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Chemical and Bacteriological Assessment of Soft Cheese Prepared from Raw Cow Milk in Ogbomoso, Nigeria

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The proximate, mineral content, total bacteria counts and specific bacteria organisms present in 25 samples of raw cow milk and soft cheese prepared from the same bulked raw cow milk were investigated. Results showed that the proximate composition of raw cow milk and soft cheese samples were 13.18 and 23.17% total solids; 3.60 and 3.35% fat; 9.58 and 19.82% solids not fat; 4.61and 2.56% crude protein; 2.89 and 3.83% lactose; 2.08 and 3.43% ash and 2.44 and 2.34% casein respectively. The mineral content values were, 1016.50 and 1602.40 mg/L for calcium; 893.00 and 971.00 mg/L for phosphorus; 126.50 and 122.30 mg/L for magnesium; 652.50 and 830.80 mg/L for sodium and 2200.10 and 2601.20 mg/L for potassium for raw cow milk and soft cheese samples, respectively. The raw cow milk samples had higher bacteria counts of 19.0 x 10^5, 9.0 x 10^6, 4.0 x 10^7 and 2.0 x 10^8 cfu/ml, while the bacteria counts in the soft cheese samples were, 3.0 x 10^5, 4.0 x 10^6 and 2.0 x 10^7 cfu/ml. In summary, soft cheese had higher concentration of nutrients than the raw cow milk and there were higher bacteria counts in the raw cow milk than in the soft cheese samples.

Key words: Raw cow milk, soft cheese, chemical composition, bacterial count.

In Nigeria, dairy herds are kept mainly by pastoral Fulani families who herd, manage and milk the lactating cows. The Fulani women are involved in the processing of the milk into various products such as cheese, butterfat and yoghurt. The significance of milk and milk products in human nutrition is now well established as they are considered as the best, ideal and complete food for all age groups (Kumar *et al*., 2011). It has also been observed that milk contains all vital nutrients like protein, fat, carbohydrates, vitamins, minerals and water (Olorunnisomo and Ibhaze, 2010). That milk is an excellent source of most essential minerals for humans and contains mostly calcium, phosphorus, magnesium, potassium and sodium has been reported by various authors (Tona *et al*. 2000; Belewu and Aiyegbusi, 2002). Though milk and milk products play important roles in human nutrition, they are highly susceptible to variety of micro-organisms because of their high nutritive value (Islam *et al*., 2009) and complex chemical composition (Grewal and Tiwari, 1990; Kumar and Prasad, 2010; Kumar *et al*. 2011). It is doubtless that milk is secreted by the cells of the udder in a sterile state but soon become contaminated by bacteria normally present in the udder (Islam *et al*., 2009). Adetunji *et al*., (2003a, b) mentioned that milk and milk products are good medium for the growth and transmission of many micro-organisms to humans. Milk could be easily contaminated with spoilage and pathogenic bacteria on animal skin, milking utensils and on the hands during milking (Alemede and Sadiq, 2008b). Other sources of milk contamination could be the soil, water and faeces that are residual on the skin of dairy cows (Woubit

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et al., 2001). The presence of *E. coli* (Belewu and Aina, 2000; Tasci, 2011; Kumar *et al*. 2011), *P. vulgaris* (Shittu *et al*. 2011) and *S. aureus* (Adetunji *et al*. 2003b; Alemede and Sadiq, 2008a) in milk products and other food items are indicative of possible presence of pathogenic or toxic micro-organisms which could constitute a public health hazard. Tasci (2011) stated that *S. aureus* is a ubiquitous organism that occurs in the mucous membranes and skin of most warm blooded animals including human beings. This author further reported that *S. aureus* is widely recognized as a major causative agent of clinical and subclinical mastitis in dairy cattle. That in food, the minimum numbers of *S. aureus* required to produce toxicity in human beings was estimated to be in excess of 10^5 cfu ml^{-1} and the staphylococcal toxins cannot be destroyed by heating, drying or freezing. The objective of our study was to evaluate the chemical composition, isolate, characterize and determine the total bacterial count (TBC) of bacterial contaminants in raw cow milk and soft cheese *prepared from the same raw cow milk*.

MATERIALS AND METHODS

Sampling location

A Fulani dairy herd in Abogunde village near Ogbomoso (8°15 2 ' and 4°152 '), Oyo State, Nigeria, was selected. The Fulani herdsmen in the village engage in the rearing of cattle, sheep and goats. The raw milk harvested from lactating ruminants is processed by the Fulani women into various products such as fermented milk, cheese, butterfat and yoghurt.

Sample collection and Processing

A total of 25 Bunaji cows were selected randomly from a Fulani dairy herd while the lactating cows were and hand milked into separate clean plastic buckets. About 20ml each of the raw cow milk samples were collected into well-labelled sterile bottles and kept in a cooler of ice-pack. The remaining raw cow milk was bulked together and processed into soft cheese. Twenty grams samples of the soft cheese was collected in triplicate into well-labelled sterile bottles and kept in a cooler of ice-pack. The raw cow milk and soft cheese samples were then transported for subsequent laboratory analysis.

Laboratory analysis

The raw cow milk and soft cheese samples obtained from the same source were analysed for proximate composition such as total solids, fat (Gerber method), protein and ash (AOAC, 2005). Percentage solids-not-fat (% SNF) was calculated by difference (% SNF = % TS - % Fat). Percentage lactose was calculated by difference (% Lactose = % SNF - % Protein - % Ash), as well as for casein (% casein = % Fat x 0.4 + 1.0) (Scott, 1981). Samples were analysed in triplicates. Mineral composition of the raw cow milk and soft cheese samples were analysed according to the methods of AOAC (2005).

Digestion of samples

Amounts of 0.5g of the raw cow milk and soft cheese samples were weighed into a set of digestion tubes and 10 ml each of perchloric and nitric concentrated inorganic acids were dispensed into the sample tubes. The samples were digested on the digestion block at 120°C for 2 hours, until the organic substances were completely decomposed. At the end of the digestion, the samples were allowed to cool to room temperature. Digested samples were made up to the 50 ml volume with deionized water and then transferred into centrifuge tubes, shaken for 10 minutes. The solutions were transferred to the centrifuge machine and centrifuged at 4500 rpm for 5 minutes. Finally, the supernatants were placed in duplicates in a set of pyrex glass vials and analysed for Ca, P, Mg, Na and K concentrations. The Ca and Mg were burnt off in an atomic absorption spectrophotometer (AAS) and the intensity of their flame was measured at the appropriate wavelength, current and pressure. Potassium and sodium were read off in the flame photometer. Phosphorus was measured calorimetrically using the Vanado-molybdate reagent (AOAC, 2005). The results were then expressed in mg/L.

Media Preparation

Eosine methylene blue agar, *Salmonella shigella* and Manitol salt agars were used to detect *Escherichia coli, P. vulgaris* and *Staphylococcus aureus* respectively. All the media were sterilized (a) 121° C and aseptic conditions were maintained accordingly. Serially diluted inocula were plated unto various media as earlier mentioned.

Isolation and culture conditions

One ml each of 10^{-4}, 10^{-5}, 10^{-6} and 10^{-7}

dilutions of the milk and cheese samples was inoculated on the sterile plate count agar (PCA) consisting of buffered peptone water at 37°C for 24 hours. The pour plate technique was used to enumerate the total bacteria count according to AOAC (2005).

Characterisation of Bacterial Isolates

Organisms were characterised by Gram-staining and using standard biochemical protocols such as the ability to produce enzyme myo-inositol gas catalase, glucose gas, sucrose gas, urease, hydrogen sulphide oxidase methyl red coagulase, haemolysis, sodium chloride and motility tests. All isolates were then identified using Bergey's Manual of Systematic Bacteriology (2004).

Statistical analysis

Analysis of the data was performed using Student's t-test (SAS, 2002). All statements of differences were based on significance at $P<0.05$.

RESULTS AND DISCUSSION

Proximate composition of raw cow milk and soft cheese samples

The proximate composition of raw cow milk and soft cheese are presented in Table 1. The proximate composition of the raw cow milk were, 13.18% TS; 4.02% fat; 9.16% SNF; 3.61% CP; 4.77% lactose; 0.78% ash and 2.61% casein. These values were comparable to the values of 4.10% fat, 3.57% CP, 5.57% lactose and 0.57% ash observed in the fresh milk from White Fulani cows reported by Olorunisomo & Ibhaze (2010). The values obtained for the proximate composition of raw milk of Bunaji cows in this study also fell within the ranges of 9.21–10.83% TS, 3.78–5.07% fat, 4.59-6.47% SNF, 3.46-3.58% CP, 6.80-9.04% lactose and 0.82-0.99% ash reported by Ndubueze *et al.* (2006) in White Fulani cows. Soft cheese had significantly ($P<0.05$) higher values of TS, SNF, CP, lactose and ash than the raw cow milk. This was expected since the raw milk had higher moisture content than the processed soft cheese, which was a more concentrated product. In other research, Olorunisomo & Ibhaze (2010) similarly reported that soft cheese samples had higher values of 33.10% fat, 24.60% CP, 5.60% lactose and 3.90% ash than the fresh White Fulani cow milk with 4.10% fat, 3.57% CP, 5.57% lactose and 0.57% ash. They attributed this to the higher moisture content in milk than in the soft cheese samples.

Table 1. Proximate composition of raw cow milk and soft cheese

%	Raw cow milk	Soft cheese
Total solids	13.18 ± 0.0019^{b}	23.17 ± 0.0016^{a}
Fat	4.02 ± 0.0002^{a}	2.85 ± 0.0022^{b}
Solids-not-fat	9.16 ± 0.0016^{b}	20.32 ± 0.0011^{a}
Crude Protein	3.61 ± 0.0016^{b}	6.88 ± 0.0016^{a}
Lactose	4.77 ± 0.0013^{b}	10.68 ± 0.0019^{a}
Ash	0.78 ± 0.0016^{b}	2.76 ± 0.0013^{a}
Casein	2.61 ± 0.0031^{a}	2.14 ± 0.0015^{b}

Values are means ± standard deviation; $^{a, b}$ values with different superscript along the same row are significantly ($P<0.05$) different from one another

Mineral composition of raw milk and soft cheese samples

Table 2 shows the mineral composition of raw cow milk and soft cheese. The mineral contents of raw cow milk were as follows: 1016.50 mg/L of Ca; 893.00 mg/L of P; 126.50 mg/L of Mg; 652.50 mg/L of Na and 2200.10 mg/L of K. These results fell within the range of 900.00 to 1055.00 mg/L of Ca; 850.00 to1050.00 mg/L of P; 100.00 to 125.00 mg/L of Mg; 430.00 to 560.00 mg/L of Na and 1700.00 to 2100.00 mg/L of K in Bunaji cows, reported by Tona *et al.* (2000). The mineral contents in the soft cheese samples were significantly ($P<0.05$) higher as follows: 1602.40 mg/L of Ca, 971.00 Mg/L of P, 830.80 mg/L of Na and 2601.20 mg/L of K but had significantly ($P>0.05$) lower value of 122.30 mg/L of Mg than that contained in the raw cow milk. This might be due to the concentration of the mineral element in the soft cheese than in the liquid raw cow milk.

Table 2. Mineral composition of raw milk and soft cheese

Mineral Contents (Mg/L)	Raw cow milk	Soft cheese
Calcium	1016.50 ± 0.016^{b}	893.00 ± 0.133^{a}
Phosphorus	1602.40 ± 0.016^{a}	971.00 ± 0.158^{a}
Ca: P	1.14:1	1.65:1
Magnesium	126.50 ± 0.013^{a}	122.30 ± 0.016^{b}
Sodium	652.50 ± 0.016^{b}	830.80 ± 0.008^{a}
Potassium	2200.10 ± 0.071	2601.20 ± 0.015^{a}

Legend: Ca: P – calcium : phosphorus ratio; values are means ± standard deviation; $^{a, b}$ values with different superscript along the same row are significantly ($P<0.05$) different from one another

These findings were consistent with the results of Gonzalez-Martin *et al.* (2009) who reported higher mean values for mineral contents in cheeses of different compositions (cow, ewe, goat) as 8060.00 mg/L of Ca; 3750.00 mg/L of P; 402.00 mg/L Mg; 7930.00 mg/L of Na and 1250.00 mg/L of K.

Total bacteria count on plate count agar

Table 3 shows the total bacteria count on Plate Count Agar (PCA) for raw cow milk and soft cheese samples. The total bacteria counts in the raw cow milk samples were higher than in the soft cheese samples. The raw cow milk samples had higher bacteria counts of 19.0×10^5, 9.0×10^6, 4.0×10^7 and 2.0×10^8 cfu/ml, in comparison with the bacteria counts in the soft cheese were, 3.0×10^5, 4.0×10^6 and 2.0×10^7 cfu/ml. The heat treatment and the bactericidal effect of the coagulant used during the processing of the raw milk into soft cheese might have caused the reduction in total bacteria counts in the soft cheese. The total bacteria counts observed in this study were however below the standard maximum of $<10^4$ cfu/ml (Barbano *et al.*, 2006). This was consistent with the findings of Joel and Adeyosoye (2010) who observed a decline in the bacterial population from raw cow milk to soft cheese in the order: unprocessed cow milk > soft cheese. These authors attributed the lower bacterial load found in cheese samples than in the raw milk samples to the bactericidal effect of the coagulant used during the cheese processing.

Table 3. Total bacteria count on plate count agar (cfu/ml)

Samples	Dilution Factor			
	$\times 10^{-4}$	$\times 10^{-5}$	$\times 10^{-6}$	$\times 10^{-7}$
Raw cow milk	19.0×10^5	9.0×10^6	4.0×10^7	2.0×10^8
Soft cheese	3.0×10^5	4.0×10^6	2.0×10^7	Nil

*Standard (maximum) $<10^4$ cfu/ml (Barbano *et al.*, 2006)

Isolated organisms from raw cow milk and soft cheese samples

The organisms *E. coli, P. vulgaris* and *S. aureus* were isolated from both the raw cow milk and soft cheese samples as presented in Table 4. Similar observations were made in earlier research works by Belewu and Aina (2000) in cheese samples and Adetunji *et al*. (2003b) in fermented milk and other milk products samples. The presence of these three bacterial organisms in the samples, even though the total bacteria counts observed in this study were below the standard maximum of $<10^4$ cfu/ml is still of concern as they are known to be pathogenic to man (Adetunji *et al*. 2003b and Shittu *et al*. 2011). Adetunji *et al*. (2003b) reported that majority of *E. coli* food borne outbreaks were due to the consumption of contaminated dairy products produced from cow milk and beef products. In this study, the isolation of *E. coli, P. vulgaris* and *S. aureus* from the raw milk samples could be due to intake of contaminated feedstuffs and water by the lactating Bunaji cows. Also, Tasci (2011) explained that *S. aureus* is a ubiquitous organism that occurs in the mucous membranes and skin of most warm blooded animals, lactating cows inclusive and that the staphylococcal toxins cannot

Table 4. Biochemical reactions of *E. coli, P.vulgaris* and *S.aureus* isolates from raw milk samples

BiochemicalTests	*E. coli*	*P.vulgaris*	*S.aureus*
Gram reaction	GNR	GNR	GPC
Catalase	+ve	+ve	+ve
Glucose	+ve gas	+ve gas	+ve gas
Sucrose	+ve gas	+ve gas	+ve gas
Urease	+ve	+ve	+ve
Motility	+ve	+ve	+ve
Myo-inositol	+ve gas	+ve gas	+ve gas
Hydrogen-Sulphide	+ve	+ve	+ve
Oxidase	-ve	-ve	-ve
Indole	NT	+ve	NT
Methyl red	+ve	NT	NT
Coagulase	NT	NT	+ve
Haemolysis	NT	NT	+ve
10% NaCl	NT	NT	+ve

Legend:GNR- Gram negative rod; GPC- Gram positive cocci; NT- Not tested; +ve (positive); -ve (negative)

be destroyed by heating, drying or freezing. The presence of these isolated organisms probably might not be totally avoided, but they should be below the standard maximum safe levels.

CONCLUSION

The soft cheese had higher concentration of nutrients than the raw cow milk. There were higher bacteria counts in the raw cow milk than soft cheese samples; although, the total bacteria counts were below the standard maximum of $<10^4$ cfu ml^{-1}. *E. coli*, a total of three bacteria (*E. coli, P. vulgaris* and *S. aureus*) were isolated from both the raw cow milk and soft cheese samples. The study thus confirmed that the unprocessed or raw cow milk was more vulnerable to bacterial contamination than the processed soft cheese, suggesting need for pasteurizing raw cow milk before consumption by humans.

Dairy farmers, particularly the Fulani herdsmen in Nigeria need to be educated on the importance of washing the teats of dairy animals and milkers' hands properly beforehand milking.

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