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ORIGINAL RESEARCH ARTICLE

Comparative Analysis of the Proximate Composition of Local Cheese Made from Soybean Milk and Cowmilk using Different Coagulants.

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ABSTRACT
This study was aimed at determining the proximate composition and the effects of two coagulants in the production of local cheese known as Awara using soybean and cow milk. Cow milk cheese using the tamarind coagulant had the highest weight at 492.7 g followed by cow milk cheese using the corn steeped liquor which was 484.3 g, while soybean cheese weighed 347.7 g and 323.4 g for tamarind and corn steeped liquor respectively. The results revealed a significant difference in the effect of different coagulants on the proximate composition of the cheese produced. Soybean cheese had the highest moisture content of (20.04 ± 0.015%) and (18.15± 0.13%), crude fiber (10.01 ± 0.01%) and (12.17 ± 0.15%), crude protein (10.33± 0.08%) and (7.88± 0.05%) and carbohydrate (46.78 ± 0.11%) and (49.16 ± 0.06%) while cow milk had the highest ash content (5.03 ± 0.03%) and (5.03 ±0.04%) and crude fat of (21.32 ± 0.28%) and (20.28 ± 0.25%) for corn steeped liquor and tamarind coagulants respectively. The sensory evaluation revealed that cheese produced from soybeans using the corn stepped liquor had the highest acceptability of 8.63±0.01, followed by cheese produced from cow milk using the corn steeped liquor 8.37±0.43, cow milk cheese using tamarind 7.50±0.54 and the cheese produced from soybeans had the least acceptability of 7.40±0.25. The results showed that cheeses made from soybeans with different coagulants were the most nutritious because they retained more of the product's nutritional content.

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INTRODUCTION
Meat substitutes are food products designed to be similar in, colour, taste, texture and appearance to meat (Hurley & Liebman, 2006). They are considered meat substitutes because they provide a good source of protein and can also be used as a puff to prolong the shelf life of meat products. Meat alternatives often contain flavors, spices and wheat gluten and are rich in fiber because they are made from plants (Klausner, 2002). Cheese which is a common meat substitute is defined as a product made from curds obtained from cow's milk, in which casein is coagulated with the aid of rennet or similar enzymes in the presence of lactic acid produced by added microorganisms to remove moisture (Adetunji *et al*., 2008). A portion is removed by cutting or pressing, it is shaped in a mould and stored for some time at appropriate temperature and humidity to ripen (Adetunji *et al*., 2008). Cheese is a staple dairy food and highly regulated in numerous countries. It is consumed by many people around the world, and not limited to a particular age or region. Processed dairy cheese types, cheese analogs, and imitation cheeses have emerged over the years and have received the attention of food processors and consumers owing to their many potential benefits (Farahmandfar *et al*., 2011; Rinaldoni *et al*., 2014).

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The principle of the process is to extract the soy protein with the water in the soy milk and coagulate it with a coagulant. In general, the cheese (tofu) manufacturing process can be divided into two main stages: protein extraction and protein coagulation into tofu products. The extraction stage itself generally involves grinding the soybeans, cooking the soybean pulp, and filtering (Yuwono & Susanto, 2016).

Soybeans are considered the ideal grain to cover the protein requirements of humans and animals. It is good for heart health because it is a source of good (unsaturated) fats, as opposed to animal-derived saturated fats (Iwe, 2003; Samuel and George, 2009).

Soymilk, a milk replacer that can be easily made from dried beans, is rapidly becoming a household staple in developing countries, including Nigeria, due to its nutritionally fortifying properties (Obiegbuna *et al.*, 2014). This study was conducted to determine the impact of different coagulants on the proximate composition and organoleptic properties of locally produced soft cheese in Nigeria.

MATERIALS AND METHODS

Sample Collections

Soybeans were purchased from Farin Gada market in Jos, Plateau state. Cow milk was also purchased from the National Veterinary Research Institute, Vom and sent to the Biology Laboratory in Federal College of Forestry, Jos.

Sample Preparation

1 kg Soybean seeds were thoroughly sorted and washed to remove impurities. The seeds were soaked in clean water for 12 hours to soften them. The washed seeds were then ground into paste.

Preparation of Coagulants

Corn broth was prepared according to (Ibironke *et al.*, 2014) by soaking corn for 12h to ferment it. Followed by grinding, sieving with a muslin cloth, allowing it to settle and decanting the supernatant. Similarly, the tamarind coagulant was prepared by soaking the tamarind in hot water for one hour and straining it through a muslin cloth.

Production of Soymilk

The ground paste was diluted 1:4 with water, sieved with a muslin cloth to extract the soymilk, and then it was boiled.

Production of Soybean Cheese and Cow milk Cheese

Three litres of soymilk and three litres of cow milk were boiled separately for 30 minutes to remove anti-nutrients, and 100 ml of corn steep liquor and 100 ml of tamarind coagulant were added to each. Milk and coagulant were boiled at 100°C for 30 minutes. Cheese was prepared by heating until a complete localized cheese shell was formed, separated from the coagulant and stored in a refrigerator (4 °C) until analysis (Ibironke *et al.*, 2016; Schuck *et al.*, 2017)

PROXIMATE ANALYSIS

Moisture Content

The water content of the samples was determined according to the Association of Analytical Chemist (AOAC) (2010). Thoroughly cleaned crucibles were placed in a drying oven at 100 °C for 1 hour. The crucibles were then cooled in a desiccator and weighed. Samples of 2 g each of soy bean cheese and cow milk cheese were placed in the crucible in triplicate and then weighed again. The samples were then dried in a constant temperature bath at a temperature of 100° C, until a constant weight was obtained. The dried sample was then cooled and weighed. The moisture content of the samples was then calculated as shown below:

$$\text{Moisture contents (\%)} = \frac{W2 - W3}{W2 - W1} \times 100$$

Where:
W_1= Initial weight of empty crucible
W_2= Weight of crucible + sample prior to drying
W_3= Final weight of crucible + sample after drying

Ash Content

Each triplicate 10 g sample was placed in the previously prepared crucible as described above and weighed. They were then placed in a muffle furnace preheated to 300°C for 3 hours until a light gray residue was obtained. The crucible was then cooled in a desiccator and the new weight of crucible plus ash was recorded. Percent ash level was calculated as:

$$\text{Ash contents (\%)} = \frac{W2 - W3}{W2 - W1} \times 100$$

Where:
W_1= weight of empty crucible
W_2=weight of crucible + sample prior to ashing
W_3= weight of crucible + Ash

Crude Fat

Two different samples of 5 g each were accurately weighed into appropriately labeled extraction thimbles and carefully stuffed with cotton. A pre-weighed boiling flask was charged with 300 ml of petroleum ether. A Soxhlet extractor was assembled with a thimble in place and refluxed for 8 hours. The heating rate was then adjusted to 50-55° C, to achieve a condensation rate of 2-3 drops/second. (AOAC, 2010). The thimble was then carefully removed and the ether recovered for reuse. When the petroleum ether was almost evaporated from the flask, it was removed and dried at 105°C for 1 hour until the solvent was completely dry. Crude lipid was calculated using the following formula:

$$\text{Crude lipid contents (\%)} = \frac{W2 - W3}{W2 - W1} \times 100$$

Where; W_1= weight of samples before extraction
W_2= weight *of samples after extraction*

Crude Fiber

Two grams of each sample was weighed into a 250 ml conical flask and 200 ml of 1.25% H_2SO4 was added. The samples were heated for 30 minutes, filtered using a poplin cloth on a Buchner funnel, and washed several times with distilled water until acid-free. The resulting residue was transferred into a 250 ml Erlenmeyer flask and 200 ml of 1.25% NaOH solution was added. The samples were reheated for 30 minutes, filtered using poplin cloth, and washed with distilled water until alkali-free. The entire residue was transferred to a crucible, dried in an oven overnight, cooled and weighed. The crucible was then placed in a muffle furnace at 550° C, ashed for 12 hours, cooled and weighed. Fiber weights were calculated by difference using the formula:

$$\text{Crude fibre contents } (\%) = \frac{\text{Wcd - Wca}}{\text{Ws}} \times 100$$

Where:

Wcd = weight of crucible + dried residue

Wca = weight of crucible + ashed residue

Ws = weight of the sample

Crude Protein

Nitrogen content in the samples was estimated using the Micro-Kelder method described by (AOAC, 2010) and modified by Onwuka (2015). Crude protein was calculated by multiplying the estimated nitrogen by 6.25. One gram of each of the different samples was accurately weighed and transferred to a digestion flask containing two tablets of selenium catalyst. 12 ml of H_2SO4 was added and the tube was heated until a clear solution was obtained. Each clear solution was transferred to a 50 ml volumetric flask and filled to the mark. Digestion of the protein produces an ammonium sulfate solution. Following 10 ml of digestion, 10 ml of 40% NaOH solution was pipetted into the Kelder distiller. An Erlenmeyer flask containing 5 ml of 2% boric acid and 3 drops of mixed indicator was placed under the outlet of the condenser. After distillation was completed, the ammonium sulfate solution produced was converted to ammonia. The generated ammonia gas was condensed and recovered as a liquid in an Erlenmeyer flask containing boric acid and a mixed indicator. $B(OH)_3$ + H_2O+ NH_3-NH^-_4 +$B(OH)^-_4$. Nitrogen in the distillate was determined by titration with 0.01 M HCl. A colour change from green to pink marks the end point. The amount of nitrogen and crude protein for each sample was calculated using the following formulas:

$$\%N = \frac{(S-B) \times \text{N acid} \times 0.014 \times D}{\text{Weight of sample x V}} \times 100$$

% Crude protein = 6.25× %N (correction factor)

Where:

S= Sample titration reading

B= Blank titration reading

N = Normality of HCl

V = Volume taken for distillation

0.014=Mill equivalent weight of Nitrogen

Total Carbohydrate

Total carbohydrate was determined by subtracting the percentage of moisture, ash, crude fat, crude fiber and crude protein from 100%. % Total Carbohydrate = 100% - (Moisture + Ash Crude Fat + Crude Fiber + Crude Protein). These procedures were performed on different Samples of cow milk cheese with tamarind coagulant, cow milk cheese with corn steep liquor, soybean cheese with tamarind, and soybean cheese with corn steep liquor.

Sensory Evaluation Test

Sensory analysis of the cheese samples was evaluated using a 9-point hedonic scale with 1 = 'does not like at all' and 9 = 'likes very much' as reported by Iwe (2010). All 10 panelists, included students from different departments at the Federal College of Forestry, Jos took part in the survey. The assessed sensory quality includes odor, colour, taste, aroma and overall acceptability. The order in which the samples were presented to panelists was random, and panelists were given portable sterile water bottles to rinse their mouths between each assessment.

Statistical Analysis

Data were acquired and analyzed using two-way analysis of variance (ANOVA). If there were differences between means, they were separated using the least significant difference test.

RESULTS

Weight of Cheese Produced.

Three litres of cow milk and three litres of soy bean milk were weighed and used to produce the local soft cheese. Cow milk cheese produced using the tamarind coagulant had the highest weight at 492.7 g followed by cow milk cheese using the corn steep liquor at 484.3 g, while soybean cheese weighed 346.7 g and 323.4 g for tamarind and corn steep liquor respectively. Cow milk had the highest production of cheese. The results of the cheese production are summarized in Table 1 below. Cluster and *p* represents the number of individuals.

Table 1: Weight of Produced Cheese from different coagulants

Coagulants	Soybean milk	Cow milk
Tamarind	346.7 g	492.7 g
Corn Steeped Liquor	323.4 g	484.3 g

At $P \leq 0.05$, there was a significant difference in proximate composition for cheeses made from cow's milk with different coagulants. The differences were observed in the proximate constitution of cheeses made using the two coagulants. Tamarind had the highest moisture content of (15.12± 0.11 %), crude protein (9.46 ±0.06%) and carbohydrates (45.70 ±0.10%) whilst corn steep liquor had the highest crude fat (21.32 ± 9.28) and crude fibre (8.04 ±0.05). There was no sizable distinction in the ash content material of the samples. Values are expressed as mean± standard deviation and values with the same superscript are not significant. The results are summarized in Table 2 below.

Table 2: Effects of different coagulants on the proximate composition of cheeses made from cow's milk.

Analyze(%)	Corn steeped liquor cow milk	Tamarind cow milk
Moisture	13.09±0.08[b]	15.12± 0.11[a]
Total ash	5.03 ±0.03[a]	5.03 ±0.04[a]
Crude fat	21.32 ±0.28[a]	20.28 ±0.25[b]
Crude fibre	8.04 ±0.05[a]	5.00 ±0.01[b]
Crude protein	8.70 ±0.03[b]	9.46 ±0.06[a]
Carbohydrates	44.31±0.10[b]	45.70 ±0.10[a]
L.S.D	0.20	
S.F	***	

At P ≤ 0.05, there is a significant difference in the proximate constitution of cheeses made from soy milk with different coagulants. There was a significant increase in the moisture content material at (20.04±0.04%), crude fat (10.12±0.10%) and crude protein (10.33 ±0.08%) using the corn steeped liquor coagulant and an increase in the ash content (4.04 ± 0.03%), crude fiber (12.17±0.15%) and carbohydrates of (49.16 ±0.06%) using the tamarind coagulant. Values are presented as mean ± standard deviation, and values with the same exponent are not significant. The results are summarized in Table 3 below.

Table 3: Effect of various coagulants on the proximate constitution of cheeses made from soy milk.

Analyse (%)	Corn steep liquor Soybean milk	Tamarind soybean milk
Moisture	20.04± 0.04[a]	18.16 ± 0.13[b]
Total ash	3.02 ± 0.02[b]	4.04 ± 0.03[a]
Crude fat	10.12 ± 0.10[a]	9.05 ± 0.04[b]
Crude fiber	10.01 ± 0.01[b]	12.17 ±0.15[a]
Crude protein	10.33 ±0.08[a]	7.88 ± 0.05[b]
Carbohydrates	46.78 ± 0.11[b]	49.16 ± 0.06[a]
L.S.D	0.14	
S.F	***	

The results of the sensory evaluation revealed that the cheese produced from soybeans milk using the steeped corn liquor as coagulant had the highest overall acceptability of 8.63±0.01, followed by cheese produced from cow milk using the steep corn liquor coagulant 8.37±0.43, cow milk cheese using tamarind coagulant was 7.50±0.54 and the cheese produced from soybeans milk had the lowest acceptability of 7.40±0.25. The results are summarized in Table 4 below.

Table 4: Sensory properties of cheeses prepared from cow's milk and soybeans using different coagulants.

Treatment	Sensory Parameters Average Scoring on a 9-Point Hedonic Scale				
	Taste	Flavour	Colour	Aroma	Acceptability
Tamarind soybeans	7.20±1.75	5.20±1.32	7.70±1.06	7.50±1.27	7.40±0.25
Tamarind cow milk	7.00±0.94	6.90±1.37	7.90±1.10	7.20±1.32	7.50±0.54
Corn steeped soy beans	8.20±1.55	6.60±2.07	7.90±1.10	7.80±0.63	8.63±0.73
Corn steeped cow milk	7.60±1.08	7.50±1.08	7.90±1.10	7.90±1.52	8.37±0.43

DISCUSSION

Results for the weight and proximate composition of cheese produced show that the samples differed significantly at (p<0.05). The moisture content of the samples ranged from 13.09± 0.08 to 20.04±0.04. This result is consistent with the findings of Agboola *et al.*, (2020) and James *et al.*, (2016), who reported significant differences in the moisture content of cheeses, made from cow milk, soy milk and coconut milk using different coagulants whereas Omotosho *et al.*, (2011) he reported that cheeses made from cow's milk showed no significant difference in moisture for all coagulants used. Ash content ranged from 3.02±0.02 to 5.03±0.03 for all samples, showing a significant difference at P ≤ 0.05, consistent with the results of other authors (Agboola *et al.*, 2020; James *et al.*, 2016; Choi *et al.*, 2015; Song *et al.*, 1997) all of which reported significant differences in ash content of different cheese samples made from cow milk, soy milk and coconut milk using different coagulants. Fat content ranged from 9.05±0.04 to 21.32±0.28 in different samples, which is relatively low compared to that reported by (James *et al.*, 2016; Caro *et al.*, 2015 and Choi *et al.*, 2015) at 38.85%, 31.22 to 33.52% and 18.80 to 31.80% respectively. The range of 5.12±0.02 to 11.97 ±0.02 was given by Agboola *et al.*, (2020) and is consistent with the same range of 5.00±0.01 to 12.17±0.15 obtained for crude fat P ≤ 0.05. with no significant difference between the cheese samples produced but agreed with Agboola *et al.*, (2020) who reported a significant difference at P≤ 0.05 that ranged from 10.53±0.03 to 24.02±0.07. Crude protein results ranged from 7.88±0.05 to 10.33±0.08, with significant differences at P ≤ 0.05. This range is lower than that reported by James *et al.*, (2016) from 40.00 ± 1.21 to 41.30±0.00, with no significant difference between the cheese samples produced but agreed with Agboola *et al.*, (2020) who reported a significant difference at P≤ 0.05 that ranged from 10.53±0.03 to 24.02±0.07. Carbohydrate content results ranged from 44.31±0.10 to 49.16±0.06, higher than what was reported by (James *et al.*, 2016; Choi *et al.*, 2015; and Agboola *et al.*, 2020), with different cheese samples made from cow milk, soy milk and coconut milk using different coagulants. Acceptable levels of sensory evaluation results ranged from 7.40±0.25 to 8.63±0.73. The results show that all cheese samples were organoleptically acceptable as scores were above average. This result is consistent with Agboola *et al.*, (2020), who reported an acceptable range of 5.75±1.06 to 8.50±0.51 and James *et al.*, (2016), who reported an acceptable range of 6.30±1.62 to 6.85±1.03 with no significant difference with the range at P≤ 0.05. On the standard scale, soy cheese had moisture content of (20.04±0.04%) and (18.15±0.13%), crude fiber (10.01±0.01%) and (12.17±0.15%), and crude protein. (10.33±0.08%) and (7.88±0.05%) carbohydrates, (46.78±0.11%) and (49.16 ± 0.06%) carbohydrates), milk has the highest ash content of (5.03 ± 0.03%) and (5.03 ± 0.04%) and crude fat (21.32±0.28%) and (20.28±0.25%) for corn steep liquor and tamarind coagulants, respectively.

The t-test results with P ≤ 0.05 (0.98) indicates that there was no significant difference in the immediate composition of the cheeses when using the two coagulants. However, from the observed results, corn steep liquor is the best coagulant for making soy cheese, and tamarind is the best coagulant for making cow milk cheese, but the cheese produced using the tamarind coagulant had flavor issues. This may be due to the sour taste of the coagulant. Soy cheese has been found to be most nutritious when used with various coagulants as it retains most of the nutritional content of the product.

CONCLUSION

The coagulants retained the nutritional content of the various cheese samples. Soymilk and cow milk cheese can be considered or assumed to be functional foods and may provide more beneficial minerals and nutrients. The results showed that the cheeses produced have high nutritional value and yield and it can be recommended as an excellent substitute for meat, especially cheeses produced from plant sources such as soymilk because apart from its high nutritional value, it is also very cheap and available locally.

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