the presence of solids in the suspension of the fat phase. The higher the solids concentration in the yoghurt mixture, the higher the viscosity and consistency of the finished product. The consistency indices of the non- cow's milk yoghurt formulations (Sample A) were typically low (250 cp). Because cow's milk has a larger total solids content than plant milk, increasing the cow's milk content should improve the consistency and viscosity of dairy products (Kpodo *et al.,* 2013). Yogurt manufacturers that wanted to generate yoghurt with higher viscosities and better consistency frequently added milk powder to the raw material to boost total solids and produce yoghurt with greater consistency and viscosity (Zanhi and Jideani, 2012). In comparison to the commercial yoghurt R, the Bambaranut-millet yoghurts analogues, fared better. Similar consistency and viscosity were obtained by Gupta *et al.,* (2022) for commercially available dairy and plant-based yogurts.

The texture of yoghurts or fresh fermented goods is described and measured by rheology, which includes terminology like viscosity and gel hardness, as well as syneresis, which refers to the yogurt's tendency to whey-off during storage. Syneresis is a common quality problem in yoghurt production. The rheology and sensitivity to syneresis of fermented milk products are important because they both have a significant influence on customer perceptions of the finished product's quality. Indeed, variations in yoghurt quality can result in a product with a too thin watery or too thick gooey texture, as well as a product with a high free whey content. Importantly, milk composition, processing techniques, and the inclusion of hydrocolloids all have an impact on yoghurt rheology and syneresis. As a result, syneresis percentages ranged from 40.28 to 18.90 percent. This is within the range reported by Oyeniyi *et al.*

(2014) for flavoured soy-yoghurt and Kale *et al.* (2011) for date yoghurt. Chacko *et al*. (2010) reported an average of 52.6% syneresis in soy-yoghurt fortified with 1.104% calcium sulphate. Therefore, in this study, the percentage synereses reported are considered satisfactory. The lower values obtained in the current experiment is attributed to the use of millet starch and full-cream powdered milk. Millet starch used in this current study caused the free water molecules within the yoghurt matrix to be better absorbed. Commercial yoghurt (sample R) exhibited the lowest levels of syneresis, whereas yoghurt sample A had the greatest amounts (18.90% and 40.28%), respectively. Commercial yogurt's (Sample R) low degree of syneresis may be related to the use of stabilisers and standardised milk in its production compared to other yoghurt counterparts. The microstructure of yogurt consists of chains and clusters of casein molecules and the susceptibility of syneresis is closely related to the space between casein clusters. In yoghurt produced with the same amount of Bambaranut-millet milk (Sample F) syneresis was reduced. Therefore, adding millet milk to the substrate bridged the gaps between casein clusters of Bambaranut-milk gel and reinforced the gel network. Similar reduction of syneresis was reported by Aydar *et al.,* (2021) for plant-based yoghurt produced from Jerusalem artichoke and almond milk.

Table 5 shows the microbial communities of the yoghurt samples. The completed product was microbiologically evaluated for the survival of starting microbes as well as the presence of undesirable spoiling and pathogenic organisms. For coliforms, there was no count. A maximum of 10.0 CFU/g coliforms is permitted in yoghurt, according to Turkish Standard Institute (TSI330) and USDA (2001). Coliforms in yoghurt are commonly thought to be a result of direct faecal contamination. The lack of coliforms indicates that all samples were free of faecal contamination; hence, the yoghurt samples' microbiological status corresponded to the accepted standard. The quantity of aerobic mesophilic bacteria was minor, with counts ranging from 6.62 to 6.20 log CFU/g, which is within an acceptable yoghurt level. Yeast and mould were found in modest amounts in all of the samples. Toxic metabolites (mycotoxin, e.g. aflatoxin) can be produced by yeast and moulds at concentrations more than 10.0 CFU/g, which can cause food poisoning and liver cancer in humans. The observed fungal count might be attributed to microorganisms present in the inoculums as a result of irresponsible handling during manufacture and analysis. The USDA (2001) advised that the amount of yeast and mould in yoghurt be limited to 50 per gramme. Microbial requirements are regulated differently in different countries (Eke and Elechi, 2020). TVC in the range of 0-10^3 CFU/g, 10^4-10^5 CFU/g, and >10^6 CFU/g is considered acceptable, slightly acceptable (tolerable), and unsatisfactory by the ICMSF. Based on the microbiological standards used, this study's findings are deemed good.

Sensory quality evaluations of yoghurt products were conducted to better understand the product qualities that influence customer preferences, allowing processors to alter product attributes to appeal to specific target consumers and to closely monitor product quality (Shimelis, 2019). The yoghurts' observable sensory characteristics are listed in Table 6. The USDA (2001) specifies a firm, pudding-like consistency with a smooth, uniform texture and a natural colour range of light white to off-white with a smooth, velvety appearance for all samples of yoghurts. The panellists deemed the appearance and consistency of all samples to be satisfactory because the mean values of the samples differed insignificantly. This is in agreement with Gupta *et al.,* (2022) who reported that sensory acceptability is affected by compositional and quality factors, such as protein source, texture, fat, sugar, or the form of the yogurt. Consumer liking, in general, positively correlates with the viscosity and smoothness of the product (Janiaski *et al.,* 2016). The results revealed that the flavour, taste, and sourness of the designed yoghurt samples differed significantly ($p<0.05$) from the control samples (i.e. commercial yoghurt R). This might be because commercial yoghurt has a lot of flavours and sweeteners. Overall acceptance of the formulated yoghurt samples was not substantially different ($p>0.05$), while overall acceptance of the commercial yoghurt was significantly greater ($p<0.05$) than that of the formulated yoghurt samples. This is contrary to the previous research that showed that some plant-based alternatives can be similarly liked to dairy yogurts in their mouthfeel profile (Greis *et al.,* 2020). For example, a recent study of commercial plant-based yogurts (soy, coconut, cashew, almond, and hemp) by Grasso *et al.,* (2020) found that soy and coconut yogurts were identical to dairy yogurts in terms of sensory acceptability and texture.

Table 3. Proximate Composition of Babaranut-Millet Yoghurt Analogues (%)

Parameter	A	B	C	D	E	F	R
Moisture	87.61±0.22[a]	83.99±0.03[b]	83.19±0.02[bc]	82.63±0.03[cd]	81.84±0.09[d]	78.26±0.16[e]	83.00±0.01[bc]
Crude protein	3.68±0.06[e]	6.65±0.02[a]	6.85±0.02[a]	6.65±0.04[a]	6.35±0.17[b]	5.67±0.03[d]	6.65±0.01[a]
Ash	0.59±0.02[f]	0.64±0.03[e]	0.66±0.01[d]	0.68±0.01[c]	0.70±0.03[b]	0.76±0.03[a]	0.71±0.03[b]
Crude Fat	2.26±0.06[e]	2.70±0.10[a]	2.56±0.03[b]	2.43±0.05[c]	2.33±0.05[d]	2.09±0.02[f]	1.81±0.02[g]
Crude Fibre	0.26±0.01[d]	0.26±0.01[d]	0.27±0.00[cd]	0.28±0.01[bc]	0.29±0.01[b]	0.34±0.00[a]	-
Carbohydrates	5.60±0.26[e]	5.87±0.05[e]	6.46±0.05[de]	7.32±0.12[cd]	8.49±0.12[b]	12.88±0.11[a]	7.83±0.04[bc]
Calories (Kcal)	57.50±0.89[f]	73.48±0.24[e]	76.31±0.26[d]	77.73±0.13[c]	80.29±0.30[b]	92.94±0.25[a]	74.21±0.09[e]

Values are means of triplicate determinations. Mean values with same superscript in a row are not significantly different ($p \geq 0.05$) Mean values having different superscript letter in a row are significantly different ($p \leq 0.05$).

KEY:
A = 100% Bambaranut milk (without Milk);
B = 100% Bambaranut milk (with Milk);
Bambaranut Milk**:** Millet Milk. C = 90:10; D = 80:20 E = 70:30; F = 50:50
R = Commercial Yoghurt
LSD = Least Significant Difference

Table 4. Physiochemical Properties of Babaranut-Millet Yoghurt Analogues (%)

Parameter	A	B	C	D	E	F	R
PH	4.64±0.10[a]	4.10±0.20[bc]	4.06±0.06[bc]	4.05±0.03[bc]	4.01±0.61[bc]	3.85±0.09[c]	4.37±0.12[ab]
TTA	0.21±0.02[d]	0.65±0.09[a]	0.21±0.08[d]	0.23±0.02[d]	0.25±0.06[d]	0.30±0.01[c]	0.90±0.10[a]
TSS	12.39±0.22[e]	16.28±0.82[d]	16.81±0.02[cd]	17.37±0.03[c]	18.16±0.09[b]	21.74±0.16[a]	17.00±0.02[c]
Syneresis	40.28±1.16[a]	38.22±1.54[b]	28.77±1.30[bc]	26.69±1.40[d]	24.69±0.60[e]	20.63±0.70[f]	18.90±0.70[g]
Viscosity (c_p)	250±29[f]	344±26[e]	385±12[d]	480±33[bc]	595±30[b]	784±32[a]	467±23[c]

Values are means of triplicate determinations. Mean values with same superscript in a row are not significantly different ($p \geq 0.05$) Mean values having different superscript letter in a row are significantly different ($p \leq 0.05$).

KEY:
A = 100% Bambaranut milk (without Milk);
B = 100% Bambaranut milk (with Milk); R = commercial yoghurt.
Bambaranut Milk: Millet Milk. C = 90:10; D = 80:20 E = 70:30; F = 50:50
LSD = Least Significant Difference.
TTA = Total Titrable Acidity
TSS = Total Soluble Solid

Table 5. Microbiological Qualities of Babaranut-Millet Yoghurt Analogues ($\log_{10}$ Cfu mL^{-1})

Types of Microbes	A	B	C	D	E	F	R
Total Plate Count	6.32±0.04[e]	6.39±0.02[d]	6.43±0.03[c]	6.46±0.02[c]	6.55±0.05[b]	6.62±0.06[a]	6.20±0.02[f]
Total Yeast and Mold count	1.94±0.03[d]	2.03±0.12[c]	2.23±0.02[a]	2.21±0.03[a]	2.17±0.02[b]	2.09±0.03[c]	1.30±0.02[e]
Coliform	ND	ND	ND	ND	ND	ND	ND

Values with same superscript in a row are not significantly different ($p \geq 0.05$) while values having different superscript letter in a row are significantly different ($p \leq 0.05$).

KEY:
A = 100% Bambaranut milk (without Milk);
B = 100% Bambaranut milk (with Milk);
Bambaranut Milk **:** Millet Milk. C = 90:10; D = 80:20 E = 70:30; F = 50:50
R = Commercial Yoghurt
ND = Not detected

Table 6. Mean Scores of Sensory for Babaranut-Millet Yoghurt Analogues

Parameter	A	B	C	D	E	F	R
Appearance	6.90^{b}	7.37^{ab}	7.37^{ab}	7.35^{ab}	6.90^{b}	6.95^{b}	8.15^{a}
Mouth Feel	6.20^{b}	6.40^{b}	6.20^{b}	6.40^{b}	6.35^{b}	6.70^{b}	8.45^{a}
Sourness	5.40^{b}	6.30^{b}	5.75^{b}	6.40^{b}	6.30^{b}	6.40^{b}	7.90^{a}
Flavour	6.10^{c}	6.20^{c}	6.85^{bc}	7.05^{b}	6.85^{b}	7.05^{b}	8.30^{a}
Consistency	6.30^{c}	6.60^{c}	6.65^{bc}	6.75^{abc}	6.85^{abc}	7.30^{a}	7.20^{ab}
Overall Acceptability	6.60^{b}	6.65^{b}	6.55^{b}	7.13^{b}	7.05^{b}	7.25^{b}	8.55^{a}

Values are means of triplicate determinations. Mean values with same superscript in a row are not significantly different ($p \geq 0.05$) Mean values having different superscript letter in a row are significantly different ($p \leq 0.05$).
KEY:
A = 100% Bambaranut milk (without Milk);
B = 100% Bambaranut milk (with Milk);
Bambaranut Milk **:** Millet Milk. C = 90:10; D = 80:20 E = 70:30; F = 50:50
R = Commercial Yoghurt
LSD = Least Significant Difference

The disparity between the overall acceptance of formulated yoghurt samples and that of commercial yoghurt samples in this study could be due to the panellists' familiarity with cow's milk yoghurt versus the reformulated products (Ijarotimi and Keshinro, 2012); additionally, the lower consumption of plant-based yogurts may be due to their different textural properties, compared to dairy yogurts (Gupta *et al.,* 2022). The cross-linking of casein proteins is an intrinsic process in the formation of dairy yogurt gels, caused by heating and acidification by bacterial cultures, which contributes to the texture of these products (Yang *et al.,* 2021). Also, astringency in plant-based milk has been scientifically reported as the most important limiting factor in consumer acceptance of plant-based milk products. The native ‘grassy’ or ‘beany’ flavour in soy caused by hexanal and other aldehydic lipid oxidation (lipoxygenase, or LOX-catalysed) products, however, faces significant acceptability issues in some countries and is perceived as an off-flavour in dairy analogues (Pua *et al.,* 2022; Zhu *et al.,* 2020; Short *et al.,* 2021). However, the overall acceptability scores of the yoghurt’s analogues were in the range of 6.55 to 7.25 on the 9-point hedonic scale indicating general acceptance of the samples by the consumers. Therefore, from this study, it is possible that the yoghurt analogues improved sensory quality may be attributable to the blending of raw materials and processing procedure, namely the heating and sterilising of the products and fermentation. Heating tends to deactivate the leptoxygenase enzyme that gives vegetable milk its beany taste. While studies showed that blending raw materials could result in improved hedonistic ratings, the mechanism behind this could be the masking of off-flavours, e.g., the beany note in legumes (Short *et al.,* 2021). Blending raw materials is also of great nutritional interest, especially for the amino acid profile of the ingredients as plant materials, unlike bovine dairy, have lesser amino acids and comparatively poorer digestibility (Cichonska *et al.,* 2022). Hence, the purposeful blending of plant materials could help to achieve a more complete amino acid profile without fortification (Pua *et al.,* 2022).

Conclusion

The possibility of making suitable yoghurt analogues from Bambaraunt and millet mixes was studied. To determine the qualitative qualities of the yoghurts and to investigate the market potential, nutritional, physico-chemical, microbiological, and sensory analyses were performed. The chemical composition of the bambaranut-millet yoghurt analogue samples and the commercial yoghurt manufactured from cow's milk was found to be almost identical in this investigation. The samples were clear of faecal contamination microbiologically. The sensory evaluation of the samples revealed that yoghurts made from bambaranut-millet milk differed significantly ($p<0.05$) in some of the sensory attributes from those made from cow's milk, especially for overall acceptability, taste and flavour. However, all the yoghurt analogues received overall mean likeable and acceptable score of > 6.00. Thus, suitable yoghurt analogues may be created with bambaranut and millet mixes with varied replacements up to 50% to make acceptable and economical yoghurt analogues. Diversification of yoghurt products should be prioritised for continued business growth and to meet unmet customer demand for safe and healthy goods. In summary, blending different raw materials could result in an ingredient with improved properties for subsequent formulation or fermentation into a plant-based dairy analogue with improved sensorial and nutritional properties. Therefore, the findings of this study demonstrated that yoghurt including grains and legumes can be processed using existing technologies, has a high nutritional content, and the processing technique produces microbiologically safe end products. More research is needed to discover remedies to prevent the gelatinization problem in millet milk production. Furthermore, research into utilising Bambaranut-millet milk mixes in the same way as cow and buffalo milk for cheese, curd manufacturing, and ice cream should be conducted. Shelf life, storage stability, and protein digestibility of yoghurt analogue products should all be researched.

Compliance with Ethical Standards

Conflict of interests: The author declares that for this article they have no actual, potential, or perceived conflict of interest.

Ethics committee approval: Authors declare that this study does not include any experiments with human or animal subjects.

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