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Effect of different biocoagulants on the microbial quality and mineral composition of West African cheese produced from sheep milk

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Abstract

West African cheese (wara) is an excellent source of protein, fats and minerals. This study seeks to investigate the effect of different biocoagulants on the microbial quality and mineral composition of West African cheese obtained from Sheep milk. The cheese produced from sheep milk was coagulated using extracts from *Calotropis procera* leaf, *Carica papaya* leaf, lemon juice and steep water from cereals. Microbial quality and mineral composition of the cheese were subsequently evaluated. The results revealed that the microbial load of cheese coagulated with lemon juice was lower than the cheese coagulated with other biocoagulants. Mineral analysis showed that milk coagulated with steep water from millet had the highest sodium content; *Calotropis procera* coagulated milk had the highest potassium, magnesium and zinc contents. Lemon juice coagulated milk had the highest calcium content and *Carica papaya* coagulated milk had the highest iron content. Findings from this study reveal cheese coagulated with lemon juice and steep water should be used for cheese production due to the antimicrobial effect of the lemon juice and the mineral composition of steep water cheese. It may be suggested that sheep milk should be used for cheese production instead of cow milk due to its high nutritional content.

1. Introduction

West African cheese (wara) is an excellent source of protein, fats and minerals such as calcium, iron, phosphorus, vitamins and essential amino acids, thus making it an important constituent of food in the diet of both young and old of West African consumers (Oladipo and Jadesimi, 2012). Wara has been observed to have an average shelf life of 2–3 days when kept at whey at the approximate temperature of 28°C or 4–5 days when stored in cool well water at about 15°C. In order to increase the shelf life, the cheese is normally deep fried in vegetable oil in order to further preserve the cheese. Due to erratic power supply and dearth of household cooling facilities in Nigeria and West Africa, several measures have been put in place, in times past to overcome this challenge, including the addition of several chemical preservatives such as propionic acid, sodium benzoate, and sorbic acid during the production of wara (Adetunji *et al.*, 2007), Alum (Omotosho *et al*., 2011), *Carica papaya* (Adetunji and Salawu, 2008).

Some of these preservatives have been shown to be effective in inhibiting the growth of microorganisms. However, they may not be readily available to the local cheese processors in West Africa. Therefore, there is a need for a simple, safe, cheap, useful and realistic alternative preservation technique.

Cattle are well recognized to be a major source of milk globally. However, in Nigeria the milk production by local cattle breeds have been reported to be low due to poor quality, insufficient feeds and feedstuffs especially during the dry season (Olafadehan and Adewumi, 2010). Milk can also be gotten from other animals such as camel, goat and sheep (Elbagermi *et al*., 2014). This milk, which is the secretion of the mammary glands, is the only food of the young mammal during the first period of life. The substance in it provides both energy and the building materials necessary for growth and development (Asif and Sumaira, 2010).

The principle of cheese processing is based on the coagulation of the protein in milk, during which about 90% of the milk fat is encapsulated. The coagulated mass is called curd; the remaining liquid is called whey

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(Oladipo and Jadesimi, 2012). Curd (cheese) consists mainly of milk proteins (casein) and milk fat; while whey mainly contains water, milk sugar (lactose), protein (serum proteins) and B vitamins (Ebing and Rutgers, 2006). Sheep milk has a higher specific gravity, viscosity, refractive index, titratable acidity, and lower freezing point compared with cow's milk (Haenlein and Wendorff, 2006).

Several studies have found lactic acid bacteria (LAB) to occur naturally as an indigenous microflora in cheese (Fox *et al*., 1998; Beresford *et al*., 2001). Microorganisms found in milk can be classified based on their biochemical types, temperature response and ability to cause infection and diseases (Marth, 1978). Milk from its natural source is a sterile product but its rich nutritional composition makes it a fertile medium for microbial inhabitation. Contamination may come from external sources like air, soil and milk handlers (Otoikhian, 2012). Sterile milk gotten from healthy udder can become contaminated by bacteria present in the storage tank and also from tubules where the milk flows through. The number of microorganisms present at the time of milking has been reported to range between several hundred and several thousand per milliliter (International Dairy Foundation, 1981).

Bacterial spoilage of raw milk depends upon various factors such as health of the animal, cleanliness of the housing area, nature of feed, the water used at farm, the milk vessels/utensils for storage and essentially the hygiene of the milker/handler (Chatterjee *et al*., 2006; Ali and Abdelgadir, 2011; Salman and Hamad, 2011). Mubarack *et al.* (2010) and Lingathurai and Vellathurai (2010) have reported the presence of pathogenic bacteria to be a major threat to public health especially for those individuals who still consume raw milk. Presence of bacteria in raw milk reduces the keeping quality of milk and certain bacteria with their associated enzymes and toxins may even survive pasteurization creating health hazards (Salman and Hamad, 2011). Therefore, the objective of the present study is to determine the effects of different biocoagulants on the improvement of microbial quality and the mineral composition of West African cheese produced from Sheep milk.

2. Materials and methods

2.1 Collection of milk

The raw milk sample was collected from sheep at Aba Baba Medinat, a Fulani farm settlement along Afao road, Ado-Ekiti, Nigeria. It was collected aseptically and subsequently transferred to the laboratory for analysis.

2.2 Collection of coagulants

The leaves of *Carica papaya* and *Calotropis procera* were collected from Erifun community around The Federal Polytechnic, Ado-Ekiti, Nigeria. Authentication of the Plants was done at the Department of Plant Science and Biotechnology, Ekiti State University, Ado-Ekiti, Nigeria. The voucher specimens of UHAE 2018/022 for *Carica papaya* and UHAE 2018/023 for *Calotropis procera* have been deposited at the University herbarium. Other biocoagulants like lemon fruits were purchased from Oba market, a local market in Ado Ekiti Metropolis, Nigeria, West Africa. Steep water (effluent from pap produced from maize, sorghum, millet) were produced by steeping the grains in water for 3 days after which it was milled and later steeped again for 2 days in the laboratory. The steep water was then collected for use as biocoagulants.

2.3 Production of West African cheese

The milk was stirred gently during the heating process with a wooden spoon. About 4mL of the leaf extract of *Calotropis procera, Carica papaya,* lemon juice, steep water was added to the warm milk and the mixture was heated for the second time with intermittent stirring to about 45-50^{o}C and was kept at this temperature until coagulation was achieved and the heating was stopped after the separation of curd and whey. The sign of coagulation was observed within the range of 10-15 mins. It was transferred into a small previously sterilized rafia basket to facilitate whey drainage and characteristic shape when the cheese was firm enough it was removed from the rafia basket and place inside a covered plastic container for analysis.

2.4 Microbiological analysis

2.4.1 Isolation of microorganisms

This was done using the modified pour plate method (Olutiola *et al*., 2000). The cheese sample was weighed 1 g and introduced into 10 mL of sterile distilled water to carry out a ten-fold serial dilution. Molten nutrient agar (NA), plate count agar (PCA) and potato dextrose agar (PDA) which have been prepared and autoclaved, were allowed to cool to about 45°C before they were dispensed aseptically into sterile Petri dishes containing 1mL of introduced inoculum from the dilutions. The plates were gently swirled and allowed to set. The inoculated plates were incubated in an incubator (Gallenkomp 9052A, England) at 37°C for 18-24 hrs for bacteria while PDA plates were incubated at 28±2°C for 72-120 hrs for fungi. All the plates were observed for growth and counted after incubation.

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2.4.2 Counting of colonies

After 24 hrs of incubation at 37°C, the discrete colonies formed on PCA, NA and PDA were counted and recorded as total bacterial counts and fungal count respectively. The results recorded as colony forming unit per volume of sample (CFU/mL) for bacteria and spore-forming unit per volume (SFU/mL) for fungi.

2.4.3 Sub-culturing method

The pure colony was obtained from plate containing the discreet colony. The microbial mixture was transferred to the edge of an agar plate with an inoculating loop and then streaked out over the surface of fresh agar plate. Pure colonies obtained were maintained on agar slants at 4°C and subsequently subcultured before use. Colonies of fungal growth observed were subcultured onto fresh potato dextrose agar (PDA) until pure cultures of the fungal isolates were obtained.

2.4.4 Cultural and Biochemical tests

The test carried out on the isolates include Gram's staining, motility, spore staining, catalase, indole production, methyl red, Voges-Proskauer test, citrate utilization, urease test, oxidase and sugar fermentation tests (Olutiola *et al*., 2000; Cheesbrough, 2000; Fawole and Oso, 2007).

2.4.5 Identification of bacteria

The appearance of the colony of each isolate on the agar media was studied. Characteristics observed include shape, edge, color, elevation and texture after 24 hrs of incubation. Staining reaction and other results of biochemical tests were interpreted for the tentative identification of bacterial isolates to species level according to Cowan and Steel (1993).

2.4.6 Morphological and microscopic identification of fungi isolates

Fungal mycelium (48-hr old) was aseptically picked with inoculating needle and placed gently on a clean slide containing a drop of lacto-phenol blue. The slide was covered with a cover slip and examined under the microscope. Microscopic identification was done according to Barnett *et al*. (2000) and Samson *et al*. (2010). The identified fungi were maintained on PDA slants at 4°C in the refrigerator for subsequent use.

2.5 Determination of mineral composition

The mineral composition (sodium, potassium, calcium, magnesium, iron, zinc) of each sample was determined by wet ashing method followed by spectrophotometric reading of the level of mineral.

Triplicate wara sample (1 g) was ashed in Muffle furnace (Labcon, RM7, India) at 600°C. The ashed samples inside the silica dishes were removed and transferred into the desiccators to cool after which the samples were dissolved with 1ml of concentrated HNO_3. Little distilled water was added, and the dissolved sample was filtered into a clean small plastic bottle using number 43Whattman filter paper. Distilled water was later used to dilute the solution up to 50ml. Atomic absorption spectrophotometer (Buck 201, VGP) was used in determining the mineral content (AOAC, 2012). The mineral content was calculated using the formula below:

$$\text{Mineral (mg/100 g)} = (R \times V \times D)/ W_t$$

When R is Solution Concentration; V is volume of sample digested; D = Dilution factor; and W_t is weight of sample.

2.6 Statistical analysis

The statistical analyses were carried out using SPSS program (Statistical Package for social Sciences version 16). The significant difference between means were calculated by one-way Analysis Variance (ANOVA) using Duncan multiple range test (DMRT) ($p \leq 0.05$).

3. Results

3.1 Occurrence of bacteria isolates in cheese sample from sheep milk

Table 1 shows the occurrence of bacteria isolated from soft cheese from sheep. In total, six genera of bacteria were isolated from the samples and these are majorly Gram-positive, *Bacillus cereus, Bacillus subtilis, Lactobacillus casei*, *Lactococcus lactis* and *Staphylococcus aureus* except *Pseudomonas aeruginosa* which is Gram-negative organism. *L. lactis* was isolated from all the cheese samples except the sample coagulated with *Calotropis procera* (SCPR). *L. casei* was present in cheese coagulated with steep water from sorghum (SSO), steep water from maize (SMA) and Lemon juice (SLJ), *S. aureus* was present in SSO, SMA, cheese coagulated with steep water from millet (SMI) and SCPR, *B. subtilis* was present in SMI, SCPR and *Carica papaya* (SCP), *P. aeruginosa* was present SCPR and SCP while *B. cereus* was present only in SCP.

3.2 Occurrence of fungi in cheese sample from sheep milk

Table 2 reveals the occurrence of fungal Isolates in wara sample from sheep milk. *Aspergillus fumigatus* was isolated from all the cheese samples except in SMI and

Table 1. Occurrence of bacteria isolates in cheese sample from sheep milk

Sample	*Lactobacillus casei*	*Lactococcus lactis*	*Staphylococcus aureus*	*Bacillus cereus*	*Bacillus subtilis*	*Pseudomonas aeruginosa*
SSO	+	+	+			
SMA	+	+	+			
SMI		+	+		+	
SLJ	+	+				
SCPR			+		+	+
SCP		+		+	+	+

Key: SSO: Sheep milk coagulated with steep water from Sorghum; SMA: Sheep milk coagulated with steep water from Maize; SMI: Sheep milk coagulated with steep water from Millet; SLJ: Sheep milk coagulated with Lemon juice; SCPR: Sheep milk coagulated with *Calotropis procera*; SCP: Sheep milk coagulated with *Carica papaya*

Table 2. Occurrence of fungi in cheese sample from sheep milk

Sample	*Aspergillus niger*	*Aspergillus fumigatus*	*Rhizopus stolonifer*	*Penicillum chrysogenum*	*Muccor hiemalis*	*Candida albican*	*Fusarium verticolor*	*Saccharomyces spp*
SSO		+		+	+		+	
SMA		+		+	+	+	+	
SMI	+		+		+		+	
SLJ		+						+
SCPR	+	+						
SCP				+		+	+	+

Key: SSO: Sheep milk coagulated with steep water from Sorghum; SMA: Sheep milk coagulated with steep water from Maize; SMI: Sheep milk coagulated with steep water from Millet; SLJ: Sheep milk coagulated with Lemon juice; SCPR: Sheep milk coagulated with *Calotropis procera*; SCP: Sheep milk coagulated with *Carica papaya*

Table 3. Mineral composition (mg/100 g) of cheese from sheep milk produced with different coagulants (ppm)

Sample	Na	K	Ca	Mg	Fe	Zn
SSO	280.00±4.00^{e}	64.00±4.00^{f}	340.00±0.00^{e}	16.80±0.00^{a}	4.50±0.50^{b}	1.50±0.30^{d}
SMA	245.00±5.00^{f}	76.00±0.00^{d}	382.00±2.00^{c}	16.60±0.60^{b}	4.00±0.00^{d}	1.80±0.10^{c}
SMI	360.00±2.00^{a}	83.00±0.20^{b}	456.00±0.00^{b}	16.70±0.00^{a}	4.30±0.10^{c}	1.70±0.20^{c}
SLJ	290.70±0.20^{d}	79.00±0.00^{c}	668.00±2.00^{a}	15.70±0.50^{c}	1.90±0.00^{f}	1.10±0.10^{d}
SCPR	350.90±0.00^{b}	160.00±0.00^{a}	385.00±2.00^{c}	16.80±0.30^{a}	3.80±0.30^{e}	2.40±0.00^{a}
SCP	330.00±0.00^{b}	69.00±3.00^{e}	369.00±0.00^{d}	16.70±0.00^{a}	5.20±0.20^{a}	2.20±0.20^{b}

Values are means of triplicate determinations ± Standard deviation. Means with different letters in the same column are significantly different ($p \leq 0.05$)

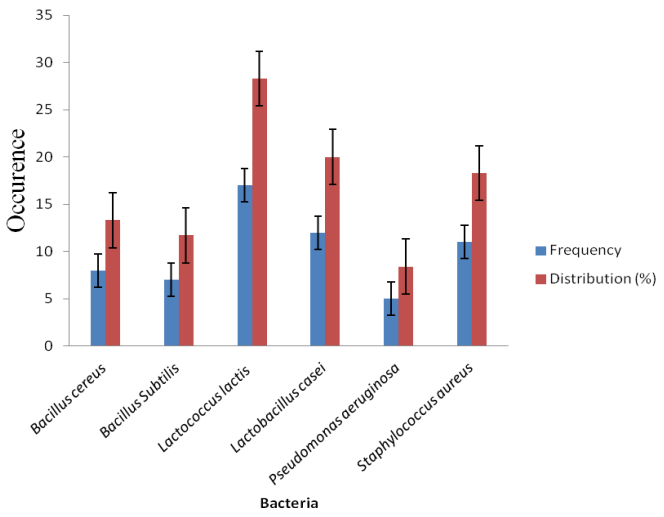

Figure 1. Prevalence of bacteria isolated from cheese samples

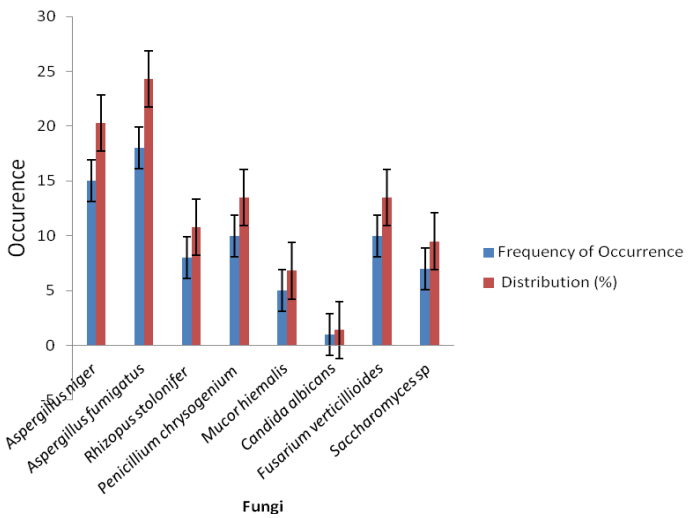

Figure 2. Prevalence of fungi isolated from cheese samples

SCP. *Fusarium verticillioides* was isolated from all the cheese samples except in SLJ and SCPR. *Aspergillus niger* was found in SMI and SCPR. *Rhizopus stolonifer* was also found in SMI only. *Mucor hiemalis* was isolated from SSO, SMI and SMA. *Penicillium chrysogenium* was isolated from SCPR, SSO and SMA. *Candida albicans* was isolated from SCP and SMA. *Saccharomyces* sp was found only in two samples which are SLJ and SCP.

3.3 Mineral composition of cheese from sheep Milk produced with different coagulants

The mineral composition of cheese sample produced from sheep milk is presented in Table 3. Sheep milk coagulated with lemon juice had the highest value of calcium among the entire mineral analysed for all the cheese samples (668 mg/100 g). Zinc was also found to be the lowest among the samples analysed with a value of 1.10 mg/100g from sheep milk coagulated with lemon juice.

3.4 Prevalence bacteria/fungi Isolated from wara samples

Figure 1 shows the prevalence of bacteria isolated from soft cheese produced from sheep milk coagulated with six different biocoagulants. It was observed that *L. lactis* had the highest percentage occurrence of 28.3%. Figure 2 shows the prevalence of fungi isolated from soft cheese produced from sheep milk coagulated with six different biocoagulants. It was observed that *A. fumigatus* had the highest percentage occurrence of 24.3% and *C. albicans* occurred least (1.4%).

4. Discussion

In the cheese samples prepared, the one coagulated with steep water from different grains had the highest population of bacteria compared with other coagulants used. The bacteria present in the steep water might have contributed to the microbial load in the sample. This can be corrected by filter-sterilization of the steep water prior to use in the preparation of cheese. On the other hand, lemon juice coagulated milk had the least occurrence of bacteria population and this can be attributed to the antimicrobial content of the lemon juice. This is in agreement with the observation of Adetunji *et al*. (2007). The decrease in the population of the microorganisms may be due to the corresponding changes in the milk pH.

The existence of mold and yeast in cheese observed in this study was not unusual. Other researchers have isolated *Penicillium* and *Aspergillus* spp. from West African cheese. In the previous study conducted by Adegoke *et al.* (1992), Olatunji *et al*. (2006) and Oyeleke *et al*. (2006) on nono, wara, mai-shanu, fufu and kamu. *L. bulgaricus*, *L. lactis, L. acidophilus, S. thermophillus* and *S. cremoris* were isolated. These support our findings that cheese harbor bacteria including LAB such as *Lactobacillu*s, *Streptococcus* as well as yeast and moulds.

B. cereus, S. aureus, A. niger, A. fumigatus, P. chrysogenium, Rhizopus spp isolated from this study is similar to the report of Uzeh *et al*. (2006) on nono and wara. *Bacillus cereus* which is known to be highly resistant to environmental stress due to its spore-forming nature was also isolated and *B. cereus* is known to be of public health importance since it is pathogenic. The detection of *S. aureus* is also of public health importance because of its ability to cause a wide range of infections especially food-borne intoxication. This organism was equally isolated by Olasupo *et al*. (2002) from cheese and kunun-zaki, a cereal based, non-alcoholic beverage.

The high bacterial load found in the cheese samples investigated during the present study agrees with the findings of Elkhider *et al*. (2011) who reported that cheese samples collected from different producers in rural areas of Eastern Sudan indicate that the level of hygiene and production methods, source of raw milk and its handling could be the main factors responsible for the high microbial loads which might affect the quality of cheese.

The microbial count of the cheese samples was found to be higher than those of the raw milk and the contaminants might be from the coagulants used. This may have accounted for the increase in microbial load found in the cheese sample compared with those found in the raw milk sample. Therefore, some preventive measures should be taken at this stage such as; washing the leaves thoroughly before extraction of the juice, covering the steep water so as to reduce contamination, monitoring the temperature of the milk and controlling the development of acidity. This is different from the results obtained by Adegoke *et al*. (1992) who observed a reduction in the population of total aerobes at the curdling point during the manufacture of the cheese.

The presence of *B. cereus*, *S. aureus*, *P. aeruginosa* and yeast indicates that the cheese was not sterile. *L. casei* had the highest percentage occurrence (28.3%) followed by *L. lactis* (20%), *S. aureus* (18.3%), *B. cereus* (13.3%), *B. subtilis* (11.7%) and *P. aeruginosa* (8.4%). This is in line with the work of Guessas and Kihal (2004) who isolated *Lactobacillus* and *Leuconostoc* from raw goat milk. LAB improves food quality and also plays an important role in preventing the growth of undesirable bacteria. Axelsson (2004) reported that LAB is generally associated with habitats rich in nutrients. It is well

known that LAB produces a variety of antimicrobial substances with potential importance for food preservation.

Lactic acid bacteria are the most extensively studied microorganisms for milk fermentation (Maragkoudakis *et al*., 2006). The presence of LAB in milk fermentation can be either as spontaneous or inoculated starter cultures. Milk itself is known as one of the natural habitats of LAB (Delavenne *et al*., 2012). The presence of certain strains of microorganisms such as *Lactobacilli* and *Streptococcus* species may be beneficial (Yantyati *et al*., 2014). Several of these have been found to have probiotic properties and immunomodulatory function. Several *Lactobacillus* strains have been reported to display stimulatory properties on cells of innate immunity in vitro and in vivo in both animal models and in humans given fermented milk products containing probiotics (Otoikhian, 2012).

Aspergillus species which were isolated are known spore formers, which therefore mean that they can easily contaminate the dairy products which are usually exposed during processing, storage and hawking. They are the major spoilage organisms of carbohydrate food. However, their growth can result in the production and accumulation of mycotoxins which are of public health and economic importance (Wouters *et al*., 2002). The yeasts and mould count in this study were lower than those reported by Soliman and Aly (2011) who isolated thirteen yeasts strains from Egyptian Karish cheese.

The effect of different biocoagulants on the mineral composition of cheese is presented in Table 3. It shows that the highest sodium (360) content was found in milk coagulated with steep water from millet (360 mg/100 g), for potassium, magnesium and zinc, *Calotropis procera* coagulated milk had the highest values of 160 mg/100 g, 16.80 mg/100 g, 2.40 mg/100 g respectively, while calcium had the highest value from lemon juice coagulated milk 668 mg/100 g, and iron had the highest value from *Carica papaya* coagulated milk 5.20 mg/100 g.

Steep water and lemon juice can compare favourably with the commonly used *Calotropis procera* in terms of nutritional composition. Mineral content of steep water coagulated cheese could probably be due to the likelihood that some of the minerals present in the steep water might have migrated into the cheese. The Fe and Zn content of cheese coagulated with *Carica papaya* obtained from this study (5.20 mg/100 g, 2.20 mg/100 g) are generally higher than the values reported by Adetunji and Salawu (2008) in his work on West African soft cheese (wara) processed with *Calotropis procera* and *Carica papaya* (4.84, 1.19). Results from this study also show that the mineral content of sheep milk is very high. This shows that sheep milk is highly nutritious. This is similar to the findings of Elbagermi *et al.* (2014), Asif and Sumaira (2010). A report of a decrease in trace elements had earlier been observed made by Coni *et al.* (1995). Some of these trace elements quantified in this study, however, were found to be high, this increase might have been contributed by the plant extract employed for the processing of the cheese.

5. Conclusion

In conclusion, this study points out that sheep milk can also be used to produce highly nutritious West African cheese in place of the commonly used cow milk. Also, other unconventional coagulants can be used for the production of cheese apart from the commonly used sodom apple leave. However, efforts should be made to ensure that high hygiene is considered in order to reduce the microbial load to the barest minimum. Cheese produced contains Lactic acid bacteria which are beneficial to the consumer because of their probiotic effect. Thus, time and temperature at this stage of processing should be established and systematically monitored to prevent possible hazard which may be posed by the microorganism isolated.

Cheese coagulated with steep water from different grains appears to contain higher microbial load though highly nutritious than other coagulants used and this may be due to the microorganism present in the steep water which might have contributed to the microbial load in the final product. Steep water appears to be the most promising coagulant with regard to the production of cheese with high mineral contents if the sensory quality is looked upon. Unconventional biocoagulants such as *Carica papaya*, lemon juice, steep water from grains could qualify as a replacement for *Calotropis procera* extract which is generally used as a coagulant for cheese production because they contain essential minerals which are beneficial to the human body.

Conflicts of interests

We declare no conflicts of interests.

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