



Microbiological and Nutritional Analyses of Soybean Cake (*Awara*) and Camel Milk Cheese (*Chukwui*) Local Snacks, Vended in Kano Metropolis-Nigeria

¹Idris, A. and ²Dabo, N.T*p.

¹Department of Microbiology and Biotechnology, Federal University Dutse, P.M.B 7156, Dutse-Nigeria.

²Department of Microbiology, Bayero University Kano, P.M.B 3011, Kano-Nigeria.

Corresponding author: ntdabo@gmail.com; adbakof@gmail.com

Abstract

A study on the microbiological and nutritional composition of soybean cake (*Awara*) and camel-milk cheese (*Chukwui*) vended in Kano metropolis was carried out between September 2014 and March 2015. Two hundred samples were subjected to microbiological evaluation using standard procedures. Selective media were used to isolate some specific pathogens, and antibiotic sensitivity profiles of the isolates were determined using the disc-diffusion method. Proximate analyses were also carried out on 50 representative samples using the Official Methods of Analysis of the AOAC. Results obtained indicated that soybean cake had mean aerobic mesophilic bacterial count of 1.37×10^3 cfu/g, mean fungal count of 2.37×10^4 cfu/g and mean coliform count of 17.65 cells/g compared to camel-milk cheese which had higher aerobic mesophilic bacterial count of 4.50×10^4 cfu/g, mean fungal count of 5.93×10^4 cfu/g and mean coliform count of 42.70 cells/g. Pathogenic bacteria isolated from the samples include *Escherichia coli* 13(50.00%), *Staphylococcus aureus* 8(30.77%) and *Salmonella typhi* 5(19.23%). *S. aureus* exhibited the highest antibiotic resistance compared to the other pathogens isolated. Proximate analysis revealed that soybean cake had higher mean percentages for moisture, protein, fat, fibre and ash than Camel-milk cheese. The isolation of some pathogenic agents from these snacks point to a potentially negative implication on public health, this suggests the need for more hygienic vigilance in the preparation and handling of these snacks.

Key words: *Awara*, Camel-Milk Cheese, *Chukwui*, Kano, proximate analysis, Soybean Cake.

INTRODUCTION

Cheese is essentially milk curd- substance formed from the coagulation of milk by rennet, pressed or molded into a solid mass (Kosikowski and Mistry, 1997). Different cheese types originate from the milks of different dairy animals (LaBarbera, 2012). Cheese contains concentrated milk solids, water, rennet, salt and sometimes bacterial cultures and calcium chloride (Farah and Fischer, 2004). Cheese can also be prepared from non dairy products such as soybean (Schaeffer, 2012). Soybean cheese becomes is a healthy, rich and less expensive source of nutrient especially for the developing countries (Nazim *et al.*, 2013).

Cheese may be analyzed for quality control purposes including the confirmation of

microbiological safety and quality (Tamime *et al.*, 2011). Soybean cake is made from a legume, and legumes are known to be very rich sources of protein (Yusuf and Ali, 2013). Soybean cake is known to have high protein content, while camel-milk cheese has high fat content (Nazim *et al.*, 2013).

Studies reported in the literature on microbial analysis of cheese-like products made from soybean have revealed the incidences of varying microbial types and loads. For instance, Adetunji and Babalobi (2011) found high aerobic microbial counts of 7.43 log cfu/ml and 7.34 log cfu/ml in soybean cake when prepared using *Cymbopogon citratus* and *Calotropis procera* extracts respectively as the coagulating agents.

Similarly, Zumbes *et al.*, (2014) observed a high mean mesophilic bacterial count of 5.16×10^5 cfu/g in Soybean cake in Jos, Nigeria. Falegan (2014) also isolated *Salmonella* spp. and *E. coli* in soybean cake from Ado-Ekiti, Nigeria. In another study, Ogbolu *et al.*, (2014) isolated *E. coli* in Soybean cake. Contaminating microorganisms in Camel-milk cheese may include pathogens such as *E. coli*, *Staphylococcus* spp., *Salmonella* spp. and *Shigella* spp. which may cause health problems to human beings (Yam *et al.*, 2014).

Onuorah *et al.*, (2007) recorded a protein content of 8.0%, a fat content of 4.38%, and an ash content of 0.79% for soybean cake produced using the traditional oriental method of production. While Oladipo and Jadesimi (2013) carried out a proximate analysis on samples of soybean cake and recorded 6.18% crude protein, 3.02% fat, 2.75% ash and 69.23% moisture.

Antibiotic sensitivity test carried out on *S. aureus* isolated from soybean cake in Osun state, Nigeria by Adeleke *et al.*, (2014) showed that some of the isolates were resistant to Erythromycin and Amoxicillin, and may have been introduced into the soybean cake due to poor hygiene during processing or handling. In a similar vein, Falegan (2014) tested the resistance of *Salmonella* spp. isolated from 20 local cheese samples to some antibiotics and reported that 10 (87%) isolates were resistant to Amoxicillin, 5 (41%) isolates were resistant to Augmentin and Gentamycin.

Soybean cake and camel-milk cheese are two snack foods that are widely accepted as casual cheese snacks by the Kano community. However, knowledge is scarce pertaining the microbiological and nutritional qualities, as well as the hygienic status of these products. A study of this nature would therefore add to the pre-existing information on the food safety of our environment thereby improving the public health.

The aim of this study was to determine the microbiological quality and proximate

composition of soybean cake and camel-milk cheese vended in Kano metropolis.

MATERIALS AND METHODS

Study Area

The study was carried out in Kano metropolis located on latitude 12.000°N and longitude 8.517°E. Samples were collected from Kano Municipal, Dala, Gwale, Fagge and Kumbotso which comprise the busiest parts of the city.

Sample Collection and Handling

A 100 sample each of soybean cake (*Awara*) and camel-milk cheese (*Chukwui*), were collected following the criteria used by Zumbes *et al.*, (2014) and Adeleke *et al.*, (2014), from different points around the study area between September 2014 and March 2015. The samples were collected in sterile polythene bags and transported immediately to the Biological Sciences research laboratory of Bayero University Kano.

Microbiological Evaluation

Aerobic Mesophilic Bacterial Count

Serial dilution was carried out on the homogenized samples and from the serially diluted tubes, one milliliter (1ml) of the dilution was transferred to each of appropriately labeled Petri dishes and followed by pouring aseptically, molten nutrient agar. The dishes were homogenized by swirling and then the media was allowed to solidify. They were then incubated at 37° C for 24 hours. Following incubation, plates that yielded between 30-300 colonies were selected and colonies counted. The number obtained was multiplied by the inverse of dilution factor to get the cfu/g.

Fungal Count

The same procedure as above was used for the fungal count but Malt Extract Agar (MEA) was used and the plates were incubated at room temperature for 3-5 days.

Coliform Count

Coliform count was carried out according to the method described by Atlas, (1997). This consists of a set up of 9 test tubes each containing 9 ml of sterilized lactose broth and an inverted Durham tube.

Serially diluted samples were used to inoculate the test tubes. The test tubes were then incubated at 37° C for 24 hours to indicate the presence or absence of gas in the tubes, based on which comparisons were made using the MPN (most probable number) table to estimate the most probable number of coliforms present in each sample.

Isolation of some Pathogens

The media used for the isolation of pathogens include Eosin Methylene Blue (EMB) agar, Salmonella-Shigella (SS) agar and Mannitol Salt Agar (MSA). Isolation was followed by microscopic examination and biochemical tests on the isolates. The biochemical tests include citrate utilization test, indole test, methyl red test, oxidase and triple sugar iron (T.S.I) test, catalase and coagulase tests.

Antibiogram of Isolates

This was achieved using disc diffusion method as described by Hudzicki, (2013). Broth culture of the test organisms equivalent to 0.5 McFarland standard were used to inoculate plates of Mueller-Hinton agar using sterile swabs. Antibiotic impregnated discs containing the antibiotics: Gentamycin, Amoxicillin, Ciprofloxacin, Streptomycin, Septrin, Erythromycin, Streptomycin, Augmentin and Nalidixic acid were then placed on the inoculated Mueller-Hinton agar plates and incubated at 37° C for 24 hours. After incubation, zones of inhibition were measured and recorded.

Proximate Analysis

Twenty five (25) representative samples each of soybean cake and camel-milk cheese were subjected to proximate analysis for moisture, crude protein, crude fat, ash and fiber.

Moisture was determined according to the method described by Helrich (1990). A porcelain crucible was oven dried at 100°C for 12 hours, weighed as W₁ and allowed to cool. Two grams (2.0g) of the ground sample was weighed (as W₂) into a porcelain crucible and placed into an oven, earlier preheated to 100°C for 3 hours and left uncovered overnight at 100°C. The container

with cover and the dried sample were cooled and weighed as W₃.

$$\% \text{ Total DM} = \frac{W_3 - W_1}{W_2}$$

$$\% \text{ moisture} = 100 - \% \text{ Total DM}$$

Ash was also determined according to the method described by Helrich (1990). The dried and ground sample was ignited in a muffle furnace at 600°C to oxidize all organic matter. An oven dried crucible was weighed in a desiccator as W₁. The crucible and 2.0g of the sample were weighed together as W₂. The sample was then ashed in a muffle furnace at 600°C for 2 hours, then the crucible and ash were cooled and weighed as W₃.

$$\% \text{ Ash} = \frac{W_3 - W_1}{W_2} \times 100$$

Crude protein determination was achieved via three successive steps including digestion, titration and distillation.

Digestion: Fifteen (15) ml concentrated sulfuric acid was added to 2.0g of the sample contained in a digestion tube. After mixing the acid and the sample, 5.0g kjeldahl catalyst was added to the mixture to boil for two hours. The content was allowed to cool and then transferred into 100ml volumetric flask and diluted with distilled water.

Distillation: Ten (10) ml of 2% boric acid and two drops of mixed indicator were measured into 100 ml conical flask. Ten (10) ml of the digest was transferred into a distillation apparatus and 15 ml of 40% NaOH was added into the mixture. The resulting mixture was then distilled into the boric acid/indicator flask for 15 minutes.

Titration: The distillate was titrated with standard 0.025 N sulfuric acid to a pink end point and the burette reading was taken (TV) (Helrich, 1990).

Finally, percentage crude protein of the samples were determined by multiplying the % nitrogen content of the samples by a factor of 6.25.

$$\% \text{ Crude Protein} = \% \text{ N} \times 6.25$$

$$\% \text{ N} = \frac{0.4 \times \text{Titer Value (TV)} \times \text{Volume of digest (100ml)} \times \text{Normality of acid (0.025)}}{\text{Weight of sample (0.2g)} \times \text{Volume of aliquot used (10ml)}} \times 100$$

In crude fat determination, a Soxhlet extractor which is composed of an extraction chamber, conical flask and a condenser was used. A filter paper thimble was weighed as W_1 . Two grams (2g) of the sample was weighed and placed into the thimble as W_2 . Two hundred and fifty milliliters (250 ml) of petroleum ether was added using glass funnel from the top of a condenser. After four hours of extraction the ether that condensed in the thimble was allowed to drain out of the thimble for 30 minutes at 70°C. The thimble was cooled in a desiccator, weighed and recorded as W_3 (Helrich, 1990).

$$\% \text{ Crude fat} = \frac{(W_1 - W_2) - W_3}{W_2} \times 100$$

In crude fiber determination, two grams (2.0g) of sample was transferred to a 9cm hard filter paper. After fat extraction, the sample was transferred quantitatively by brushing into a 600 ml beaker. Two hundred milliliters (200 ml) of 1.25 % sulfuric acid was added to the beaker and boiled for 30 minutes. The contents were then filtered through a California Buchner funnel, rinsed with 75 ml of boiling water and washed through funnel, and then with three 50 ml portions of water, and suck dry. The residue was returned to beaker and treated with NaOH solution the same way as the acid. The fiber mat was washed with 25 ml of boiling 1.25% sulfuric acid solution, three 50 ml portions of water, and 25 ml of alcohol. The fiber mat and the residue were dried at 130±2° C for two hours. They were then cooled in a desiccator, weighed as W_2 and ignited at 600° C to constant weight for 30 minutes, cooled again in a desiccator and weighed as W_3 (Holst, 1982).

$$\% \text{ Crude fiber} = \frac{W_2 - W_3}{W_1} \times 100$$

Statistical Analysis

Statistical analysis was performed using GraphPad® InStat® software (version 3.0) to determine means, standard deviations and differences between means was scored by t-

test. Significant difference was set at $p < 0.05$.

RESULTS AND DISCUSSION

Results for aerobic mesophilic, fungal and coliform counts are presented in table 1. Mean aerobic mesophilic count for soybean cake (*Awara*) was 1.37×10^3 cfu/g and lower than that for camel-milk cheese which was 4.50×10^4 cfu/g. This observation was similar to that obtained for fungal and coliform counts. They were lower in soybean cake (2.37×10^4 cfu/g and 17.5 cells/g respectively) than those obtained for camel-milk cheese which were (5.93×10^4 cfu/g and 42.70 cells/g respectively). There were statistically significant differences in microbial counts between the two sample types.

The mean total bacterial count from soybean cake in this study (1.37×10^3 cfu/g) is lower than that of a study in Kano, Nigeria by Bukar *et al.*, (2010), who obtained a mean aerobic mesophilic count of 5.80×10^5 cfu/g in soybean cake. Exposure of the food to the open environment and the consequent contact with air, soil and dirty hands of sellers and buyers, as well as the use of contaminated containers and other post production processes might have contributed to a high contamination level. According to the Food and Agricultural Organization, FAO (1979), the standard limit for aerobic mesophilic bacterial count in food should be less than 10^5 cfu/g/ml. In this study, several samples exceeded the FAO standard limit of bacterial count. However, the total means of the bacterial counts from the unit samples was just below the standard limit.

The microbial load obtained from the analysis of camel-milk cheese (4.50×10^4 cfu/g) proved to be the highest of the two sample types. But the load is lower than the 9.44×10^5 cfu/g detected by Derar and El-Zubeir (2013) from camel-milk cheese samples. Dried samples were used in this study, whereas Derar and El-Zubeir (2013) used samples stored in whey for 21 days prior to examination.

Table 1: Aerobic Mesophilic, Coliform and Fungal Counts for Soybean Cake and Camel-Milk Cheese Samples Vended in Kano Metropolis.

Food Item	N	Aerobic Mesophilic count (cfu/g)	Fungal count (cfu/g)	Coliform count (cells/g)
Soybean cake	100	$1.37 \times 10^3 \pm 2.59^a$	$2.37 \times 10^4 \pm 2.20^a$	17.65 ± 0.57^a
Camel-Milk cheese	100	$4.50 \times 10^4 \pm 2.59^b$	$5.93 \times 10^4 \pm 1.96^a$	42.70 ± 0.27^b

$p < 0.05$ *Means with common superscript alphabet letters are not significantly different from each other within the same columns.

Key: N= Number of samples analyzed

A total of 13(50.00%) *E. coli* isolates were found in both samples, seven were from soybean cake and six were from camel-milk cheese. Whereas five *Salmonella* spp. isolates were recorded, four of these were from soybean cake while one was from

camel-milk cheese. Moreover, eight *S. aureus* (30.77%) isolates were found, five from soybean cake and three from camel-milk cheese (Table 2).

Table 2: Occurrence of the Pathogenic Bacteria Isolated from Soybean Cake and Camel-milk Cheese Samples from Kano Metropolis.

Type of sample	<i>E. coli</i> % Occurrence	<i>S. typhi</i> % Occurrence	<i>S. aureus</i> % Occurrence
SBC	26.92	15.38	19.23
CMC	23.08	3.85	11.54
Total	50.00	19.23	30.77

Key: SBC = soybean cake, CMC = camel-milk cheese

The frequency of isolation for *E. coli* in this work was 50%. Zumbes *et al.*, (2014) recorded a frequency of isolation of 40% (20 samples) for *E. coli* from the six food types they investigated which includes soybean cake. Zumbes *et al.*, (2014) examined an assortment of ready- to-eat food items. Falegan (2014) reported a lower rate of occurrence of 25% (5 isolates) for *E. coli* in local soybean cake in Ado-Ekiti, Ekiti State. A higher or lower rate of isolation could be due to differences in handling practices among vendors, for example the use of contaminated equipment or careless attitude toward hand washing (Kawo and Abdulmumin, 2009). This might equally explain a high rate of isolation for *Salmonella* spp. It is possible that microbial load increases in the stages between production and hawking (Ogbolu *et al.*, 2014). The number of *Salmonella* spp. positive samples from this work accounted for 19.23% of the total number of pathogens isolated. Falegan (2014) recorded the rate of occurrence of 60% for *Salmonella* spp. from 20 samples of soybean cake. Poor condition

of handling and a lower sample size may explain the higher rate of isolation in the latter work. *S. aureus* on the other hand was isolated with a frequency of 30.77% from the samples examined in this work, which was higher than what was obtained by Adeleke *et al.*, (2014). Adeleke *et al.*, (2014) studied a lower sample size (30). But nonetheless, this suggests a contamination brought about by a compromised hygienic standard.

Two isolates of *S. aureus* species were resistant. The first was resistant to Erythromycin (and it was isolated from camel-milk cheese) while the other was resistant to Amoxicillin (and it was isolated from soybean cake). One *E. coli* isolate was resistant to Gentamycin (and it came from soybean cake). Eight isolates among the three different bacterial species were within an intermediate zone of inhibition for some antibiotics. All the other isolates obtained from soybean cake and camel-milk cheese samples were found to be sensitive to all the antibiotic discs used (Table 3).

Adeleke *et al.*, (2014) found most organisms from local cheese to be sensitive to Gentamycin, yet showed multiple resistance to Amoxicillin and Erythromycin. On the other hand, all the isolates of *Salmonella* spp. in this study were not resistant to any of the antibiotics tested, but Falegan (2014)

found a *Salmonella* spp. isolate from a soybean cake to be resistant to many antibiotics including Amoxicillin and Augmentin. The difference might be as a result of differences in the two study areas, since genetic basis for resistance might occur at one place and not in another.

Table 3: Antibiotic Sensitivity Test for the Bacterial Isolates from Soybean Cake and Camel-Milk Cheese Samples from Kano Metropolis.

S/No	Name of Isolate	Resistance	Intermediate
1	<i>E. coli</i>	CN	AM, SXT,
2	<i>S. typhi</i>	E	AM, SXT, NA
3	<i>S. aureus</i>		CN
4	<i>S. aureus</i>	AM	CN

Key: E= Erythromycin, AM= Amoxicillin, CN= Gentamycin, SXT= Septrin, NA= Nalidixic Acid, CPX= Ciprofloxacin, S=Streptomycin, AU= Augmentin

Result of the proximate analysis showed that soybean cake had mean moisture content of 23.18%, mean crude protein (CP) of 35.58%, mean fat of 28.42%, mean crude fiber of 2.32% and mean ash content of 2.49% (Table 4). Camel-Milk cheese being a dry product had mean moisture content of 4.85%, mean crude protein (CP) of 18.34%, mean fat of 18.95%, and mean ash content of 0.97% (Table 4). There was a significant difference

($p < 0.05$) in mean composition between soybean cake and Camel-milk cheese. Nazim *et al.*, (2013) recorded higher values of 65.43% for moisture and 3.52% for ash, a lower value of 3.21% for fat and 20.38% for protein from soybean cake. Differences in composition between various ready-to-eat food samples could be due to differences in products or processing methods used (Sanni *et al.*, 1999).

Table 4: Mean Proximate Compositions of Soybean Cake and Camel-Milk Cheese from Kano Metropolis.

Sample	Mean % Ash	Mean % Moisture	Mean % Cp	Mean % Cf	Mean % Fat
Soybean cake	2.49 ^a ±0.15	23.18 ^a ±0.78	35.58 ^a ±0.35	2.32±0.25	28.42 ^a ±0.51
Camel-Milk cheese	0.97 ^b ±0.04	4.85 ^b ±0.32	18.34 ^b ±0.18		18.95 ^b ±4.02

$p < 0.05$ *Means with different superscript alphabet letters are significantly different from each other within the same columns, degrees of freedom is 8.

Key: Cp= Crude protein, Cf= Crude fiber,

CONCLUSION

From the results of this study, it can be concluded that camel-milk cheese had higher microbiological counts compared to soybean cake, since it recorded higher numbers of aerobic mesophilic, coliform and fungal counts. Camel-Milk cheese thus carries more microbial load while soybean cake had the

highest number of bacterial pathogens isolated from the two snack types, including *E. coli*, *S. aureus* and *S. typhi*. Moreover, the proximate constituents were higher in soybean cake, than in camel-milk cheese suggesting the latter to have a better nutritional quality than the former

RECOMMENDATIONS

There is the need for food/snack vendors to apply basic food hygienic practices to minimize the introduction of coliforms and other pathogens while selling these snack

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- foods. Finally, as data from the proximate analysis had proven the high nutritive quality of soybean cake, the public should patronize it more since it is more readily available in our community than animal protein.
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