



Evaluation of the nutritional, sensory and microbial status of processed and preserved soft cheese types

Abdulhameed Badmos^{1,*}, Kazeem Adeyemi¹, Afolabi Yusuff¹, Nabeel Uthman¹, Abdurashid Yusuf¹, Helen Oyedepo¹, Samson Oyeyinka², Kafayat Belewu³, and Olusegun Lawal⁴

¹ Department of Animal Production, University of Ilorin, Ilorin.

² Department of Home Economics and Food Science, University of Ilorin, Ilorin.

³ Department of Agricultural Economics and Farm Management, University of Ilorin, Ilorin.

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ABSTRACT

*The high nutrient value and the high demand for cheese worldwide has necessitated the adoption of cheese alternatives including Soybean cheese, and different methods of processing it. This study was designed to determine the replacement value of these cheese types by comparing the nutritional, sensory, economic and bacterial attributes of differently processed cheese from cow milk and soybean (cow cheese and soya cheese respectively). Thirty samples each of cow cheese (boiled and fried) and soya cheese (boiled, fried, pepper-coated and egg-coated) constituted six treatments (T1 to T6). The cheese values and bacterial attribute of these six treatments were compared using a Completely Randomized Design. Results revealed that protein was highest in fried soya cheese, then the egg coated soya cheese, but lowest in the pepper coated soya cheese. The sensory values of egg coated soya cheese sample was highest. The pepper coated soya cheese had the highest content of tannins, isoflavones, steroids and phenols, while the fried cow milk cheese had the highest content of alkaloids and terpenoids. The pepper coated soyabean cheese (T5) had lowest bacterial count for the first two days of storage on the shelf. The fried cow cheese (T2) was also low in bacterial count, but highest in cost. The widely prevalent bacteria species in the cheese samples studied include *Enterobacter aerogenes*, *Staphylococcus aureus* and *Lactobacillus plantarum*. It was concluded that the freshly prepared cow cheese supplies calcium and sodium abundantly and is low in anti-nutritive factors, but the nutrients contained depreciates fast with storage while the highly nutritious and acceptable egg coated soya cheese and the phytochemicals-rich pepper coated soyabean cheese are highly recommended as lower cost cheese substitutes to enhance protein supply and bioavailability.*

Introduction

Great number of cheese varieties are produced worldwide with the United States (US) alone producing more than 1700 different cheese. Cheese-making is an economic backbone for many US states, and it serves as a major source of revenue (Donnelly, 2014). Cheese is traditionally produced from cow milk, but the dire need for this nutritious food material has compelled its sourcing from many vegetative sources, including soybean. Soybean cheese is a popular product from soybean which is highly proteinaceous, cheese-like in taste and is particularly appealing to vegetarians. Vegetable soybean is rich in phytochemicals beneficial to human being and is therefore considered a

neutraceutical or a functional food crop (Dillard and German, 2000; Rokayya *et al.*, 2013).

A cheese-like soyabean product is called *Tofu* in the Orient while it goes with different names in tropical Africa, where it has been used as meat or cheese substitutes in both rural and urban areas (Nakayama and Osho, 1996; Hassan, 2013). Jeewanthi and Paik (2018) reported the good potential of Soy cheese for fulfilling global nutritional demand and developing the global market consumption around the world.

The protein content of soybean is very high compared to other plant proteins, and is inferior to animal protein only because it is deficient in sulfur-containing amino acids (Liu, 1997; Hassan, 2013) as well as for the presence of several antinutritional

* Corresponding author.

Email address: badmos111@yahoo.com

factors (ANFs) including trypsin inhibitors, saponins and phytic acid (Liu, 2000). These anti nutritional factors are the phytochemicals, or natural protective mechanisms of plants against pests and microbes. Previous researches have however shown that processing methods such as soaking, cooking, fermentation and germination can drastically reduce the anti-nutrients (Ene-obong and Obizoba, 1996; Liu, 2000 and Yasothai, 2016). These processes take place in the traditional process of soya cheese production. The presence of the residual phytochemicals in soya cheese might however enhance the cheese shelf life. Nascimento *et al.* (2014) earlier observed that phenolic compounds (including soybean) possess antimicrobial properties. It is important to determine the presence (if any) of these phytochemicals in soybean cheese, and their impact on the microbial status of the cheese. It is equally important to determine the value of soybean cheese nutritionally and economically, in comparison to the cheese from cow-milk. Determination of the replacement value of variously processed cow and soya cheese for freshly prepared West African soft cheese in terms of microbial stability, the market cost, the sensory and nutrient values in storage will enhance more strategies in protein bio availability.

This study is designed to compare the nutritional value of the differently processed cheese from cow milk and soybean (cow cheese and soya cheese respectively), the sensory value and the market cost of each. The study will also determine the phytochemical content in these cheese types and their microbial status in storage.

Materials and Methods

Study location

The study was conducted at the Animal Production laboratory, University of Ilorin, Nigeria, located approximately on the latitude 08030' N and longitude 04035' E.

Cow milk and soymilk

Fresh cow milk used for this study was collected by supervised hand milking of lactating cows (of mixed breed, but predominantly white Fulani, and with age ranging between 3 and 6 years). Soy Milk was obtained as reported by Oboh (2006) and Adejuyitan *et al.* (2014). Soyabean grains purchased from Ilorin central market were cleaned, soaked, drained and dehulled manually by rubbing against the palms. The grains were milled and the paste strained to reduce the water content. The resulting soy milk was collected in a vessel.

Experimental Design

Six types of differently processed cheese constituted the treatments to be denoted as T1, T2, T3, T4, T5 and T6 (described below). The study used a Completely Randomized Design.

Table 1. Treatment plan of the study

Treatment	Cheese Type	Cheese Processing	Number of Samples
T1	Cow cheese	Boiled	Thirty (30)
T2	Cow cheese	Fried	Thirty (30)
T3	Soya cheese	Boiled	Thirty (30)
T4	Soya cheese	Fried	Thirty (30)
T5	Soya cheese	Pepper Coated	Thirty (30)
T6	Soya cheese	Egg coated	Thirty (30)

Preparation of coagulants

Sodom apple juice and Alum solution were used as coagulants for coagulating cow milk and soy milk respectively. Stems of *Calotropis procera* were harvested, cleaned, air dried and crushed with mortar and pestle. The crushed mash (10 g) was weighed, sieved with distilled water (20 ml) and labeled as Sodom apple juice (Badmos and Joseph, 2012). Alum crystals were obtained from Ilorin market and 1.0 % solution was prepared by dissolving 10 g of alum in 1 litre of distilled water (Adejuyitan *et al.*, 2014, James *et al.*, 2016).

Cheese preparation and processing

Cow milk and Soymilk of 100ml (as in the treatment plan above) were separately dispensed into sixty (60) heating vessels, being ten replicates of the six treatments and the vessels were immersed and heated in a water bath. Gentle heating of the milk ensued until the milk reached 50 °C when 3ml of coagulant (Sodom apple for cow milk and Alum solution for Soy milk) was added for coagulation of the milk samples. The cow cheese curds were then ladled into raffia basket for more whey expulsion and to obtain the desired shape while the soy cheese curds were filtered using an autoclaved linen cloth (Badmos and Joseph, 2012; Adejuyitan *et al.*, 2014). The cheese curds were allowed to cool for fifteen minutes and then processed as described in the treatment plan above.

Parameters evaluated

Nutritional Assay: The proximate value and mineral content of the cheese samples were analysed on the day of cheese production (day 0) according to the methods of AOAC (2005). Three replicates were selected for analysis from every treatment group. The protein determination used the micro kjeldahl method where samples was mixed with concentrated sulphuric acid (H₂SO₄) and heated to digest in

precense of Selenium catalyst, mixed with equal volume of 45% sodium hydroxide (NaOH) solution, distilled and titrated. The conversion factor of 6.25 was used to determine the protein content. Cheese fat of the sample was determined by the soxhlet extraction where sample was loosely wrapped with a filter paper and put in a thimble fitted into a clean round bottom flask and extracted with petroleum ether in a reflux for 5 hours. Cheese ash was determined by heating sample for 3 hours in moisture extraction oven, then transferred to a muffle furnace until the sample was free of carbon and cooled in a dessicator.

Sensory Assay: The category type hedonic scale of 1 to 9 was used. The appearance, aroma, taste, texture and general acceptability of each cheese sample was determined (on day 0) according to the methods of Lammond (1977) and Badmos *et al.* (2017). Thirty panelists who were familiar with cheese sensory quality were invited to sample and taste the cheese of the six treatment samples.

Phytochemical Screening: The method reported by Ogunlade *et al.* (2019) was used to determine the phytochemical content of cheese samples. The total phenolic content was determined by weighing the samples in Triple Distilled Water (TDW). Gallic acid was used as a positive control and the calibration curve was used to determine the phenolic content. Flavonoid determination used the Aluminium chloride calorimetric method. The total alkaloids was determined by the alkaline precipitation gravimetric method (Mboso, 2013; Ogunlade *et al.*, 2019)

Cheese Cost: Market costing of each cheese type at Ilorin central market was carried out. The market cost was converted to the US dollar equivalent. The cost of the particular cheese type in Dollar/ 100 g cheese was compared.

Microbial Assay: Three replicates of each group was kept on the shelf, and from each group, samples were taken for microbial analysis on the day of cheese production, the third day and the fifth day (day 0, day 2 and day 4) in line with the methods of Fawole and Osho (2007).

Statistical analysis

The data collected in the analysis above were subjected to analysis of variance using a completely randomised design and the means separated by the Duncan Multiple Range Test (Steel and Torrie, 1980).

Results

Proximate analysis

Moisture was highest for boiled soya cheese and pepper coated soya cheese, while it was lowest for

fried cow cheese and fried soya cheese. There was a tendency for lower moisture with frying of cheese. Protein was highest in fried soya cheese and fried cow cheese, but lowest in the pepper coated soya cheese. Ether extract was highest in fried cow cheese, then boiled cow cheese but lowest in pepper coated soya cheese. Ash was highest in fried cow cheese but lowest in boiled soya cheese. Mineral Assay revealed that boiled cow milk cheese contained the greatest amount of sodium and calcium while egg coated soya cheese had the highest content of magnesium, manganese and iron.

Table 2. Nutritional composition (%) of cheese samples.

Nutrient	Treatments					
	T1	T2	T3	T4	T5	T6
Moisture	61.12 ^b	43.8 ^d	67.27 ^a	55.91 ^c	66.07 ^a	60.01 ^b
Crude protein	22.18 ^c	24.61 ^b	22.57 ^c	27.89 ^a	19.75 ^d	24.65 ^b
Ether extract	16.55 ^c	29.18 ^a	11.95 ^c	18.10 ^b	9.77 ^c	12.54 ^d
Ash	5.20 ^b	7.13 ^a	3.00 ^d	4.00 ^c	4.00 ^c	4.20 ^c

a,b,c,d,e,f means having different superscripts along the row are significantly different ($p < 0.05$). T1- boiled cow milk cheese; T2-fried cow milk cheese; T3 - boiled soya cheese; T4 - fried soya cheese; T5 - pepper coated soya cheese; T6 - egg coated soya cheese.

Table 3. Mineral composition (%) of cheese samples.

Mineral	Treatments					
	T1	T2	T3	T4	T5	T6
Calcium	3.50 ^a	2.80 ^b	1.19 ^{cd}	1.3 ^c	1.3 ^c	1.10 ^d
Magnesium	1.10 ^c	0.5 ^d	2.75 ^a	1.7 ^c	2.26 ^b	3.09 ^e
Sodium	0.92 ^a	0.66 ^c	0.49 ^f	0.54 ^c	0.6 ^d	0.74 ^b
Pottasium	1.54 ^c	1.98 ^a	1.6 ^d	1.4 ^f	1.71 ^c	1.84 ^b
Manganese	0.09 ^d	0.11 ^d	0.26 ^d	0.25 ^c	0.69 ^a	0.55 ^b
Iron	2.45 ^c	2.44 ^c	5.20 ^a	5.26 ^a	5.48 ^a	4.19 ^b
Copper	0.22 ^c	0.24 ^c	0.42 ^b	0.46 ^b	0.64 ^a	0.48 ^a
Zinc	5.46 ^a	5.62 ^a	0.71 ^c	0.76 ^c	1.25 ^b	0.88 ^c

a,b,c,d,e,f means having different superscripts along the row are significantly different ($p < 0.05$). T1- boiled cow milk cheese; T2-fried cow milk cheese; T3 - boiled soya cheese; T4 - fried soya cheese; T5 - pepper coated soya cheese; T6 - egg coated soya cheese.

Phytochemical Content: The pepper coated soya cheese had the highest content of tannins, isoflavones, steroids and phenols, while the fried cow milk cheese had the highest content of alkaloids and terpenoids. The presence of anti nutritive factors did not follow a particular trend among the cheese samples.

Sensory Analysis: The egg coated soya cheese sample was most preferred, with preference for its appearance, aroma, texture and general acceptability. The boiled cow cheese was also acceptable, but less preferred to the egg coated soya cheese. The pepper coated soya cheese was adjudged most tasty. This is apparently due to the pepper contents. The boiled soya cheese was least preferred for all sensory

characters. This apparently shows that the boiling process did not reduce the bitterness of the bitter soybean phytochemicals.

Cost of cheese: The market cost survey, as reported in Table 6, shows that the cost per 100 g cheese is highest for the fried cow cheese, then the boiled cow cheese. The egg coated soya cheese costs less, but the boiled soya cheese was the cheapest.

Table 4. Phytochemical content of treated cheese samples (%).

Phytochemical	Treatments					
	T1	T2	T3	T4	T5	T6
Alkaloid	3.51 ^c	6.16 ^a	1.07 ^f	5.76 ^b	1.61 ^e	2.25 ^d
Tannins	0.05 ^c	0.04 ^c	0.86 ^{ab}	0.68 ^b	0.96 ^a	0.84 ^{ab}
Phytates	0.00 ^c	0.00 ^c	0.86 ^{ab}	0.68 ^b	0.96 ^a	0.84 ^{ab}
Isoflavones	7.0 ^b	7.47 ^b	9.64 ^{ab}	9.72 ^{ab}	10.94 ^a	10.02 ^{ab}
Steroids	0.00 ^c	0.00 ^c	0.26 ^b	0.45 ^b	0.90 ^a	0.36 ^b
Flavonoids	4.47 ^b	5.55 ^a	3.08 ^c	3.23 ^d	2.73 ^f	3.68 ^c
Saponins	0.00 ^d	0.00 ^d	0.44 ^a	0.25 ^c	0.33 ^b	0.44 ^a
Terpenoids	3.86 ^b	10.72 ^a	4.27 ^b	4.37 ^b	2.62 ^b	3.83 ^b

a,b,c,d,e,f: means having different superscripts along the row are significantly different ($p < 0.05$). T1- boiled cow milk cheese; T2-fried cow milk cheese; T3 - boiled soya cheese; T4 - fried soya cheese; T5 - pepper coated soya cheese; T6 - egg coated soya cheese.

Table 5. Sensory value of treated cheese samples.

Sensory Value	Treatments					
	T1	T2	T3	T4	T5	T6
Colour	6.15 ^{cb}	6.38 ^b	5.31 ^c	4.77 ^d	7.15 ^a	6.15 ^b
Taste	5.23 ^b	7.15 ^a	7.62 ^a	7.62 ^a	7.46 ^a	5.85 ^b
Aroma	6.00 ^b	7.00 ^{ab}	6.23 ^b	5.38 ^b	8.00 ^a	5.92 ^b
Texture	6.08 ^b	7.07 ^{ab}	7.31 ^{ab}	6.69 ^{ab}	7.62 ^a	6.69 ^{ab}
General Acceptability	6.08 ^c	7.69 ^{ab}	7.46 ^b	5.85 ^c	7.92 ^a	6.46 ^{bc}

a,b,c,d,e,f: means having different superscripts along the row are significantly different ($p < 0.05$). T1- boiled cow milk cheese; T2-fried cow milk cheese; T3 - boiled soya cheese; T4 - fried soya cheese; T5 - pepper coated soya cheese; T6 - egg coated soya cheese.

Table 6. Equivalent market cost of cheese samples (USD/kg).

	Treatments					
	T1	T2	T3	T4	T5	T6
Cheese Cost	4.03 ^b	4.93 ^a	1.03 ^f	1.50 ^d	1.27 ^e	2.50 ^c

a,b,c,d,e,f: means having different superscripts along the row are significantly different ($p < 0.05$). T1- boiled cow milk cheese; T2-fried cow milk cheese; T3 - boiled soya cheese; T4 - fried soya cheese; T5 - pepper coated soya cheese; T6 - egg coated soya cheese.

Bacterial Assay: The Total Bacterial Count in Table 7 showed that the pepper coated soya cheese (T5) had lowest bacterial count for the first two days of storage on the shelf. The fried cow cheese (T2) was also low in bacterial count and by the fourth day, gave the bacterial lowest count. Cheese samples that decreased in bacteria count in the first two days of storage include the boiled and the fried soya cheese samples (T3 and T4), while the other cheese samples increased in bacterial count between day 0 and day 2. Bacterial growth between day 2 and day 4 was however in reverse of the earlier days, as only the boiled soya cheese increased in bacterial population.

The bacterial population was constant for the pepper coated soya cheese (T4) and decreased for the other cheese samples.

Table 7. Total bacterial count (10^5 cfu/ml) of cheese samples during storage.

Storage Days	Treatments					
	T1	T2	T3	T4	T5	T6
Day 0	2.80 ^a	0.90 ^b	3.80 ^a	3.20 ^a	0.70 ^b	2.80 ^a
Day 2	3.20 ^a	1.20 ^b	3.50 ^a	3.00 ^a	1.00 ^b	3.20 ^a
Day 4	2.70 ^b	0.60 ^c	4.50 ^a	2.40 ^b	1.00 ^c	3.10 ^{ab}

a,b,c,d,e,f: means having different superscripts along the row are significantly different ($p < 0.05$). T1- boiled cow milk cheese; T2-fried cow milk cheese; T3 - boiled soya cheese; T4 - fried soya cheese; T5 - pepper coated soya cheese; T6 - egg coated soya cheese.

Table 8. Distribution of bacterial isolates on treated cheese samples.

Bacterial Isolates	Treatments					
	T1	T2	T3	T4	T5	T6
<i>Lactobacillus plantarum</i>	+	-	+	+	+	+
<i>Bacillus subtilis</i>	-	+	+	-	+	+
<i>Streptococcus lactis</i>	+	+	-	-	+	+
<i>Enterobacter aerogenes</i>	+	+	+	+	+	+
<i>Staphylococcus aureus</i>	+	+	+	+	+	+
<i>Proteus vulgaris</i>	+	+	+	-	-	-

Discussion

Moisture content is apparently reduced with the frying of cheese, and this explains the moisture content of fried soya cheese. The frying process appear to have reduced the moisture of cheese. Krokida et al. (2000) reported that frying can be considered a dehydration operation in which a simultaneous heat and mass transfer occurs resulting in two counter-fluxes, i.e. a water outlet from the food to the hot oil and oil inlet in the food, leading to series of physical and chemical changes in the final product. The high protein content of fried soya cheese and fried cow cheese apparently resulted from the increased dry matter content of cheese with frying. The high ether extract in fried cow cheese and the boiled cow cheese appear to be due to the higher fat content of cow milk. Nazim et al. (2013) earlier reported higher ether extract in cow cheese than soya cheese. The highest ash content in fried cow cheese and the lowest ash in boiled soya cheese was apparently due to the mineral content of the milk sources. Hajirostamloo (2009) reported that the total ash content of cow milk and soya milk from a cup of 245g milk is 75g ash for cow milk and 66 g ash for soya milk. Mineral assay revealed that boiled cow cheese contained the greatest amount of calcium and sodium while egg coated soya cheese had the highest content of magnesium, manganese and iron. Hajirostamloo (2009) reported that cow milk contain more than three hundred times the amount of

calcium as does soymilk. Nazim et al. (2013) reported higher calcium content of cow cheese, but lower content of manganese, copper and other minerals. These reports corroborate the findings of this study.

The presence of phytochemicals in the cheese samples, particularly the soybean based and pepper coated cheese appear to have been moderated by the processing methods. The pepper coated soya cheese appears to have benefited from the phytochemicals of pepper. Bell peppers are reported to contain several hydroxycinnamate glucosides, flavonols and numerous flavones. (Marin et al., 2004). Soya cheese is moreover, expected to be rich in phytochemicals as soybean grain contains isoflavones, phenolic compounds, tocopherols, soy peptides, lecithin and saponins (Bae et al., 2012). The high temperatures of boiling, frying as well as the soaking-cum-fermentation process might be responsible for degradation of some of these phytochemicals (Rungapamestry et al., 2007).

The relatively low market cost of soy cheese in this study is expected to enhance global protein bioavailability. More nutrients are made available at low cost by the consumption of soy cheese in low-income areas. This corroborates the report of Jeewanthi and Paik (2018) that the high nutritional quality and lower cost associated with developing Soy cheese types ameliorates the increasing milk shortages and consumer awareness in poor economies. The use of cheese substitutes as a means to cheaper proteins has been severally reported regarding their cost-effectiveness, the simplicity of their production and the replacement value of selected cow milk nutrients by cheaper vegetable-based nutrients (Eymery and Pangborn, 1988; Hassan, 2013).

The sensory preference for the egg coated soya cheese might be peculiarly due to the flavor produced from soyabean and egg. The presence of egg appeared to have improved the fatty acid profile and flavor of the egg coated soya cheese. Tserveni-Gousi (2001) reported that odor and flavor in eggs are important both aesthetically and physiologically as they stimulate the secretion of digestive juices. The low preference for the boiled soya cheese was apparently due to the presence of bitter phytochemicals in soybeans (Annongu, 2012) and also that the boiling process did not reduce the bitterness of the soybean phytochemicals. The pepper coated soy cheese is high in general acceptability. This is apparently due to the flavor improvement by pepper. Sharma et al. (2015) suggested the addition of spices such as bay leaf,

cumin seeds and pepper to soymilk to improve the flavor of soy cheese.

The phytochemicals present in soybean as well as those of pepper appear to give a combined antibacterial effect to the pepper coated soya cheese, despite some destruction of phytochemicals by processing. Kumaran and Citarasu (2015) reported that the methanolic extract of Glycine max (Soybean) inhibited the growth of *Escherichia Coli*, *Pseudomonas Aeruginosa* and *V. Harveyi* and produced percentage inhibition ranging between 67.6% to 82.8%. The report explained that the anti-bacterial activity demonstrated by the plant extract may be due to the presence of the phytochemicals present in the plant. Nascimento et al. (2014) observed that besides the antioxidant activity of phenolic compounds that can protect the human body from free radicals, these compounds also possess antimicrobial properties, and they are natural inhibitors of microbial growth. The most prevalent bacteria species isolated from this study are *Enterobacter aerogenes* and *Staphylococcus aureus*, as they were present in all the treatment samples. The bacteria detected were apparently the result of milk contamination during the milking process. *Lactobacillus plantarum* was also widely prevalent, but was absent in fried cow cheese.

Conclusions

The boiled cow cheese, particularly when freshly prepared is comparatively richest in nutritive calcium and sodium, but unfortunately depreciates in quality as storage progresses. The egg coated soya cheese was nutritious and most acceptable and the cost is lower than that of cow cheese. The pepper coated soya cheese contained potent antinutritive factors like Tannins, Isoflavones, Steroids and Phenols. It is costs less than cow cheese, had the lowest bacteria count during storage and is considered to have the longest shelf life among the cheese types. This study recommends the egg coated and pepper coated soya cheese as cheese alternatives with high nutritional and preservative qualities.

Declarations of interest

The author(s) declare that there is no conflict of interest with regards to the research, authorship and/or publication of this article.

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