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Partial Substitution of Cow's Milk with Soymilk in the Production of Fulani Cheese (Wagashi) in Benin

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ABSTRACT

Background: Fulani cheese is a soft, nutritious cheese traditionally produced through the heat coagulation of fresh cow's milk using calotropain, a plant-derived enzyme extracted from *Calotropis procera*. However, the availability of fresh milk is increasingly constrained by adverse climatic conditions and parasitic diseases affecting livestock. This situation highlights the need to explore alternative milk sources to sustain cheese production. The present study aimed to develop a hybrid cheese of high nutritional and health value by partially substituting cow's milk with soy milk.

Methods: The ingredients included soybeans, stems and leaves of *Calotropis procera*, sorghum panicles and fresh cow's milk. The production process comprised filtration, preheating, coagulation, heating, cooking, draining and molding. Three formulations were tested, corresponding to different levels of cow's milk substitution with soy milk: F1 (0%), F2 (15%) and F3 (20%). The analysis were carried out according to the standards of analysis in microbiology, physicochemistry and nutritional.

Result: The results showed that increasing the proportion of soy milk led to higher fat (from 32.62% to 36.92%), ash (from 4.13% to 4.32%) and protein contents (from 20.38% to 26.05%). Microbiological analyses confirmed that all variants complied with safety standards. Sensory evaluation indicated that F2 was the most preferred formulation, offering a promising alternative to help address protein-energy malnutrition in developing countries.

Key words: Benin, Cow's milk, Fulani cheese, Soy milk, Substitution.

INTRODUCTION

In Africa and particularly in Benin, the agri-food industrial sector remains poorly developed (Tchekessi *et al.*, 2021a). Traditional cheese making, known locally as *Wagashi*, is a well-established practice that plays a vital role in the Fulani household economy, often contributing more than half of their annual income (Aisso *et al.*, 2016). *Wagashi* is a soft cheese of high nutritional value, produced by the hot coagulation of fresh whole milk using calotropain, a plant-derived enzyme extracted from *Calotropis procera* (Dossou *et al.*, 2016). *Calotropis procera*, which belongs to the *Asclepiadaceae* family (Murshed *et al.*, 2025), grows well in dry habitats and is frequently observed in open environments where competition is low (Murshed *et al.*, 2025). Highly appreciated not only in Benin but also in Togo, Nigeria and other countries of the West African sub-region, Wagashi is an integral part of the local culinary tradition. The results of Bekihal *et al.* (2025) show no disadvantage of substituting a plant coagulant "cyprosin" for rennet during the coagulation phase of milk intended for the manufacture of a soft cheese of the "J'ben Elgafs" type.

Calotropis procera, a plant of family asclepiadaceae, is widely used in traditional medicine to cure some diseases such as colds, fever, or as protection and also to manufacture wagashi in Benin by the Fulani people (Tossou *et al.*, 2018). Egounlety *et al.* (1994) observed that despite the content of *Calotropis procera* in toxic substances (heterosides cardiot Oxic acid, especially calotropin), no case of food poisoning due to the consumption of cheese has been reported to date. In addition, Tossou *et al.* (2018) after testing phytochemicals in cheese, this study showed that toxins were not detected in cheese and in whey which might be due to heat reaction

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during production process. The absence of phytochemicals especially the cardiac glycosides (toxic component) in cheese Wagashi and whey could be due to the fact of the temperature of Wagashi production (e”100ºC), the toxins may be decomposed by the cheese’s boiling (Tossou *et al.*, 2018).

In these hot tropical regions, where ambient temperatures range between 30ºC and 45ºC, rapid milk spoilage poses a major challenge. In this context, *Wagashi* production serves as an effective strategy for milk preservation. Cheese making relies on milk as a raw material rich in essential nutrients vitamins, amino acids, fats, minerals, proteins and sugars which also provide a favorable substrate for microbial growth (Bokossa *et al.*, 2011; Tchekessi *et al.*, 2021b). However, in developing countries and particularly in tropical areas of sub-Saharan Africa, dairy production is limited and costly due to climatic constraints and livestock diseases, leading to heavy reliance on imported dairy products (Bokossa *et al.*, 2011). In Benin, per capita milk consumption is estimated at 100 to 110 liters per year, with domestic production meeting only about 40% of this demand (Atia *et al.*, 2019). This milk deficit stems from adverse climatic conditions, suboptimal husbandry practices and diseases affecting livestock (Katinan *et al.*, 2012).

Given these challenges, exploring alternative milk sources for cheese production has become imperative. Soy milk emerges as a promising substitute, due to its high nutritional value and increasing availability on the Beninese market (Konnon and Ahoueya, 2017; FAO, 2021). Economically, soy milk is more affordable than cow’s milk and nutritionally it is rich in protein and essential amino acids (Bokossa *et al.*, 2011). Moreover, soybeans are widely promoted as a food-based strategy to combat malnutrition in rural areas and offer diverse processing opportunities for human consumption (FAO, 2021; Tchekessi *et al.*, 2021b). Its economic importance stems from its multiple uses, notably as a source of protein (40%) and oil (20%) for human and animal consumption and as a versatile raw material in various industries (Channakeshava *et al.*, 2025).

By combining cow’s milk with soy milk under hygienic processing conditions, it is possible to develop a novel hybrid cheese that is both protein-rich and of good sensory and nutritional quality, thus addressing growing consumer demand. The objective of this study was to formulate and evaluate a hybrid cheese of high health and nutritional value through the partial substitution of cow’s milk with soy milk.

MATERIALS AND METHODS

Collection of raw material

The materials used in this study included both plant- and animal-based ingredients. The plant materials comprised soy milk and sorghum panicles, purchased at the Dantokpa International Market (Cotonou District, Republic of Benin) and *Calotropis procera*, collected from the campus of the University of Abomey-Calavi (Benin) (Fig 1). The animal material consisted of fresh cow’s milk obtained from a farm in Akassato (Benin) (Fig 2). Nutritional and microbiological analyses were performed using conventional laboratory equipment and standard analytical techniques. Various tools and utensils were employed during the production process, including a mortar and pestle for grinding *Calotropis procera* leaves and stems; a sieve for filtering the milk and the *Calotropis procera* -milk mixture; a knife for cutting; colanders for draining the curds; a ladle for stirring the milk mixture during heating; a saucepan for cooking; bowls for collecting whey during draining; a gas cylinder for heating; a food thermometer for monitoring temperature during processing; a WeiHeng mechanical scale for weighing ingredients and curds; a stopwatch for timing unit operations; and potable water supplied by SONEB (National Water Company of Benin).

Period and Place of research

The research was conducted between January 2024 to March 2025 in Benin. It was carried out in the two laboratories in Benin, Food Safety Research Unit of the Laboratory of Microbiology, Food Technology and Phytopathology in the Department of Vegetable Biology of the Faculty of Science and Technology located at the University of Abomey-Calavi (Benin) and Laboratory of Food Science, Bioresources, Technology and Human Nutrition of National University of Agriculture (Benin).

Soy milk extraction step

The method for preparing soy milk from whole soybeans was carried out as follows. The soybeans were weighed,

Fig 1: Plant material used.

sorted and thoroughly washed. They were then soaked for 18 hours at 28-30°C, at a ratio of 1 kg of seeds to 5 liters of water. After soaking, the seeds were drained, washed again and blanched in hot water at 100°C for 20 seconds. The blanched seeds were ground using a disc mill to produce a paste. Water was added to the paste at a ratio of 1 kg of paste to 7 liters of water and the mixture was filtered through a white cloth with an 18 µm mesh to obtain soy milk (Bokossa *et al.*, 2011). The soy milk was then heated and packaged. The production process is illustrated in Fig 3.

Formulation test

The formulation tests involved substituting fresh cow's milk with soy milk at varying proportions, as summarized in Table 1.

Fig 2: Fresh cow's milk.

Process for manufacturing mixed cheese

Following filtration and preheating of the mixed milk, the filtrate obtained from the stems and leaves of *Calotropis procera* was added to the milk to initiate coagulation. Once the coagulum formed, it was cooked at a controlled high temperature for a specified duration until the curds rose to the surface of the whey. The coagulum was then drained and molded to shape the cheese. The overall process is illustrated in the flow diagram presented in Fig 4.

Evaluation of yields

Yields were calculated using the following formulas (Aisso *et al.*, 2016):

$$\text{Soy milk yield (\%)} = \frac{\text{(weight of milk)}}{\text{(weight of slurry)}} \times 100$$

$$\text{Cheese yield (\%)} = \frac{\text{(weight of cheese)}}{\text{(weight of milk)}} \times 100$$

(Aisso *et al.*, 2016).

Physicochemical analysis

Physicochemical analysis were performed on the most accepted cheese formulations and included measurements of pH, dry matter, titratable acidity, ash content, fat content, carbohydrate content and protein content. Dry matter was determined following the AACC method (1984). Protein content was measured by the Kjeldahl method and calculated as nitrogen content multiplied by a factor of 6.25, according to the AACC protocol (1984). Titratable acidity

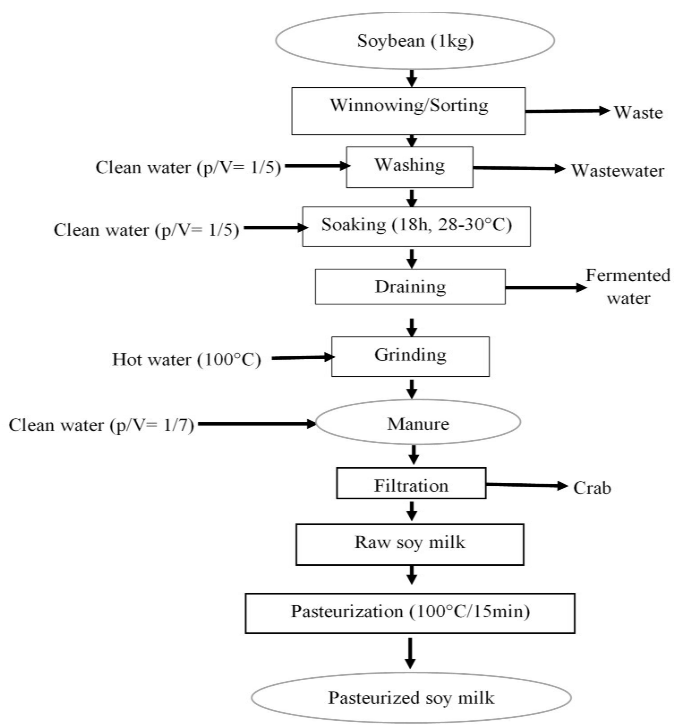

Fig 3: Technological diagram of soy milk production (Bokossa *et al.,* 2011).

and pH were assessed following the method described by Nout *et al.* (1989). Total carbohydrate content was determined using the phenol-sulfuric acid method of Dubois *et al.* (1956). Ash content was evaluated according to the AACC method (1984). Fat content was measured by Soxhlet extraction in accordance with the international AACC standard (1984). The energy value of the samples was estimated using Atwater coefficients, which quantify the kilocalories provided per gram of protein, carbohydrate and fat, as described by Zannou-Tchoko *et al.* (2011)which express the quantities of kilocalories provided by:

$$\text{VE (Kcal/100 g)} = 4 \times \text{Proteins (\%)} + 4 \times \text{Carbohydrates (\%)} + 9 \times \text{Lipids (\%)}$$

Microbiological analysis

The microbial flora, natural or native, plays an important role in the quality of the raw milk cheese, in particular in its taste (Dahou *et al.*, 2021). The microbiological analysis targeted both pathogenic microorganisms and indicator organisms reflective of hygiene practices in food processing (Hadrya, 2009).

The culture media were prepared according to the manufacturer's instructions and maintained in supercooled until the time of inoculation except Baird Parker Agar (BPA) enriched with egg yolk and potassium tellurite, which was pre-poured into Petri dishes. For sample preparation, 10 g of each cheese sample was aseptically collected using a sterile spatula, transferred into a sterile Stomacher bag and homogenized with 90 mL of tryptone salt broth using a stomacher mixer. Successive decimal dilutions were made from the stock solution.

Culture media were prepared according to the manufacturer's instructions and stored refrigerated until inoculation, with the exception of Baird Parker Agar (BPA) supplemented with egg yolk and potassium tellurite, which was pre-poured into Petri dishes. 10 g of each cheese sample was aseptically collected using a sterile spatula, transferred into a sterile Stomacher bag and homogenized with 90 mL of tryptone salt broth using a stomacher mixer. Serial decimal dilutions were then prepared from this stock solution.

The inoculation was performed using mass inoculation technique except for BPA and OGA which was inoculated on the surface. Total bacteria was counted on the Plate Count Agar (PCA) after incubation at 30°C for 72 h ±2 h (ISO 4833-1, 2013), the total coliforms on the Violet Red Bile Glucose (VRBG) after incubation at 30°C for 24h ±2h (NF V08-050, 2009), the thermotolerant coliforms on VRBG at 44°C for 24±2 h (NF V08-060, 2009); staphylococci on BPA with egg yolk and potassium tellurite after incubation at 37°C for 48h ± 2h (ISO 6888-1, 2021). The search for *Escherichia coli* â-glucuronidase was carried out using coliform dishes on Violet Red Bile Lactose Agar (VRBLA) by performing the Mac-Kenzie test (indole and oxidase) using Kovacs and oxidase reagents (ISO 16649-2, 2001). The enumeration of the anaerobic sulfite-reducing bacteria was carried out on Tryptone Sulfite Neomycin Agar (TSNA) after incubation at 46°C for 20h±2h (ISO 15213, 2003). Finally, the search for salmonella was carried out on Salmonella Shigella Agar (SSA) according to standard ISO 6579-1 (ISO 6579-1, 2017). Yeasts and molds were counted on Oxytetracycline Glucose Agar (OGA) supplemented with oxytetracycline after incubated at 25°C for 3 to 5 days (ISO 21527-2, 2008). Lactic acid bacteria were enumerated by

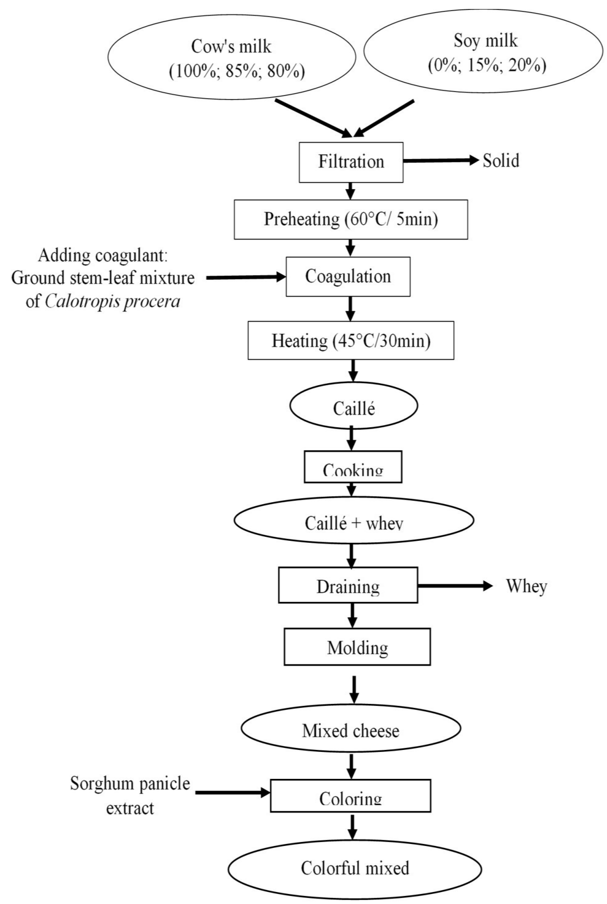

Fig 4: Technological diagram of mixed cheese production.

Table 1: Milk incorporation rate in %.

Cow's milk (ml)	1000	950	900	850	800	750	700	650	600	550	500	0
Soy milk (ml) 0	50	100	150	200	250	300	350	400	450	500	1000	
Mixing quantity (L)	1	1	1	1	1	1	1	1	1	1	1	1
Cow's milk (%)	100	95	90	85	80	75	70	65	60	55	50	0
Soy milk (%) 0	5	10	15	20	25	30	35	40	45	50	100	
Mixing quantity (%)	100	100	100	100	100	100	100	100	100	100	100	100

deep plating on Man, Rogosa, and Sharpe (MRS) agar, with incubation at 30°C for 48 h (NF ISO 15214, 1998). All microbiological analysis were performed in triplicate for each sample. Microbial counts were expressed as colony-forming units per gram (CFU/g).

Sensory analysis of the developed cheeses

Sensory evaluation involves the systematic assessment of food products by human panelists, focusing on organoleptic attributes such as taste, aroma, texture and appearance (Konfo *et al.*, 2024). Sensory analysis enables food manufacturers to better understand consumer preferences and optimize product quality with respect to flavor, odor and mouthfeel (Mihafu *et al.*, 2020).

In this study, a panel of 40 trained tasters was assembled to evaluate key sensory parameters, including color, texture, taste, aroma and overall acceptability. Each attribute was rated using a 9-point hedonic scale, where 1 corresponded to "extremely inferior", 5 indicated "identical to the reference sample" and 9 represented "extremely better" (Larmond, 1977; Tchekessi *et al.*, 2014). This scale facilitated a nuanced assessment of the cheese variants relative to a control or standard reference.

Statistical analysis of data

The collected data were initially processed using Microsoft Excel 2016. Subsequently, statistical analyses were performed using R software. An analysis of variance (ANOVA) was conducted to evaluate differences among the physicochemical and microbiological parameters. Differences were considered statistically significant at a confidence level of $p<0.05$.

RESULTS AND DISCUSSION

Cheese yield products

The calculated average yield of soy milk was 81.46%, whereas the cheese yield was notably higher, reaching approximately 103.1% (Table 2). The highest yields were observed for formulations F2 and F3, reaching approximately 103%. This result can be attributed to the incorporation of soy milk, consistent with the findings of Bokossa *et al.* (2011), who reported yields exceeding 100% in yogurts produced by partially replacing powdered milk with soy milk.

Cheese production

To illustrate the results of our study visually, we present below a series of photographs of the cheese samples produced using different proportions of cow's milk and soy milk. These images provide a better understanding of the visual characteristics and texture of the cheeses obtained from the different experimental formulations (Fig 5).

Nutritional characteristics of the different cheeses produced

The results, summarized in Table 3, demonstrate that the physicochemical properties of the cheeses vary according to the proportion of soy milk incorporated. The pH values of cheeses F2 and F3 (6.795 and 6.645, respectively) were slightly lower than that of the control cheese F1 (6.825). Titratable acidity decreased with increasing soy milk content, declining from 0.013 meq in F1 to 0.009 meq in F3. Moisture content ranged from 57.42% to 65.43%, with the highest water content observed in F1, composed entirely of cow's milk.

Protein content increased proportionally with soy milk substitution, with F3 (20% soy milk) exhibiting the highest level at 26.05%, compared to 20.38% in F1. Similarly, fat content ranged from 32.62% in F1 to 38% in F3, indicating an increase in fat concentration associated with soy milk incorporation. All cheese variants (F1, F2 and F3) showed elevated total ash content, while carbohydrate levels remained comparatively low across formulations. Despite the low carbohydrate content, all cheeses exhibited high energy values.

Table 2: Production yield.

Cheese	Weight of cow's milk (g)	Weight of soy milk (g)	Weight after preheating (g)	Weight after cooking (g)	Yields (%)
F1	100	0	93	87	60.65
F2	100	72.46	169.46	157	98.59
F3	100	81.46	172.46	161	103.01

Fig 5: Cheeses produced.

These findings suggest that partial substitution of cow's milk with soy milk significantly influences the nutritional composition of the cheese, particularly by enhancing protein and fat contents, while maintaining overall nutritional quality.

The physicochemical analysis of the cheeses revealed a moisture content ranging from 57.42% to 65.43%, well within the limit of ≥75% set by the Beninese Standard for pressed Soy Cheese (2021). This variability may be linked to differences in processing conditions, environmental factors and the intrinsic properties of the raw materials used. The reduction in moisture content might be due to the heat applied during Wagashi processing (Ogunlade *et al.,* 2017). The pH values (6.645-6.825) were slightly higher than those reported by Aïsso *et al.* (2016) and Dossou *et al.* (2016), while the titratable acidity (0.009% to 0.013%) was lower. These differences could be explained by the milk type and the interactions between soy and cow milk components. Protein content varied from 20.38% to 26.05%, exceeding the values (8.18%) obtained by Tossou *et al.* (2018). Ash content (4.13%-4.46%) was higher than values (1.41%) reported by Tossou *et al.* (2018), likely reflecting the mineral richness of soy milk.

Microbiological characteristics of the different cheeses produced

The microbiological analysis results, summarized in Table 4, showed the absence of thermotolerant coliforms, Staphylococci, *Escherichia coli* and sulfite-reducing anaerobic bacteria in all cheese samples. Mesophilic aerobic bacteria were detected, with counts ranging from 2 × 10t to 3 × 10t CFU/g, which remain below the acceptable limits established by standards. Total coliforms were present at low levels, ranging from 5×10^1 to 6×10^1 CFU/g, while Enterobacteriaceae counts ranged between 1.03×10^1 and 2×10^1 CFU/g. Yeasts and molds were found in minimal quantities. However, lactic acid bacteria counts, ranging from 3×10^2 to 4×10^2 CFU/g, exceeded 10^2 CFU/g fixed by standards.

Microbiological analysis confirmed the presence of total aerobic mesophilic flora (2×10^4 to 3×10^4 CFU/g), below the threshold of 5×10^4 CFU/g, indicating satisfactory microbiological quality. Lactic acid bacteria counts (3×10^2 to 4×10^2 CFU/g) exceeded the AFNOR standard (1996) limit of 10^2 CFU/g, likely due to fermentation activity. Yeasts and molds were present at low levels, consistent with the Quebec standard (2019). The absence of thermotolerant coliforms and Staphylococci suggests adherence to good hygienic practices, in line with observations by Abakar (2012). These microbiological profiles indicate good hygienic practices during production and suggest the cheeses are safe for consumption, with the elevated lactic acid bacteria levels reflecting their potential beneficial role fermentation. Figure 6 showed some aspects of microorganism colonies after culture.

Table 3: Physicochemical characteristics of the cheeses produced.

Parameters	F1 (100%)	F2 (15%)	F3 (20%)
pH	6.825 ± 0.05^{a}	6.795 ± 0.05^{a}	6.645 ± 0.05^{a}
Acidity titrable (meq /100 g)	0.013 ± 0.004^{a}	0.013 ± 0.004^{a}	0.009 ± 0.00^{a}
Material content dry (%)	34.57 ± 0.13^{a}	39.84 ± 0.76^{b}	42.58 ± 0.45^{a}
Water content (%)	65.42 ± 0.13^{a}	60.16 ± 0.76^{a}	57.43 ± 0.45^{b}
Protein content (%)	20.38 ± 0.39^{b}	21.38 ± 0.05^{b}	26.05 ± 0.07^{a}
Fat content (%)	32.62 ± 0.34^{a}	36.92 ± 0.20^{a}	38.00 ± 0.32^{a}
Ash content (%)	4.13 ± 0.78^{a}	4.32 ± 0.62^{a}	4.46 ± 0.34^{a}
Carbohydrate content (%)	1.00^{a}	1.00^{a}	1.00^{a}
Energy value (kcal/100 g)	375.1^{b}	417.8^{a}	446.2^{a}

Values with the same letters on the same line are not significantly different at the 5% level.

Legend: F1 = Cheese made from 0% soy milk; F2 = Cheese made from 15% soy milk; F3 = Cheese made from 20% soy milk.

Table 4: Microbiological characteristics of the cheeses produced.

Microbiological parameters sought (CFU/g)	Cheeses produced			Standards
	F1	F2	F3	
Total aerobic mesophilic flora	2×10^4	3×10^4	3×10^4	5×10^4
Lactic acid bacteria	4×10^2	3×10^2	4×10^2	10^2
Sulfite- reducing anaerobic bacteria	Absence	Absence	Absence	-
Escherichia coli	Absence	Absence	Absence	Absence
Enterobacteriaceae	Absence	1.03×10^1	2×10^1	$<10^2$
Total coliforms	Absence	5.10^1	5×10^1	$<10^2$
Thermotolerant coliforms	Absence	Absence	Absence	$<10^0$
Yeasts and molds	Absence	1×10^1	1×10^1	10^2
Staphylococcus aureus	Absence	Absence	Absence	Absence

Legend: F1 = Cheese made from 0% soy milk; F2 = Cheese made from 15% soy milk; F3 = Cheese made from 20% soy milk.

Table 5: Sensory characteristics of cheeses.

Settings / Samples	Smell	Taste	Color	Texture	Overall acceptability
F1	5±00^{a}	5±00^{a}	5±0 0^{a}	5±00^{a}	5±00^{a}
F2	5.769±0.58^{a}	6.615±0.29^{a}	5.5±0.42^{a}	6,769±0.07^{a}	7±0.38^{a}
F3	5.461±0.57$^{a\ a}$	6.538±0.30^{a}	5±0.42^{a}	4,381±0.08$^{b\ b}$	4.230±0.53^{b}

Mean values with the same letters in the same column are not significantly different (p<0.05).

Legend: F1 = cheese made from 0% soy milk; F2 = cheese made from 15% soy milk; F3 = cheese made from 20% soy milk.

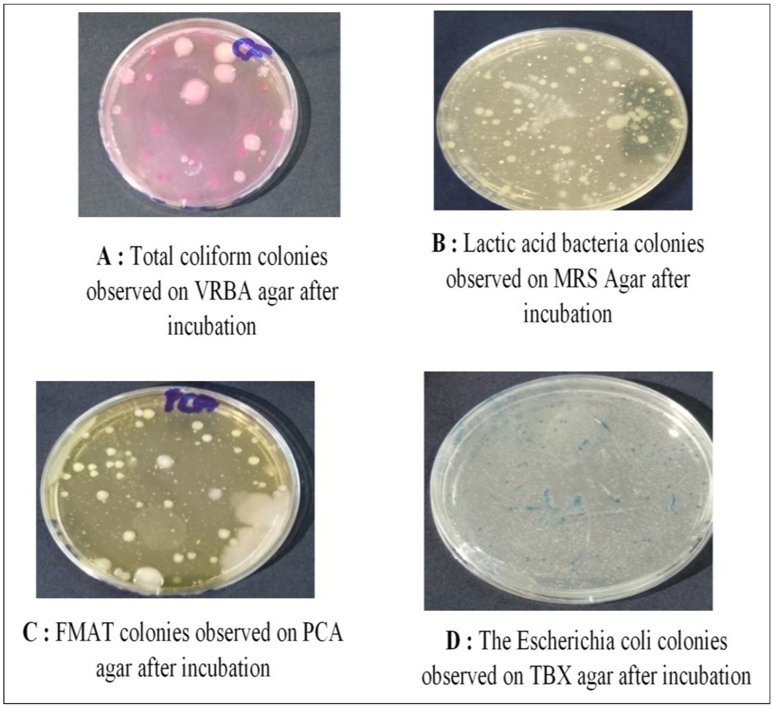

Fig 6: Petri dish and germs sought.

Sensory characteristics of the different cheeses produced

The results of the sensory evaluation indicate that cheeses F2 (15% soy milk) and F3 (20% soy milk) exhibited similar sensory profiles across the assessed parameters. Among these, F2 received the highest scores, particularly for overall acceptability. Panelists preferred F2, followed by F1 (100% cow's milk) and then F3. Nutritional analyses revealed that F2 possessed elevated levels of protein, fat and energy, which likely contributed to its favorable texture and enhanced sensory appeal, thereby explaining its preference among tasters (Table 5).

Sensory evaluation showed that cheese F2 (15% soy milk, 85% cow's milk) was most preferred, with high ratings for texture (6.77), taste (6.62) and aroma (5.77), outperforming scores reported by Aïsso *et al.* (2016) for Fulani cheese. Finally, incorporating soy milk into cheese production represents a promising strategy for enhancing nutritional value and product diversity while maintaining acceptable quality standards. The 15% soy milk formulation, in particular, offers a viable alternative for developing innovative dairy products with improved health benefits.

CONCLUSION

This study focused on the valorization of soy through the development of a mixed cheese combining soy milk and cow's milk. Soy milk, rich in nutrients comparable to those of cow's milk, provided a valuable basis for this innovation. The cheeses formulated with 15% (F2) and 20% (F3) soy milk stood out for their high protein content and satisfactory microbiological quality. Among these, F2 cheese was the most appreciated by the tasting panel, particularly for its texture, flavor and overall acceptability. Microbiological analyses revealed a predominance of lactic acid bacteria and confirmed the absence of pathogenic microorganisms, attesting to the good hygienic quality of the products. This work highlights the potential of mixed cheeses as a means of promoting local resources while offering an effective strategy to combat protein-energy malnutrition in developing countries. The mixed cheese, particularly the formulation with 15% soy milk, is suitable for all age groups and is especially recommended for individuals with increased protein requirements.

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Disclaimers

The views and conclusions expressed in this article are solely those of the authors and do not necessarily represent the views of their affiliated institutions. The authors are responsible for the accuracy and completeness of the information provided, but do not accept any liability for any direct or indirect losses resulting from the use of this content.

Conflict of interest

All authors declare that they have no conflict of interest.

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