Chemical and Nutritional Compositions of Cheese-Like Products from Tiger Nut and Soybean Fermented with Lactobacillus Plantarum Strain AMA2A

Abu, M. A.*, Amakoromo, E. R. and Eruteya. O. C.

Department of Microbiology, Faculty of Science, University of Port Harcourt, Choba, Port Harcourt, Rivers State, Nigeria.
**Correspondence Author*

***Abstract*: Cheese has been known for long a time in human diet. It is a rich supply of protein and fat which make it nutritious and energy rich food that is suitable for people at all ages. Current advances in food science have revealed the role of cheese in nutrition and health. Apart from protein and energy, cheese has a high contribution of vital nutrients such as amino acids, vitamins, bioactive peptides, fatty acids, and minerals. The aim of this study was to evaluate the chemical and nutritional compositions of cheese-like products from tiger nut and soybean milk fermented with *Lactobacillus plantarum* strain AMA2A. Tiger nut milk and soymilk were produced from tiger nut tubers and soybean seeds respectively, using Standard methods. The milk was mixed for cheese production at different ratios, 100:0, 80:20, 60:40 and 0:100. The four samples were each inoculated with 4%v/v *Lactobacillus plantarum* strain AMA 2A. *Calotropis procera* extract serving the purpose of coagulant was cautiously added to the mixture and allowed to ferment for 24 hours for cheese formation. The chemical and nutritional compositions were determined using standard methods. Analysis of the cheese-like samples revealed the following ranges of proximate compositions: Moisture (36.54±0.67 - 50.55±0.64%), Carbohydrate (4.65±1.04 – 29.88±2.12%), protein (12.50±1.13 - 34.86±1.07%), ash (0.22±0.00 - 1.91±0.06%), fat (7.13±0.18 - 24.43±2.02%) and crude fibre (0.200±0.00 – 0.815±0.40%). The mineral contents of the products had the following range (mg/kg): Calcium (23.93±1.17 – 55.74±0.79), magnesium (93.08±4.50 – 124.11±0.42) potassium (282.55±3.61 – 509.86±0.21), sodium (19.40±0.92 -34.26±1.17) iron (0.23±0.03 – 4.75±0.26) and manganese (15.71±1.00 – 59.45±2.26). The study revealed that the chemical and nutritional compositions of tiger nut-soybean cheese are such that can substitute dairy cheese in human nutrition.**

***Keywords*: Cheese, fermentation, Lactobacillus plantarum, soybean, tiger nut**

I.INTRODUCTION

Cheese is a semi-solid product obtained when dairy milk is fermented and gelatinized through the activities of microorganisms, heat or enzymes. It could be formed by whole milk or skimmed milk by removing large portion of whey while retaining the coagulum and the entrapped milk solids [1, 2]

Cheese which is customarily made from cow milk comes in a wide range of textures and flavours [3]. Apart from the conventional cow milk, milk from buffalos, sheep and goats are also used in cheese making. Cheese is seen to have reasonable amounts of protein, fats and minerals like iron, calcium, and phosphorus, essential amino acids, and vitamins. Due to these nutritional endowments of cheese, it is therefore an all-important food for individuals of all ages. In fact, its protein content especially, makes one feel more satisfied after eating a meal of it [4] - [7], [1], [8,9].

It is important to note that though dairy cheese comes with a lot of nutritional benefits, its elevated fat content is a big concern in nutrition. Because of this challenge, there is an on the rise demand for vegetable milk such as tiger nut and soymilk and their products with lower fat content. Apart from the fat content as mentioned, plant-based milk and their products are currently in high demand due to incidence of animal milk protein allergies [10, 11].

Soymilk is an excellent milky extract obtained from soybean (*Glycine max)* containing high quality protein. It is lactose free and as such, may be employed in the formulation of diet for lactose intolerant individuals. Also, soymilk can effectively be combined with cereals in food formulation due to its high protein high protein intensity and balanced amino acid pattern [12]

Tiger nuts, though called 'nuts,' are tubers rich in starch and fat, (20-30%) and (20-28%) respectively. It has a diminutive amount of protein which is roughly twice that of cassava. The tubers have high fat content and gross energy, as a result, they are better compared with nuts than cereals which also belong to the same order [13] – [16].

Lactobacillus species are members of the family Lactobacillaceae. They are non-spore forming, psychrophilic, rod shaped, non-motile, Gram positive and facultative anaerobes lacking catalase enzyme [17]. *Lactobacillus plantarum,* a member of Lactobacillaceae is commonly used in fermentation of products such as meat, dairy and plant. It is found in fermented food products such as sauerkraut, cheese, yogurt, pickles, sourdough, and kimchi. The stability between volatile and non –volatile organic acids in this bacterium brings about the aroma and taste in fermented foods. The ability of *L. plantarum* to secret bactreiocins (antimicrobial compounds) makes it to be considered as a probiotic. The bacteriocins produced by the bacterium inhibit pathogenic and spoilage agents from proliferating [18, 19].

The aim of this study was to evaluate the chemical and nutritional compositions of cheese-like products from tiger nut and soybean fermented with *Lactobacillus plantarum* strain AMA2A.

II. MATERIALS AND METHODS

II.1 Sample collection

Dry yellow tiger nuts (*Cyperus esculentus)* tubers and soybean seeds (*Glycine max*) were purchased from Choba Junction Market, Rivers State, Port Harcourt. The samples were taken in sterile nylon bags to Microbiology Laboratory, University of Port Harcourt for analysis. The starter culture, *Lactobacillus plantarum* AMA2A used in this study was previously isolated by the authors.

2.2.1 Coagulant Preparation

The coagulant used in the manufacture of cheese-like samples in this study was prepared using the method of [20]. Sodom apple (*Calotropis procera)* leaves were collected, weighed, washed, squeezed by hand and comprehensively mixed with fresh clean water. The ratio of the leaves weight (g) to water (ml) was 1:1. The resulting extract was collected after five minutes through filtration of the mixture. The extract was kept in the refrigerator to sediment for 72 hours. The supernatant of the extract separated and then used as coagulant.

2.2.2 Production of milk from tiger nut and soybean

The dry yellow tiger nut tubers and the soybean seeds were sorted by hand-picking stones, broken seeds and other foreign particles. The tubers and the seeds were thoroughly washed and soaked overnight to swell, after which the method of [21] was used in tiger nut milk preparation, whereas soymilk preparation was carried out using the method of [22].

2.2.3 Production of Cheese-like Samples

The extracted tiger nut milk (TM) and soymilk (SM) were combined at different ratios, (TM: SM); 100: 0, 80:20, 60:40 and 0:100 to obtain tiger nut-soymilk mixture (TSM). The combo was blended in a food blender operated at full speed for 10 minutes. The final blends were heated with stirring to 95^oC for 20 minutes and allowed to cool to 43^0C. A 4% (v/v) of the starter culture (*Lactobacillus plantarum* strain AMA2A) was inoculated into the various blends and allowed for fermentation for 18 hours. A 100 ml coagulant was added to each of the blends and stirred with heating. With alternating stirring, heating continued until boiling point. The heating of the curds was maintained at boiling point for about 10 minutes until it totally coagulated and there was observable separation of whey and curd. The free curds were poured into a sieve to facilitate whey drainage and to mold into shapes [20].

2.3 Proximate composition

The moisture, fat, crude fibre and ash contents were determined using the method of [23]. Protein content was determined by Kjeldahl method (6.25×N) and total carbohydrate was calculated by difference.

2.3.1 PH and TTA

The pH of the samples was determined using a pH meter (Jenway 3505, Bibby Scientific, London, UK), while the titratable acid (TTA) content was determined using the method of [24]. Four (4) drops of phenolphthalein indicator were added to the sample and 0.1 M NaOH was added until there was a colour changed to pink, suggestive of the endpoint. The percentage titratable acidity of the lactic acid was obtained by multiplying the volume of NaOH added by a factor of 0.09.

2.3.2 Mineral composition

The mineral content of the cheese was determined as described by [25], using the Inductively Coupled Plasma (ICP) spectroscopy. Samples were acid-digested by the addition of 1 mL of 55% (v/v) HNO_3.

III. RESULTS

The result of the proximate composition of cheese-like products from tiger nut and soybean milk fermented with *Lactobacillus plantarum* strain AMA 2A is presented in Table 1. The moisture content of the various samples ranged from 36.54 ± 0.67 to 50.55 ± 0.64%. There was no significant difference (P< 0.05) between 100% tiger nut cheese (TNC), 80% tiger nut + 20% soybean cheese (TSC1) and 60% tiger nut + 40% soybean cheese (TSC2) in moisture content. Pure soybean cheese (SBC) sample was significantly lower than other samples in moisture content.

Carbohydrate content ranged from 4.65 ± 1.04 to 29.88 ± 2.12%; protein ranged from 12.50 ± 1.13 to 34.86 ± 1.07%; ash 0.22 ± 0.00 to 1.91 ± 0.06%; fat 7.13 ± 0.18 to 27.86 ± 1.15%; crude fibre 0.200 ± 0.00 to 0.815 ± 0.04% and finally energy content ranged from 232.90 ± 3.39 to 330.63 ± 0.66kcal. There was significant difference (P<0.05) in carbohydrate and protein compositions between the cheese samples. The range of protein content between samples in this study was (12.50 ± 1.13 to 34.86 ± 1.02%).

The result of the mineral composition of the cheese-like products fermented with *Lactobacillus plantarum* AMA 2A is presented in Table 2. The minerals evaluated in this study were calcium, magnesium, potassium, sodium, iron and manganese. The ranges of the evaluated minerals were as follows: Calcium (23.93±1.17 to 55.74±0.79 mg/kg), magnesium (93.08±4.50 to 124.11±0.42 mg/kg), potassium (282.55±3.6 to 509.86±0.21 mg/kg), sodium (19.40±0.92 to

34.26±1.17 mg/kg), iron (0.23±0.03 to 4.75±0.26 mg/kg) and manganese (15.71±1.00 to 59.45±2.26 mg/kg). The most abundant among the evaluated minerals was potassium, followed by magnesium, then calcium. The least abundant mineral was iron (Table 4). The result of the pH and titratable acidity of the cheese-like products fermented with *Lactobacillus plantarum* strain AMA2A is presented in Table 3. From the result, the pH and the titratable acidity ranged from (5.15 ± 0.71 to 5.55 ± 0.00) and (0.35 ± 0.01 to 0.48 ± 0.02 g/l), respectively. The highest pH value was obtained for TSC2 sample, whereas the lowest pH value was observed for TNC sample. The pH of the cheese samples was generally within acidic region (5.15 ± 0.71 to 5.55 ± 0.00). The highest titratable acidity value was recorded for SBC sample. There was no significant difference (P>0.05) among the cheese samples in terms of pH. Also, in terms of titratable acidity, there was no significant difference (P>0.05) between TNC and TSC1 samples, likewise TSC2 and SBC samples.

Table1: Proximate composition of cheese-like products

Sample	Moisture (%)	CHO (%)	Protein (%)	Ash (%)	Fat (%)	Crude fibre (%)	Energy(kca)
TNC	50.25±1.06[a]	29.88±2.12[a]	12.50±1.13[a]	0.22±0.00[a]	7.13±0.18[a]	0.200±0.00[a]	232.90±3.39[a]
TSC1	50.55±0.64[a]	14.77±0.21[b]	15.25±0.35[b]	0.85±0.01[b]	18.33±0.10[b]	0.250±0.01[a, b]	285.05±3.15[b]
TSC2	50.53±2.87[a]	4.65±1.04[c]	18.21±0.85[c]	1.91±0.06[c]	24.43±2.02[c]	0.285±0.04[b]	311.26±2.93[b, c]
SBC	36.54±0.67[b]	26.01±0.69[d]	34.86±1.07[d]	1.90±0.13[c]	27.86±1.15[d]	0.815±0.04[c]	330.63±0.66[c]

Key: TNC = (100% tiger nut cheese), TSC1= (80% tiger nut + 20% soybean composite cheese), TSC2= (60% tiger nut + 40% Soybean composite cheese) and SBC = Soybean cheese (100%). Values in Table- represent means and standard deviation from triplicate experiments. [a,b,c,d]…, mean values with the same superscript within a given column are not significantly different (P > 0.05).

Table 2: Mineral composition of the cheese-like products

Sample	Ca(mg/kg)	Mg(mg/kg)	K(mg/kg)	Na(mg/kg)	Fe(mg/kg)	Mn(mg/kg)
TNC	23.93±1.17[a]	93.08±4.50[a]	282.55±3.6[a]	19.40±0.92[a]	0.23±0.03[a]	32.61±1.22[a]
TSC1	32.86±1.77[b]	107.28±1.10[b]	313.10±3.68[b]	22.35±0.49[a]	0.95±0.00[a]	41.29±1.01[b]
TSC2	40.58±1.44[c]	115.00±6.36[b, c]	488.77±18.25[c]	30.30±3.54[b]	3.41±0.63[b]	59.45±2.26[c]
SBC	55.74±0.79[d]	124.11±0.42[c]	509.86±0.21[c]	34.26±1.17[c, b]	4.75±0.26[c]	15.71±1.00[d]

Key: TNC = (100% tiger nut cheese), TSC1= (80% tiger nut + 20% soybean composite cheese), TSC2= (60% tiger nut + 40% Soybean composite cheese) and SBC = Soybean cheese (100%).

Values in Table- represent means and standard deviation from triplicate experiments. [a,b,c,d]…, mean values with the same superscript within a given column are not significantly different (P > 0.05).

Table 3: Physico-chemical parameters of the cheese-like products

Sample	pH	Titratable Acidity(g/l)
TNC	5.15±0.71[a]	0.35±0.01[a]
TSC1	5.40±0.00[a]	0.36±0.00[a]
TSC2	5.55±0.01[a]	0.48±0.01[b]
SBC	5.35±0.21[a]	0.48±0.02[b]

Key: TNC = (100% tiger nut cheese), TSC1= (80% tiger nut + 20% soybean composite cheese), TSC2= (60% tiger nut + 40% Soybean composite cheese) and SBC = Soybean cheese (100%). Values in Table- represent means and standard deviation from triplicate experiments. [a,b,c,],.. mean values with the same superscript within a given column are not significantly different (P > 0.05).

IV. DISCUSSION

The low moisture content of SBC sample compared to other samples could be as a result of high protein level of soybean which may be the reason for the decreased moisture content of the sample. This result is similar to the reports of the previous authors [26] The range of carbohydrate content of the samples in this study (4.65±1.04 to 29.88±2.12), is lower than the one obtained by [27] in a related study which was found to be 45.73±0.035. This could be since soybean has low carbohydrate, hence the low carbohydrate content of the product.

The protein range in this study (12.50 ± 1.13 to 34.86 ± 1.07%) is substantially higher than the protein content (8.07%) reported by [28] in a related study. The increase in the protein level may be contributed by fermentation time and the activities of the starter cultures used both singly and as consortium which may have improved the protein level of the cheese by reducing the levels of anti- nutritional factors (e.g., chymotrypsin and trypsin inhibitors) that inhibit digestive enzymes. This promotes protein cross linking, resulting in increased product protein level.

Of the six minerals analyzed, iron had the lowest content with the range (0.23±0.03 to 4.75±0.26). Balogun [26] also reported lowest iron content (11.03-14.67 mg/kg), though higher than the one obtained in this study. Fe is a component of hemoglobin, and it helps in cognitive development and immune function [29]. Calcium and potassium are necessary for the formation of bone and teeth in growing children. The high potassium level can also help in lowering high blood pressure and take care of osteoporosis as reported by [26] and [30]. Tiger nut is rich in potassium and calcium, and as a result, the consumption of tiger nut is of great benefit as it supplies these very important minerals in the body.

There was no significant difference (p≥0.05) in pH between the cheese samples. The pH values of all the samples were within acidic medium, ranging from5.15±0.71 to 5.55±0.01. This could be since tiger-nut milk is acidic, the pH range in this study agrees with the findings of [26].

The TTA values in this study were slightly higher than the values reported by [1] for cow-coconut milk cheese. The pH and Titratable acidity are two unified concepts in food examination that deal with acidity. While pH is vital for microbial growth evaluation, titratable acidity reveals how organic acids in the food impact flavour [31].

V. CONCLUSION

Lactobacillus plantarum strain AMA 2A was used in the production of cheese-like products using tiger nut and soybean milk. The chemical composition result of the produced cheese showed that possible fermentation leading to substantial improvement in chemical composition may have been because of the activities of the fermenting organism (*Lactobacillus plantarum* strain AMA 2A). The cheese samples produced had no significant difference (p≥0.05) in moisture except SBC (100% soybean cheese) sample which was significantly lower than other samples in moisture content. The protein content was highest in SBC (100% soybean cheese) and lowest in TNC (100% tiger nut). The most abundant among the evaluated minerals was potassium, followed by magnesium, then calcium. The least abundant mineral was iron.

Producing cheese from tiger nut and soybean milk blend may be a positive step in the right direction as this may bring value addition and to further promote the utilization of tiger nut and soybean among other indigenous crops beyond conventional usage.

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