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Production and quality evaluation of soy cheese (*tofu*) using various coagulants

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ABSTRACT

Food formulations that incorporate soy ingredients are being exploited as veritable alternatives. Cheese analogues are healthy alternatives to obtain the benefits of consuming functional foods. There is a need to enhance the production and quality of soy cheese. In this study, soy cheese (tofu) samples were prepared from soybean using four selected coagulants - *Moringa oleifera* seed extract (MC), tamarind seed extract (TC), lime juice (LC) and alum (AC). The effects of the coagulants on the physicochemical, microbial and sensory qualities of the soy cheese samples were evaluated. The results of the physical properties of the samples showed that MC had higher yield (12.84%) and acidity (0.37%), while AC had the highest values in hardness (84.55%) and dry matter (36.72%) content. The result of the proximate composition revealed that MC had the lowest moisture content (60.52 %) and the highest protein content (22.51%). The ash content of the soy cheese samples from plant coagulants MC (3.00%), TC (2.80%) and LC (3.5%) were lower than that of the alum coagulant AC (3.89%). The cheese from alum coagulant (AC) had more minerals than those from plant coagulants. TC had the highest vitamin C (2.69 mg/100 g), while MC had the highest vitamin B1 (6.07 mg/100 g) and B2 (29.21 mg/100 g) contents. There was no detection of coliform in the soy cheese samples. There were no significant differences (p>0.05) in the appearance and overall acceptability of MC sample and LC sample MC sample from *Moringa oleifera* seed extract coagulant had the highest sensory scores for flavour (8.35), texture (8.40) and overall acceptability (8.63). The results of quality properties of the cheese samples differed with the coagulants used. *Moringa Oleifera* coagulated cheese (MC) had superiority in nutrients and sensory qualities Hence, it is recommended for *tofu* production.

Introduction

Cheese is the curd formed by the coagulation of milk by rennet or enzymes in the presence of lactic acid produced by added micro-organisms (Johnson et al., 2001; Achachlouei et al., 2013). It is produced with a wide range of flavours, textures and forms by coagulation of the milk protein casein. Usually the milk of buffalo, goat, or sheep is used. During production, the milk is usually acidified by adding the enzyme rennet which causes coagulation. The solids produced are separated and pressed into the final form (Achachlouei et al., 2013).

Cheese is very nutritious due to the high protein quality (Butler et al., 2010; Ndife, 2016) and is also a good source of dietary calcium. Cheese has fewer digestive problems compared to other dairy products (Oboh and Omotsho, 2005; Onwuka, 2014). However, the high saturated fat content, from the nutritional quality point of view, poses a health challenge to consumer perception (Onwuka, 2014).
Soybean, a highly nutritious plant legume contains well balanced amino acids and desirable fatty acids. It serves as an important protein source for many people around the world (Amadou et al., 2009, Barnes, 2010; Butler et al., 2010). Food formulations that incorporate

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soy proteins into different products are being exploited by food experts and manufacturers as functional foods (Nanri et al., 2010; Ndife, 2016). The inclined acceptance of soy foods by the general population is due to the increase recognition of the health benefits of soy foods, especially by sceptics, who want to reduce their consumption of animal products.

Tofu is the Japanese name for bean curd products. Tofu is low in calories, rich in essential amino acids, contains beneficial amount of iron, low in saturated fats, high in vitamins and it contains antioxidants (Achachlouei et al., 2013). Tofu is popularly consumed in Nigeria because of the various nutritional and medicinal attributes associated with soybeans products, such as the reduction of cardiovascular diseases, osteoporosis and cancer risks (Butler et al., 2010; Nanri et al., 2010). Furthermore, the cost of producing cheese analogues is less than products obtained from animal sources (Oboh and Omotsho, 2005; Velasquez and Bhathena, 2007; Rinaldoni et al., 2014).

Different types of coagulants are used locally for the coagulation of milk. These coagulants are reported to impart both physical and chemical properties as well as the sensory effects on the quality of the curds (Achachlouei et al., 2013; Gopalakrishnan et al., 2016). Thus, the objective of this study was to examine the potentials of selected coagulants, which included *Moringa oleifera* seed cake extract, tamarind pulp, alum salt (aluminum sulphate) and lime juice, for the production of soy cheeses and to evaluate the physicochemical properties, microbial qualities and sensory acceptability of the products. This will help determine which coagulant is the most suitable for tofu production and development.

Materials and methods

Raw material procurement

The soybeans and coagulants (*Moringa oleifera* seeds, lime fruit, tamarind seeds, and alum (potassium aluminum sulphate) used for this study were purchased from Kaduna Central Market, Nigeria. The chemicals and reagents used were of scientific standards. The equipment and utensils used were properly cleaned and kept in good sanitary conditions.

Preparation of coagulants

Alum was milled into powdered form and 200 g of the salt was dissolved in 250 mL water, filtered and the filtrate was used as alum coagulant. For lime juice, lime fruits were cut into equal halves and their juices were squeezed out, the extracts from the juice (200 mL) were sieved to get rid of seeds and fibres. The filtrate obtained served as lime coagulant. In the preparation of tamarind seed extract, tamarind seeds were sorted to remove extraneous materials and soaked in water for 2 h, dehulled and wet milled in equal ratio with water and then filtered by squeezing through a muslin cloth. The extract served as tamarind coagulant. For moringa seed extract, the seeds were dehulled and sorted to obtain the kernels. The kernels (300 g) were wet milled with 300 mL of water. The slurry was filtered with muslin cloth and the filtrate extract was used as moringa coagulant.

Production of cheese samples

Soybeans were sorted to remove extraneous materials, washed to remove dirt before blanching the seeds for 20-25 min in boiling water. The soybeans were then steeped in water twice the volume of the soya beans for 6 h before dehulling manually. The soybeans were drained and wet milled into paste with an attrition mill. The soy paste was strained through a sieve and folded muslin cloth by squeezing out as much filtrate as possible. The soy milk obtained was divided into four portions (10 L each) and uniformly mixed with spices. The different liquid coagulants comprising alum water, tamarind seeds extracts, *Moringa oleifera* seed extracts and lime juice (200 mL each) were added to the separate soy-milk portions and allowed to coagulate for 4 h before cooking for 30 minutes. The curds were drained and pressed to expel the whey before cutting them into uniform sizes. The soy cheeses were labelled according to their coagulants as: AC (alum), TC (tamarind seeds extracts), MC (*Moringa oleifera* seeds extracts), and LC (lime juice) respectively (Table 1) and kept in the refrigerator at 5°C for subsequent analysis.

Methods of analysis

Some physicochemical, microbial and sensory parameters were analysed to ascertain the quality of the soy cheeses using standard procedures.

Physical properties

Percentage (%) yield and compression as well as acidity were determined by using procedures described by Onwuka (2018).

Table 1. Formulation of soy cheeses

Ingredients	AC	MC	TC	LC
Coagulants (mL)	200	200	200	200
Soymilk (L)	10	10	10	10
Boulioun (g)	25	25	25	25
Onion (g)	100	100	100	100
Pepper (g)	100	100	100	100
Salt (g)	10	10	10	10

Key: AC (alum), TC (tamarind seeds extract), MC (*Moringa oleifera* seeds extract); LC (lime juice).

Chemical properties

The determination of the chemical composition of the samples on dry wet basis: moisture, ash, protein, fat, fibre and carbohydrate contents were determined by methods described by AOAC (2005). The vitamin and mineral contents were also determined by procedures described by AOAC (2005).

Mineral contents

Each sample was digested by wet ashing method prior to mineral content determination using atomic absorption spectrophotometer for Ca, Mg and Fe and Corning 400 flame photometer for K and Na (AOAC, 2005). The phosphorus content was determined colourimetrically with Jenway 6100 spectrophotometer using the method described by Nielsen (2003).

Vitamin contents

The spectrophotometric method described by Jacobs (1999) was used. The absorbance of the sample and standard solutions was measured with a UV-Spectrophotometer at their respective wavelengths. The concentrations of the vitamins in the prepared samples were determined against known standards calibrated curve.

Microbial contents

The determination of the microbial quality (mesophilic aerobic bacteria, coliforms and fungi counts) of the cheese products was performed by the method outlined in compendium of methods for the microbiological examination of foods (APHA, 1992) with some modifications.

Sensory evaluation

The protocol described by Iwe (2010) was used. The organoleptic properties of soy cheeses were evaluated by 20 semi-trained panellists, randomly chosen from the staff and students of the University. Quality attributes such as appearance, aroma, texture, taste and general acceptability of the products were scored on a 9-point Hedonic scale with 9 as dislike extremely, 5 as neither like nor dislike and 1 as like extremely.

Experimental design and statistical analysis

The experimental set-up was of a completely randomized design. Results are presented as mean ± standard deviations. The data obtained from the various analyses were subjected to analysis of variance using the statistical package for social sciences (SPSS), version 16.0. One way analysis of variance (ANOVA) was used for the comparison of the means. Differences between means were considered to be significant at $p<0.05$ using the Duncan multiple range test.

Results and discussion

Physical properties of soy cheese samples

Table 2 shows the results of the physical properties of soy cheese samples. Coagulation of the protein and oil (emulsion) suspended in the boiled soy milk is the most important step in the production of high yield tofu (Cai and Chang, 1999). The soy cheese produced from *Moringa oleifera* seed extract (MC) had the highest yield (12.84%) and acidity (0.37%), while the alum-salt coagulated cheese (AC) had the highest values in hardness (84.55%) and dryness. There were significant differences ($p<0.05$) in some of the physical properties of cheese samples. This is an indication that the selected coagulant under consideration differs substantially in their coagulating abilities. The difference could be the result of their chemical components (Johnson *et al.*, 2001; Ghebremichael et al., 2005). Firm tofu contains low amount of moisture and has firmness comparable to cooked meat and a somewhat rubbery feel similar to that of paneer. Salt coagulants are mostly used in the production of Chinese-type brittle tofu. Acid coagulants have been reported to give higher and firmer yields of curds than salt and enzyme precipitated curds (Gopalakrishnan et al., 2016). The production techniques employed help to create *tofus* with unique physical and chemical characteristics (Butler et al., 2010).

Table 2. Physical properties of soy cheeses

Parameters (%)	AC	MC	TC	LC
Yield	11.35[b]±0.55	12.84[a]±0.48	10.42[c]±0.40	11.20[b]±0.63
Hardness	84.55[a]±0.31	80.46[b]±0.35	66.28[d]±0.35	75.92[c]±0.40
Acidity	0.28[b]±0.03	0.37[a]±0.06	0.23[b]±0.05	0.32[a]±0.04
Dry Matter	38.72[a]±0.01	36.81[a]±0.04	28.56[c]±0.06	34.40[b]±0.08

*Means within a row with different letters are significantly different at $p < 0.05$: AC (Alum-potassium aluminum sulphate), Key: TC (tamarind seeds extracts), MC *(Moringa oleifera* seeds extracts), and LC (lime juice).

Table 3. Proximate content of soy cheeses

Parameters (%)	AC	MC	TC	LC
Moisture	63.40[b]±1.23	60.52[b]±0.53	68.41[a]±0.20	65.23[a]±0.25
Protein	17.36[c]±1.20	22.51[a]±1.34	19.60[b]±1.10	20.16[b]±1.25
Fat	5.53[c]±0.58	7.56[a]±0.80	6.80[b]±0.53	6.12[b]±0.47
Ash	3.89[a]±0.10	3.00[b]±0.53	2.80[b]±0.70	3.5[a]±0.20
Carbohydrate	6.34[b]±1.24	7.46[a]±1.40	4.82[c]±0.98	6.28[b]±1.15

*Means within a row with different letters are significantly different at $p < 0.05$: AC (Alum-potassium aluminum sulphate), TC (tamarind seeds extracts), MC (*Moringa oleifera* seeds extracts), and LC (lime juice).

Chemical compositions of soy cheese samples

The results of the proximate composition of the different soy cheese samples, as indicated in Table 3, shows the moisture content to be the lowest in sample MC (60.52%) and the highest in sample LC (65.23%). The results were in contrast to the hardness of the samples, showing the extent of coagulation. The protein and fat contents of the soy curds produced ranged from 17.36-22.51% and 5.53-7.56%, respectively. There was a significant difference ($p<0.05$) in the protein content of the soy cheeses. The high protein content of the moringa coagulated sample MC (22.51%) could possibly be attributed to the likelihood that the protein in the moringa seeds and the other plant extracts (TC and LC) might have been transferred into the soy curd unlike the alum salt coagulant AC (17.36%). The reduction of the moisture content of the cheese samples could lead to increased protein concentration. Furthermore, the protein content of the produced soy cheeses was higher than that of the commercial soy cheese (12%) reported by Rinaldoni et al. (2014). This variation could be the result of the difference in the variety of soybean, coagulants used and the condition under which the coagulation was carried out (Moller et al., 2008; Amadou et al., 2009).

The fat contents of samples MC (7.56%) and TC (6.80%) were higher than that of AC (5.53%) and LC (6.12%). The fat contents of these soy cheese samples (5.53-7.56%) were however lower than the average value (9.0%) reported by Amadou et al. (2009) for some commercial soy cheeses.

The ash contents of the soy cheeses were AC (3.89%), MC (3.00%), TC (2.80%) and LC (3.5%), respectively. The highest ash content was obtained in AC (3.89%), followed by the moringa sample MC (3.00 %). The inorganic nature of the alum coagulant must have contributed to the increase in the mineral and ash contents of sample AC. The increase in mineral content of tofu may be attributed to the use of inorganic coagulants as demonstrated in previous studies (Amadou et al., 2009; Achachlouei et al., 2013).

Mineral contents of soy cheese samples

The mineral content of the soy cheese samples is presented in Table 4. The mineral contents ranged from 0.11 to 41.03 ppm. Sample LC had the highest calcium content (41.03 ppm), while sample AC had the lowest phosphorus content (9.02 ppm). The highest iron and manganese values were recorded in sample MC (3.66 ppm) and sample AC (0.64 ppm), respectively.

The percentage mineral content is considered a quality criterion for the nutritional adequacy of foods for wellbeing. The differences in mineral content would majorly depend on the type of substrate and the nature of the coagulants (Achachlouei et al., 2013). The use of salt coagulants has been reported to make tofu that is rich in minerals with tender but slightly brittle texture (Amadou et al., 2009).

Vitamin contents of soy cheese samples

Table 5 shows the results of the vitamin contents of the soy cheese samples. The vitamin contents were generally low. The vitamins analysed were essentially water soluble vitamins which are known to be heat labile. The samples were subjected to cooking to hasten the coagulation process during the production. Sample TC had the highest vitamin C (2.69 mg/100 g) content, while sample MC had the highest vitamin B2 (29.21 mg/100 g) and B1 (6.07 mg/100 g) contents.

The soy cheeses must have derived some of the vitamins from the different coagulants, especially the plants based coagulants. It has been reported by several researchers that moringa is very rich in water soluble vitamins. The alum salt coagulant (AC) produced the least vitamin content in the cheeses, as there was no contribution of vitamin from the salt coagulant.

Microbial contents of soy cheese samples

The mean bacteria and fungi (yeasts and mould) counts of the soy cheese samples that were studied, as shown in Table 6, ranged from 7.8 × 102 to 6.0 × 104 cfu/g and 1.0 × 101 to 5.5 × 101 cfu/g. There were significant differences ($p<0.05$) in the bacteria and fungi counts of the soy cheeses. Sample LC had the highest bacteria (6.0 × 104 cfu/g) and fungi counts (4.0 × 101 cfu/g), while TC and MC had the lowest bacteria (1.1 × 104 cfu/g) and fungi (1.0 × 101) loads, respectively. There were no coliform bacteria detected in the soy cheese samples. The microbiological quality of the samples is an indication of the hygienic conditions under which the product was handled and processed and points to the type of fermentation and subsequent spoilage envisaged when stored (Oboh and Omotsho, 2005; Onwuka, 2014).

Sensory evaluation of soy cheese samples

The mean sensory scores on the organoleptic preference for different soy cheese samples are shown in Table 7. There were significant differences ($p<0.05$) in the sensory attributes analysed between the different types of soy cheeses. Samples MC and AC had the highest scores of 7.76 and 7.31, respectively, for appearance attribute. Similar trends were obtained in the panellists' scores for attributes of flavour and texture. The difference in scores for texture among the soy cheeses implies that the coagulants had noticeable effect on the procedures.

Table 4. Mineral content of soy cheeses

Parameters (ppm)	AC	MC	TC	LC
Calcium	$41.03^{b}\pm0.65$	$29.00^{c}\pm0.74$	$31.02^{c}\pm0.59$	$58.01^{a}\pm0.60$
Phosphorus	$9.02^{c}\pm0.01$	$14.06^{a}\pm0.01$	$12.06^{b}\pm0.02$	$10.07^{c}\pm0.02$
Iron	$1.13^{b}\pm0.20$	$3.66^{a}\pm0.51$	$1.73^{b}\pm0.18$	$4.29^{a}\pm0.32$
Manganese	$0.6^{a}\pm0.03$	$0.11^{b}\pm0.04$	$0.26^{b}\pm0.01$	$0.34^{b}\pm0.01$
Calcium	$41.03^{b}\pm0.65$	$29.00^{c}\pm0.74$	$31.02^{c}\pm0.59$	$58.01^{a}\pm0.60$

*Means within a row with different letters are significantly different at $p < 0.05$: AC (Alum-potassium aluminium sulphate), TC (Tamarind seeds extracts), MC (*Moringa oleifera* seeds extracts), and LC (Lime juice).

Table 5. Vitamin contents of soy cheeses

Parameters (ppm)	AC	MC	TC	LC
Vitamin C	$1.07^{c}\pm0.65$	$2.43^{a}\pm0.74$	$2.69^{a}\pm0.59$	$1.88^{b}\pm0.60$
Vitamin B2	$2.31^{c}\pm0.01$	$29.21^{a}\pm0.01$	$22.34^{b}\pm0.02$	$19.62^{b}\pm0.02$
Vitamin B1	$1.10^{c}\pm0.20$	$6.07^{a}\pm0.51$	$4.82^{b}\pm0.18$	$3.70^{b}\pm0.32$

*Means within a row with different letters are significantly different at $p < 0.05$: AC (Alum-potassium aluminium sulphate), TC (Tamarind seeds extracts), MC (*Moringa oleifera* seeds extracts), and LC (Lime juice).

Table 6. Microbial content of soy cheeses

Parameters (cfu/g)	AC	MC	TC	LC
Total bacteria counts	$5.5^{c}\times10^{3}$	$1.1^{b}\times10^{4}$	$7.8^{d}\times10^{2}$	$6.0^{a}\times10^{4}$
Total fungi counts	$1.6^{c}\times10^{1}$	$1.0^{c}\times10^{1}$	$4.0^{b}\times10^{1}$	$5.5^{a}\times10^{1}$
Total coliform counts	Nil	Nil	Nil	Nil

*Means within a row with different letters are significantly different at $p < 0.05$: AC (Alum-potassium aluminium sulphate), TC (Tamarind seeds extracts), MC (*Moringa oleifera* seeds extracts), and LC (Lime juice).

Table 7. Mean sensory scores of soy cheeses

Parameters	AC	MC	TC	LC
Appearance	$7.31^{b}\pm0.63$	$7.76^{a}\pm0.45$	$5.54^{c}\pm0.70$	$7.56^{a}\pm0.50$
Flavour	$8.04^{b}\pm0.71$	$8.04^{b}\pm0.71$	$8.35^{a}\pm0.82$	$7.16^{c}\pm0.65$
Texture	$8.31^{a}\pm0.55$	$8.40^{a}\pm0.60$	$7.33^{b}\pm0.74$	$7.50^{b}\pm0.63$
Overall acceptability	$8.17^{b}\pm0.48$	$8.63^{a}\pm0.53$	$7.40^{c}\pm0.61$	$7.53^{c}\pm0.48$

*Means within a row with different letters are significantly different at $p < 0.05$; AC (Alum-potassium aluminium sulphate), TC (Tamarind seeds extracts), MC (*Moringa oleifera* seeds extracts), and LC (Lime juice).

Soy cheese samples MC and AC had the best overall acceptance scores of 8.63 and 8., respectively. The low acceptability of TC (7.40) and LC (7.53) coagulated with tamarind and lime, respectively, could be attributed to the impact these coagulants had on the appearances and texture of the soy curd (Barnes, 2010; Butler et al., 2010). Cai and Chang (1999) reported that acid coagulants tend to affect the taste and texture of tofu more than tofu coagulated by alkaline coagulants. The alum coagulant had no strong perceivable smell and taste in the soy cheese (AC). Salt coagulants have been reported to produce tofu that is tender but slightly brittle in texture (Gopalakrishnan et al., 2016).

Conclusion

The result of the study showed that *Moringa oleifera* seed cake extract was a better coagulant when compared with other coagulants used in the production of soy cheeses. *Moringa oleifera* coagulated soy cheese (MC) had superior sensorial quality and higher nutrient contents compared to the other soy cheeses. The alum salt coagulated soy cheese (AC), though lower in nutrient values, was organoleptically better than the tamarind (TC) and lime (LC) coagulated soy cheeses. Due to the high nutritional and health benefits of *Moringa oleifera*, the soy cheese produced could serve as functional food. Further research should be made to identify the contribution of each coagulant type on the nutritional quality of soy tofu. The production of soy tofu with probiotic advantages will be a welcome development.

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