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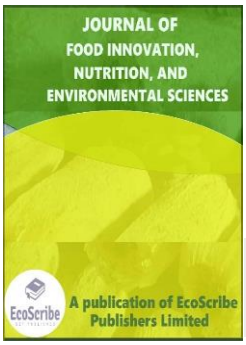

ORIGINAL ARTICLE

Utilization of Bambara (*Vigna subterranea*) Milk in Cheese Production: An Evaluation of the Product Quality and Sensory Attributes

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ABSTRACT

The increasing global demand for plant-based protein meals, driven by concerns about carbon footprints and health-related challenges associated with animal protein consumption, has spurred scientific exploration into plant-based milk alternatives. Bambara (*Vigna subterranea*) milk, an underutilized resource, in cheese production, was the focus of this study. Cow milk was substituted with varying percentages (0%, 25%, 50%, 75%, and 100%) of Bambara milk for cheese production. The resulting products underwent analysis for proximate contents, microbial stability, and gas formation during three weeks of refrigerated storage at 6±2°C. Sensory parameters, including texture, flavor, mouthfeel, color, and general acceptability, were assessed using a nine-point hedonic scale (where 1 = dislike extremely, 5 = neither like nor dislike, and 9 = like extremely). The results were analyzed using one-way ANOVA at a significance level of $P \leq 0.05$.

Proximate analysis revealed significant increases ($P \leq 0.05$) in the percentage of ash, crude fiber, and carbohydrate content (3.75-4.38, 0.85-2.16, and 28.1-48.37, respectively) due to Bambara milk substitution. Protein and fat contents experienced significant reductions. Throughout refrigerated storage at 6±2°C, substituted products demonstrated substantial stability in microbial load and gas production compared to unsubstituted samples. Gas formation in the substituted products occurred only after the third week. Sensory evaluation indicated significant improvements ($P \leq 0.05$) in acceptability for color (6.55-7.26), mouthfeel (6.25-7.70), and texture (6.46-6.85) resulting from Bambara milk substitution. The study suggested that Incorporating Bambara milk improved the quality of cheese products, potentially expanding its use as a plant protein.

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1. Introduction

The increased global consumption of plant-based foods, particularly plant-based milk products, reflects growing environmental concerns, ethical considerations, and the desire for better health results. plant-based milk products provide a distinctive role in plant-based diets, allowing a variety of alternatives derived from legumes, nuts, seeds, cereals, and pseudo-cereals (Hidalgo-Fuentes *et al*., 2024). Food systems now account for roughly one-third of global greenhouse gas (GHG) emissions, with animal-based diets accounting for two-thirds of these emissions (Xu *et al*., 2021). According to projections, if no shift toward alternative protein sources occurs by 2050, economic and population development will result in a 21% increase in per capita meat consumption and a 63% increase in overall meat consumption and associated GHG emissions.This significant environmental impact, combined with the rising global prevalence of diet-related noncommunicable diseases (NCDs) like obesity, type II diabetes, and cardiovascular disease, highlights the critical need for dietary changes that benefit both human and environmental health (Revell, 2015).

Cheese, a fermented dairy product, is traditionally derived from animal milk. However, the rising popularity of plant-based alternatives, such as soy milk, oat milk, and almond milk, has prompted innovative research in the field. Cheese serves as a valuable source of protein, fat, and essential nutrients, making it a crucial component in various diets (Oladipo and Jadesimi, 2012). Cheese, a semi-solid fermented dairy product, is crafted from milk coagulated by various agents such as enzymes (e.g., rennin), microorganisms, acids (e.g., lactic acid), heat, or other components (Nazim et al., 2013). Milk, sourced from animals like cows, is processed in whole, part-skim, skim, or cream forms, extracting the liquid portion while retaining the coagulum and milk solids (Balogun et al., 2016). Due to its rich content of protein, fat, and essential nutrients like calcium, iron, phosphorus, vitamins, and amino acids, cheese holds vital significance in the diets of both young and old individuals (Oladipo and Jadesimi, 2012). The challenge of maintaining adequate protein and fat levels in milk finds a resolution in the production of cheese. Through the breakdown of milk's components into smaller chunks, cheese becomes a storage-friendly form, addressing the goal of preserving perishable food by transforming it into a stable stored product. This not only enhances food variety but also contributes to the extended shelf life of dairy products (Hashim *et al.,* 2011)

Cheeses are classified into categories such as hard, soft, ripened, or unripen based on factors like moisture content and whether they undergo ripening facilitated by bacteria, mold, or not at all. Alternative methods involving a combination of heat and acid as coagulating agents can also yield different types of cheese. An example of such cheese is Queso Blanco, a prominent white cheese widely consumed in many Latin American households, produced through this specific method (Hashim *et al.,* 2011). For the purpose of manufacturing cheese, acid coagulation can be accomplished by adding a starter culture, high-acid whey, or an organic acid such as citric, acetic, or tartaric acid. As in the case of cottage cheese, pH lowering can take place at room temperature without adding acid to the milk. On the other hand, at temperatures above 80°C, the pH can be decreased by adding acid, as is the case with Italian ricotta. Cottage cheese requires a 4.6 pH level in the milk. Lowering the pH to 5.6 is adequate for Ricotta (above 80°C) (Cross and Overby, 1988). When milk is acidified, calcium and phosphorus are gradually eliminated from the milk until it reaches the pH 4.6 isoelectric point, at which point the casein is completely devoid of salts and coagulates (DeMan, 1999).

Unlike certain countries in Asia and South America, cheese does not play a prominent role in the culinary culture of Africa. The primary reason for this is the cost of milk derived from animal sources, leading to a decline in its utilization for cheese production. To address protein-energy malnutrition in underdeveloped nations, there is an ongoing quest for alternative protein sources (Al-Tamini, 2016). This exploration has led to research endeavors dedicated to uncovering alternative protein sources, with a primary focus on plant-based options. The motivation behind this shift arises from concerns such as the prevalence of allergies to animal milk proteins, the nutritional value of alternatives, and the reduction of carbon footprints. Legumes, including Bambara milk, emerge as a viable substitute for protein (Siddhuraju *et al.,* 1996). Apart from being a cost-effective food option, Bambara milk offers nutritional and health advantages due to its absence of cholesterol, lactose, and minimal levels of saturated fatty acids (Nazim *et al.,* 2013). While cheese production is well-established using traditional animal milk sources, this study explores the utilization of Bambara (*Vigna subterranea*) Milk, derived from the Bambara groundnut, in cheese production. The Bambara groundnut, a neglected indigenous African crop (Atiku *et al.,* 2004), holds nutritional significance, offering carbohydrates, protein, and oil (Goli, 1997). Despite its economic importance, there has been limited industrial use in West Africa, with research predominantly focusing on agronomic aspects rather than processing (Akani *et al*., 2000).

Despite its valuable traits, the underutilized Bambara groundnut crops are excluded from classification as commodities in the global trading system, primarily due to their low production rank, a factor that has led researchers to allocate limited attention to them (Halimi *et al.,* 2019). However, the Bambara groundnut showcases notable characteristics, including drought tolerance, favorable nutritional composition, and the capability to fix nitrogen from the atmosphere. These unique qualities position the Bambara groundnut as a promising crop for safeguarding future food and dietary needs, especially in the face of challenges posed by climate change. Its natural resilience to adverse conditions, along with its ability to thrive in soils unsuitable for cultivating drought-sensitive species like peanuts, underscores

its importance as a vital component of a more diverse and resilient crop landscape (Thammarat, 2015).

Akani *et al.* (2000) and Atiku *et al.* (2004) highlight the versatile applications of Bambara groundnut seeds, emphasizing their suitability for producing baby food, human food, industrial products, and animal feed. Notably, Brough *et al*. (1993) found that milk derived from Bambara nuts exhibited a flavor preference over milk sourced from cowpea, pigeon pea, and soybean. Despite the economic significance of the crop, its substantial annual production in West Africa has not translated into discernible industrial utilization. Interestingly, the creation of a cheese-like substance has been explored using unconventional sources such as soymilk, melon milk, and coconut milk, as reported by Adejuyitan *et al.* (2014). The resulting product, resembling cheese, was generally regarded as both palatable and healthful. The incorporation of cow milk and Bambara milk in cheese production emerges as a potential strategy to reduce dependence on animal milk.

One of the biggest obstacles to using Bambara nuts as a source of vegetable milk has been the lack of scientific attention and inconsistent findings. The objective of this study is to create a cheese substitute by combining different ratios of cow milk and Bambara milk. Specifically, the study aims to examine the impact of various levels of Bambara milk substitution on the Proximate, Microbial, and Sensory properties of the resulting cheese-like product. This exploration not only addresses the growing demand for plant-based alternatives but also seeks to overcome challenges related to the underutilization of Bambara nuts in vegetable milk production.

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2. Materials and Methods

2.1 Materials

Bambara seeds were procured from Ubani market, and full cream powdered milk along with lime were obtained from Isi-gate market in Umuahia. The chemicals utilized in the process were of food and analytical grade, sourced from the Department of Food Science and Technology at Michael Okpara University of Agriculture, Umudike, Abia State.

Production of Bambara milk

The procedure for extracting Bambara milk was adapted from the method outlined by Igyor *et al*. (2006) designed for the production of milk substitutes. The 'Nav Red' variety of Bambara nuts was manually sorted, cleaned with potable water, and allowed a 24-hour soaking period in water at a ratio of 4 (parts of water) : 1 (part of Bambara seed) (w/v). During soaking, the water was changed every 6 hours. After the 24-hour soaking period, the seed coat was removed by rubbing the nuts between the palms, and the husks were separated from the water. Subsequently, the nuts were wet-grinded using a locally fabricated hammer mill. The milk was then extracted from the resulting Bambara mash through cheesecloth, triple-filtration was done, and finally stored in a sterile white container for future use.

Extraction of Lemon Juice

Lemons were thoroughly washed with water to remove any dirt and debris. The lemons were then cut in half, and the juice was extracted manually by pressing the halves. The extracted juice was filtered using cheese cloth to remove any solid particles (Heiru,2021).

2.2 Cheese Production using Lemon Juice

The Percentage Composition of cheese samples used were presented in Table 1. The method described in Heiru (2021) was used for the production of cheese using lemon juice with slight modification. Fresh whole milk was used for cheese production. The milk was heated to 85°C to eliminate most bacteria and increase cheese yield by precipitating whey proteins. Subsequently, 20mL of lemon juice was added per liter of milk. The lemon juice was added carefully while stirring the milk, causing the curd to precipitate almost immediately. Stirring continued for about three minutes, after which the curd was allowed to settle for 15 minutes. The curd was separated from the whey by draining through a muslin cloth. Salt was added to the curd at a ratio of about 4 g per 100 g of curd and mixed thoroughly. The quantity of salt could be adjusted to suit consumer preferences. To enhance the appearance of the cheese, the curd was pressed overnight using metal weights placed on a wooden follower. The curd was cut into smaller sizes and transferred to a refrigerator for storage at 6±2°C for further analysis.

Table 1: Formulations of the cheese samples

Sample raw materials	B0	B1	B2	B3	B4
Cow milk (%)	100	75	50	52	-
Bambara milk (%)	-	25	50	75	100
Lime (ml)	20	20	20	20	20

Key: B0 (control): 100% Cow Milk; B1: 25% Bambara milk +75% Cow milk; B2: 50% Bambara Milk + 50% Cow milk; B3: 75% Bambara milk +25% Cow milk, B4: 100% Bambara milk.

2.3 Proximate analysis

The moisture content, crude protein, crude fat, crude fiber, ash, and total solids of the cheese samples were all determined using AOAC standard techniques (AOAC, 2000).

2.3.1 Ash content analysis

For each sample, a two-gram portion was carefully weighed into a silica crucible. The crucible, containing the sample, underwent heating in a Muffle Furnace for approximately 5-6 hours at a temperature of 500°C. Subsequently, the crucible was allowed to cool in a desiccator and was then re-weighed. It was heated again in the furnace for half an hour, cooled and weighed. The process was repeated till the weight became

constant (AOAC, 2000)

Ash content is calculated using the formular below:

$$\% \text{ Ash (dry basis)} = \frac{W3-W1}{W2-W1} * 100 \quad \text{- eqn. 1}$$

Where $W_{1=}$ weight of empty crucible
$W_{2=}$ Weight of crucible +sample before ashing
$W_{3=}$ Weight of crucible +Ash

2.3.2 Moisture content analysis

Two aluminum dishes each were washed thoroughly and dried in the oven, cooled inside the desiccators and weighed. Exactly 5g of the sample was weighed inside the pre-weighed aluminum dishes (in duplicate). The samples were dried in the oven at 80^0C for 2h and at 100 ^{o} C for another 4h of until a constant weight is achieved. The samples were cooled in the desiccators and the dry weight of sample and dish was taken. Analysis was done duplicate. The moisture content was calculated using as shown in equation 2.

$$\% \text{ moisture} = \frac{W2-W1}{Weight\ of sample} * 100 \quad \text{- eqn. 2}$$

Where:
$W_{1=}$ Initial weight of empty dish
$W_{2=}$ Final weight of empty dish + sample after drying.

2.3.3 Fat content determination

A 250 ml clean boiling flask was dried in the oven at 110°C for 30 minutes before being transferred into desiccators, then allowed to dry A labeled thimble was filled with exactly 2 g of sample. Weighed conical flasks were filled with 200 ml petroleum ether (boiling point 40-60°C). The extraction thimble was lightly plugged with cotton wool, and the Soxhlex apparatus was assembled and allowed to reflux for about 6 hours. The thimble was removed, and petroleum ether was collected in the top container of the setup and drained into a container for reuse when the flask was removed. It was removed and dried at 110°C for 1 hour, then cooled in the dedicator until it was almost free of petroleum ether, after which it was weighed (AOAC, 2000).

The fat content was calculated using the formula:

$$\% Fat = \frac{W3-W1}{W2-W1} * 100 \quad \text{- eqn.3}$$

Where: $W_{1=}$ weight of sample, $W_{2=}$ weight of flask, $W_{3=}$ weight of flask + fat

2.3.4 Crude protein analysis

A Kjeldahl flask was filled with exactly 2 g of a sample after being weighed, and Kjeldahl catalyst tablets were added. Exactly, 25 ml of concentrated Sulphuric acid and 5 glass beads were added (to avoid bumping during heating). The sample was heated in a fume cupboard, first slowly with periodic shaking, then more quickly until the solution took on a green color (the temperature of digestion was 420°C for 30 min). The sample was now cooled, and black particles that had accumulated at the flask's tip or middle were flushed away with distilled water. It was first gently cooked until the green color clears out, then it was allowed to cool. The digest was poured into a volumetric flask measuring 100 ml, and distilled water was added.

The Markham distillation apparatus was used to distill the proteins, and it was steam-heated for 15 minutes. Two drops of screened methyl red indicator and precisely 100 ml of boric acid were added to the condenser such that the tip was positioned just below the boric acid solution. The small funnel opening was used to pipette in exactly 5ml of the digest, which was then followed by 5ml of a 60% NaOH solution and distilled water. This was allowed to steam for around 5-7 minutes to collect enough NH_3SO_4, after which the conical flask was withdrawn and the nitrogen concentration was measured by titration against 0.0IN hydrochloric acid. The transition from purple to green marks the end of the titration process. Then the amount of NaOH used was noted (AOAC, 2000). The crude protein percentage was then calculated using the formula:

$$\text{Protein Content (\%)} = \frac{N\ content\ (\%) X\ Factor}{Sample\ mass} \quad \text{- eqn. 4}$$

Where:
N content (%) is the percentage of nitrogen present in the sample; Factor is the nitrogen-to-protein conversion factor (= 6.25); Sample mass is the mass of sample from which the protein content is being determined.

The N content (%) was calculated using the formula;

$$\text{N content (\%)} = \frac{V\ X\ N\ X\ 14.01}{M\ x\ 1000} \quad \text{- eqn. 5}$$

Where:
V = Volume (mL) of the titrant used to neutralize the distillate; N = Normality of the titrant acid (= 0.1N); M = mass of sample in grams; 1000 = conversion factor to adjust the units to percentage.

2.3.5 Crude fiber analysis

The hydrolysis method was used to analyze crude fiber. Two (2) grams of dried sample were weighed and placed it in a Three-neck Rounded Flask. Exactly 50 ml of H_2SO_4 solution was added and refluxed for 30 minutes while stirring with a magnetic stirrer at 400 rpm. After 30 minutes, 50 ml of 3.25% NaOH solution was added, followed by 30 minutes of reflux while stirring with a magnetic stirrer at 400 rpm. The hot sample was immediately filtered through filter paper that had been dried and weighed. The precipitate in the filter paper were washed with 10ml each of 1.25% H_2SO_4, hot water, and 96% ethanol.

2.3.6 Total carbohydrate estimation

The carbohydrate content was determined by difference (AOAC, 2000).

Total Carbohydrate (%) = [100 − % (Protein + Fat + Moisture + Ash + Fiber)] - *eqn. 6*

2.4 Gross energy value

The factors for protein (4 Kcal/g), fat (9 Kcal/g), and carbohydrate (4 Kcal/g) were used to estimate the gross energy values (Kcal/100 g samples) of the cheese samples (AOAC, 2000). The formula is:

Food energy = (%Crude protein×4) + (%Fat content×9) + (%Carbohydrate×4) - *eqn. 7*

2.5 Total solid content

This was obtained using the method described by Okon and Ojimelukwe (2017). A continuous oven temperature of 100°C was used to dry two (2) g of shredded cheese for three (3) hours until to a consistent weight. After drying, the sample's weight was calculated as a percentage of its original weight. The results were the mean of duplicates determination.

2.6 Sensory Evaluation

A 30-Man, Semi-trained sensory panelists comprising of regular cheese consumers who were students on industrial training and staff from Central Service Laboratory and Post-Harvest Unit of National Root Crops Research Institute Umudike were used. Consent forms were issued to them which they completed and signed before getting involved in the sensory evaluation. A 9-Point hedonic scale (9" like extremely, 5 "neither like nor dislike", 1 "dislike extremely") was used to rank level of acceptability (general acceptability, flavor, flavour, colour, texture, mouth feel and taste) of the cheese. Panelists were also asked to give any comment about the samples. The samples were presented to the panelists in a sterile container coded with 3 random number digits and served simultaneously. Necessary precautions were taken to prevent carry over flavor during tasting by ensuring that the panelists rinse their mouth with clean water after tasting each sample.

2.7 Microbiological analysis

The pour plate inoculation method described by the bacteriological analytical manual (BAM, 1992) and Compendium of methods for the microbiological examination of food (CMMEF, 1991) was adopted. Exactly 1g of cheese samples was serially diluted to 10^3 and poured into petri-dish containing 12ml of the molten nutrient agar (cooled to 45^0C) within 15min from the time of preparation of original dilution. The dilution was mixed with the media by gently clock wise, anti-clockwise, to and fro Process. This was allowed to set. At incubation, the dishes were inverted and kept at 35^0C for 48h. Following incubation, all colonies on two dishes containing 30-300 colonies were counted and recorded per dilution counted. The actual number in both plate of a dilution having 30-300 colony range was recorded as the result per dilution counted and calculated as: $\frac{\Sigma C}{(N1+0.1N2)D}\ (cfu)$ - *eqn. 8*

Where:
ΣC = Sum of colonies counted on all the dishes;
N1 = No of dishes retained in the first dilution
N2 = No of dishes retained in the second dilution
D = Dilution factor corresponding to the first dilution

2.8 Gas production analysis

The method of Silvetti *et al.* (2018) was used for the determination of gas production. The liquid culture was prepared using 10g of yeast extract in 20ml of distilled water. The liquid medium was dispensed into the vial (bottles) then Durham's tube was dropped into each vial. The vials were autoclaved for sterilization. One (1) gram of sample was extracted in 10ml of distilled water and the liquid was added to the vials containing the media. This was incubated at 37^0C for 18h. Gas formation in Durham's tube shows a positive test. The vials without gas formation were continued until gas production occurred.

2.9 Statistical Analysis

One-way analysis of variance (ANOVA) was used to statistically examine the data to see whether there were any significant differences between the samples with a p-value of 0.05. To identify where the differences occurred, Duncan's least significant difference (LSD) test was used. The Statistical Product and Service Solutions (IBM SPSS version 20.0) software were used for all of the analyses. The results were determined in triplicate**.**

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3. Results and Discussion

3.1 Proximate composition

The results of the proximate composition of cheese samples are detailed in Table2. The moisture content of the cheese samples ranged from 33.00% to 40.00%, with sample B0 (control) having the lowest moisture content and sample B4 having the highest. The differences were statistically significant ($P \leq 0.05$), attributed to the lower moisture level of reconstituted powdered milk. The incorporation of Bambara milk led to an increase in moisture content. A similar observation was reported by Balogun *et al.* (2016), who found lower moisture levels in pure cow milk compared to samples with increasing levels of coconut milk incorporation. This was attributed to the higher moisture content of the coconut milk used in the formulation. This increase can be controlled by regulating the water quantity added during extraction of plant milk to enhance the product's shelf-life. High moisture content in foods increases susceptibility to perishability (Ilodibia et al., 2014).

The ash content ranged from 3.75% to 4.38%, with the control (B0) having the lowest and B4 having the highest ash content. The ash content increased with an increase in the added level of Bambara milk, and the differences were statistically significant (P≤0.05). This increase indicates that cheese samples are rich in certain minerals. The higher ash content is consistent with the results reported by Balogun (2017) and Okorie and Adedekun (2013), suggesting that the addition of Bambara milk contributes to mineral richness in cheese.

The crude fiber content of the cheese analogue samples ranged from 0.85% to 2.16%, with sample B4 having the highest fiber content. This result aligns with the findings of Okorie and Adedekun (2013). Higher fiber content enhances the digestibility of the cheese (Dhillon et al., 2016).

For fat content, the control (B0) recorded the highest fat (14%), and as the percentage of added Bambara milk increased, there was a significant decrease in the fat content in the cheese products. The lower fat content of the Bambara milk cheese presents a nutritional advantage, indicating a reduction in cholesterol levels. This reduction is beneficial as a diet high in saturated fat can lead to elevated cholesterol levels, increasing the risk of heart attack or stroke. The differences among the samples were significant (P ≤ 0.05), and the lower fat content in Bambara milk cheese can be attributed to the lower fat level in Bambara milk itself.

The protein content in the sample blends ranged from 20.30% to 3.44%. Sample B4 had the lowest protein content, and B0 (control) had the highest. This decrease in protein content with an increase in Bambara milk level was statistically significant (P ≤ 0.05). It can be attributed to the higher digestibility of protein in cow milk compared to plant-based milk, as noted by Otunola *et al.* (2012) and Al-Tamini (2016). The difference in molecular structure between plant and animal proteins contributes to these variations (Jeske *et al*., 2018).

The carbohydrate content of the cheese ranged from 28.1% to 48.37%, with B0 having the lowest and B4 having the highest carbohydrate content. This increase in carbohydrate content with added Bambara milk was statistically significant (P≤0.05). The results align with Adedokun *et al.* (2013), who reported a similar increase in carbohydrate content with increased Bambara milk substitution.

3.2 Gross energy content

The gross energy content result is shown in Fig. 1. it decreased as the level of added Bambara milk increased, ranging from 209.09 to 319.60 kcal. However, this range remained within the acceptable limit for plant-based milk, according to USDA Smart Snacks in School Regulation (2019-2020). The nutritional evaluations demonstrate the positive influence of adding Bambara milk as a plant milk source, as it is low in calories, lactose-free, and cholesterol-free.

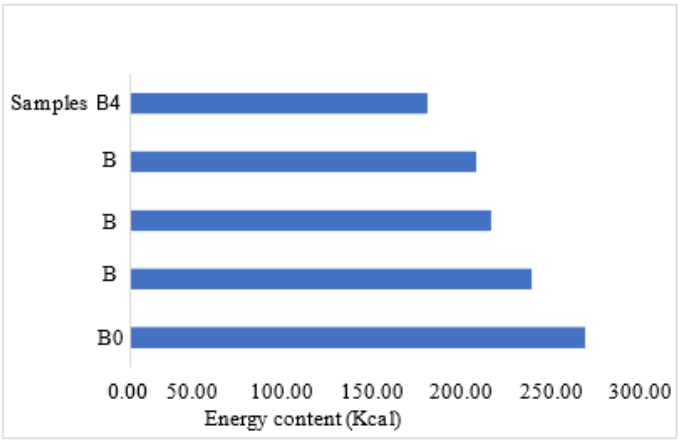

Fig.1. Energy Content (Kcal) of Cheese with added Bambara milk. B0 = 100% Cow Milk (control), B1=25% Bambara milk +75% Cow milk, B2 = 50% Bambara Milk + 50% Cow milk, B3= 75% Bambara milk +25% Cow milk, B4=100% Bambara milk.

3.3 Total solids content

The total solid content, as shown in Fig.2 was highest in the control (B0) and lowest in B4. The addition of Bambara milk reduced the total solids, which is advantageous as it indicates a well-compacted nutrient profile. A lower total solid for the substituted samples is desirable as it can reduce shipping and packaging costs.

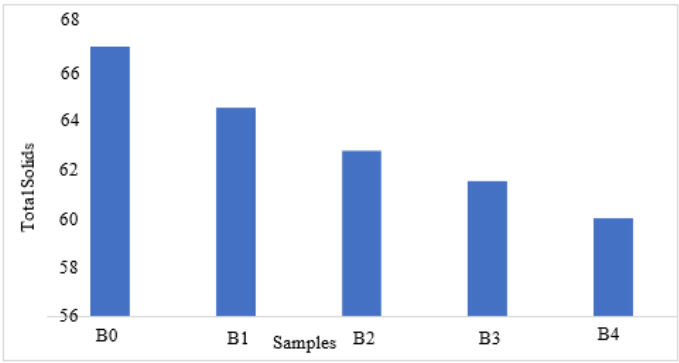

Fig.2. Total Solid Content of Cheese with added Bambara milk. B0 = 100% Cow Milk (control), B1=25% Bambara milk +75% Cow milk, B2 = 50% Bambara Milk + 50% Cow milk, B3= 75% Bambara milk +25% Cow milk, B4=100% Bambara milk.

3.4 Microbial load

The microbial load results of the cheese samples under study during a 3-week refrigerated storage at 6±2°C were presented in Figure 3. Sample B4 exhibited the highest microbial load, followed by B0 (control). The incorporation of Bambara milk into the cheese demonstrated stability in the rate of microbial multiplication. This observed stability could be attributed to the natural antimicrobial compounds in Bambara groundnut, such as

phenolics and tannins **(**CMMEF, 1991**).** This finding aligns with a study by Okon and Ojimelukwe (2017), where coconut milk substitution reduced the microbial load of cow milk from 5.0-0.5*cfu* with a 30% coconut addition. Similarly, the work of Adebayo-Oyetoro *et al*. (2016) on the shelf-life of Bambara milk cheese with lime as the coagulant reported a total plate count in the range of 0.2-2.1 x 10^4 throughout a four-week room temperature study, showing the preservative effect of lime.

The lower microbial loads observed in samples B1, B2, and B3 suggest an advantage in terms of a longer shelf-life. This outcome addresses the challenge of cheese deterioration due to its high moisture content and instability after a few days, as reported by Bonazzi (2003). Maintaining cheese in a stable state has been a persisting issue, and the results of this study offer a potential solution.

According to the International Commission for Microbiological Specification for Foods (ICMSF, 2005), ready-to-eat foods with plate counts between 0–10^3*cfu* are considered acceptable, between 10^4 - ≤ 10^5*cfu* is tolerable, and 10^6*cfu* and above is deemed unacceptable. The microbial load results of this study fell within the acceptable level (10^3), indicating that the cheese samples maintained a satisfactory microbial quality during the refrigerated storage period.

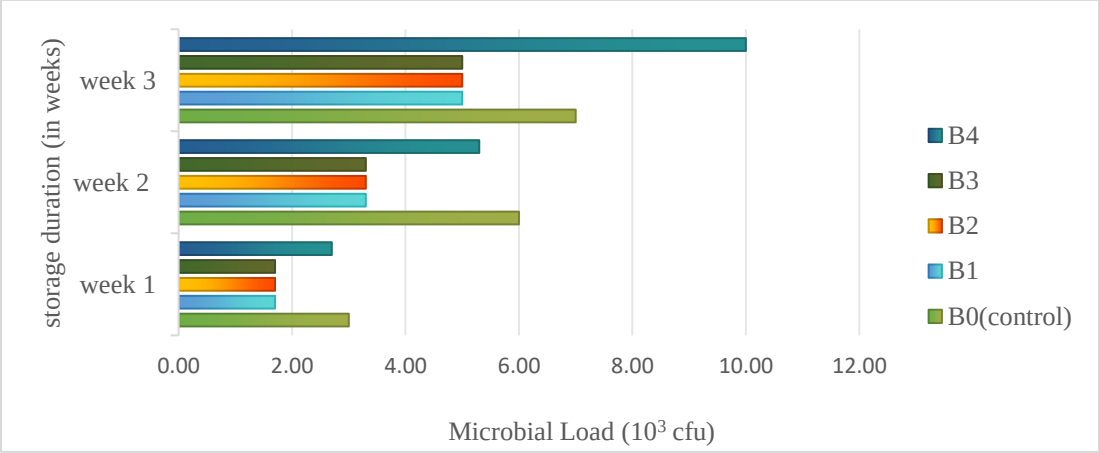

Fig 3. The Microbial Load of Cheese with added Bambara milk during 4 weeks of refrigerated storage
Key: B0 = 100% Cow Milk (control), B1=25% Bambara milk +75% Cow milk, B2 = 50% Bambara Milk + 50% Cow milk, B3= 75% Bambara milk +25% Cow milk, B4=100% Bambara milk.

Table 2. Proximate composition (%) of Cheese with added Bambara milk

Sample	Proximate composition					
	Moisture	Ash	Fat	Protein	Crude Fiber	Carbohydrate
B0	33.00^{e}	3.75^{d}	14.00^{a}	20.30^{a}	0.85^{d}	28.10^{e}
B1	35.50^{d}	3.98^{c}	8.75^{b}	17.44^{b}	0.91^{c}	33.42^{d}
B2	37.25^{c}	4.10^{b}	4.75^{c}	12.49^{c}	1.12^{b}	40.29^{c}
B3	38.50^{b}	4.32^{a}	4.13^{cd}	10.36^{d}	1.53^{a}	41.16^{b}
B4	40.00^{a}	4.38^{a}	1.65^{e}	3.44^{e}	2.16^{a}	48.37^{a}
LSD at 5%	0.0178	0.0793	0.01789	0.0168	0.0179	0.0178

Means with different superscripts are significantly different at P ≤ 0.05.B0 = 100% Cow Milk (control), B1=25% Bambara milk +75% Cow milk, B2 = 50% Bambara Milk + 50% Cow milk, B3= 75% Bambara milk +25% Cow milk, B4=100% Bambara milk.

3.5 Sensory evaluation results

The result of mean sensory scores for cheese samples incorporated with varying proportions of added Bambara milk, are detailed in Table 3. Notably, Bambara cheese products demonstrated favorable comparisons with the control, which comprised 100% cow milk (B0, control). However, for general acceptability, panelists found B3 and B4 substitutions to be the least satisfactory. In terms of mouthfeel, all cheese samples received general acceptance, with B1 scoring the highest. Texture results indicated that samples B2 and B1 were better accepted than the B0 (control), possibly due to the soften texture of Bambara milk on the cheese-like product.

The flavor of all cheese samples was well accepted by the panelist, with sample B1 competing favorably with the B0 (control). This improved flavor could be attributed to the breakdown of carbohydrates into lactic acid during cheese ripening, releasing flavor components such as acetaldehyde or the decomposition of fat into volatile fatty acids, as suggested by Blagden and Gilliland (2005). The ripening process's metabolic activities contribute to fundamental changes in flavor and texture (Moghaddas Kia et al., 2018). Furthermore, the elimination of undesirable contents in plant-based milk, such as aldehydes and ketones, during ripening may enhance texture and flavor (Blagden and Gilliland, 2005).

Regarding color, all substituted samples were well accepted compared to the B0 (control). The sensory scores exhibited a significant difference ($P \leq 0.05$).

3.6 Gas formation

The results of gas formation in the studied cheese analogues during a 4-week refrigerated storage are outlined in Table 4. Notably, Sample B4 and the B0 control displayed early gas formation in the 3rd week of refrigerated storage. In contrast, all Bambara milk-substituted samples exhibited a delay in gas formation, with this occurrence observed in the 4th week under identical storage conditions. The substitution with Bambara milk demonstrated a notable effect in delaying gas formation during the refrigerated storage of cheese products. Various compounds present in cheese, such as lactate, lactose, citrate, and urea, serve as major substrates involved in gas formation. Undesirable gas production can result in cracks and fissures in cheese products, as noted by Mullan *et al*. (1983). Specifically, carbon dioxide produced by propionic acid bacteria contributes to the formation of holes, often referred to as "eyes," in hard cheeses (Guggisberg *et al.,* 2015). The delayed gas formation observed in Bambara milk-substituted samples suggests a potential impact on the overall quality and structural integrity of the cheese analogues during refrigerated storage.

Table 3. Sensory characteristics of cheese with added Bambara milk

Samples	Texture	Flavour	Mouthfeel	Colour	General acceptability
B0 (control)	6.46^{c}	7.05^{a}	6.25^{b}	6.55^{d}	7.65^{a}
B1	6.85^{a}	6.50^{b}	7.70^{a}	7.26^{a}	6.65^{b}
B2	6.65^{b}	6.20^{c}	6.05^{c}	6.90^{b}	6.00^{c}
B3	6.15^{d}	5.25^{e}	5.90^{d}	6.70^{b}	5.90^{d}
B4	5.15^{d}	5.30^{d}	4.90^{e}	6.95^{c}	5.90^{d}
LSD at 5%	0.0178	0.0178	0.0178	0.0178	0.0178

Means with different superscripts are significantly different at $P \leq 0.05$Key: B0 = 100% Cow Milk (control), B1=25% Bambara milk +75% Cow milk, B2 = 50% Bambara Milk + 50% Cow milk, B3= 75% Bambara milk +25% Cow milk, B4=100% Bambara milk.

Table 4. Gas formation of cheese with added Bambara milk during 4 weeks of refrigerated storage

Weeks	B0 (control)	B1	B2	B3	B4
1	-	-	-	-	-
2	-	-	-	-	-
3	+	-	-	-	+
4	+	+	+	+	+

B0 = 100% Cow Milk (control), B1=25% Bambara milk +75% Cow milk, B2 = 50% Bambara Milk + 50% Cow milk, B3= 75% Bambara milk +25% Cow milk, B4=100% Bambara milk

Conclusion

The results of this study revealed that different percentages of Bambara milk can be added to cow milk to achieve a cheese product with good nutritional quality and sensory attributes. The proximate analysis of these products sowed that the use of Bambara milk as milk alternative improved the Ash content from 3.75% to 4.38%, Crude Fiber content from 0.85% to 2.16 %, and Carbohydrate from 28.1% to 48.37%. For microbial load, a combination of cow milk and Bambara milk in cheese production showed improvement in the microbial load and better shelf-life stability of the product. Sample B1, B2, and B3 showed similar microbial stability during storage compared to BO, and B4. Sensory Evaluation shows remarkable improvement in the level of acceptability of products in terms of Texture, Flavour, Mouthfeel, Colour, and general acceptability. Sample with 100% cow milk scored the highest in terms of General Acceptability followed by B3 before B1. A combination of cow milk and Bambara milk for cheese production in this work will help in meeting nutrient recommendations for patients with lactose intolerance, meet daily protein and amino acid recommendations and improve sustainability in diet in climate change vulnerable regions. Sample B1 and B2 which contains 25 and 50% Bambara milk respectively are therefore recommended for use in the production of Bambara milk substituted cheese. This work will improve the utilization of Bambara nut which has been neglected and underutilized in developing and under-developing countries of Africa where cow milk is not easily affordable.

Nutritional Boundaries of the study

Adding Bambara milk to cheese can boost mineral and fiber content while lowering fat. However, it also reduces protein and can make the cheese more watery and high in carbohydrates and energy. Samples containing lower portions of Bambara milk up to 25% were the more acceptable options in these parameters. However, it depends on personal dietary and health needs. For consumers without metabolic dietary restrictions, it will be beneficial and sustainable by promoting the use of Bambara nut which has been neglected and underutilized in developing and under-developing countries of Africa where cow milk is not easily affordable.

Data Availability statement

The data used in this study is available upon request from the author

Conflict of interest

The authors declare that there is no conflict of interest.

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