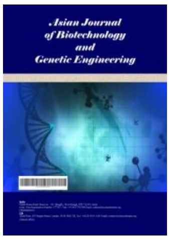

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Effect of Different Coagulants on the Yield and Microbiological Quality of Soft Cheese (WARA) Produced from Tigernut

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Authors' contributions

This work was carried out in collaboration between both authors Both authors read and approved the final manuscript.

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ABSTRACT

The milk and the soft cheese (wara) produced from tigernut were stored in the refrigerator and samples for microbiological analyses were collected at day one, seven and fourteen. Bacterial load of tigernut milk during storage increased from 1.5×10^1 to 1.8×10^1 cfu/mL while the bacterial load of cheese ranged from 9.7 cfu/g to 1.2×10^1 cfu/g. There was no significant increase ($p \le 0.05$) in the bacterial load of the cheese during storage. The bacterial isolates from freshly prepared tigernut milk were tentatively identified as *Lactobacillus casei, L. plantarum* and *Streptococcus lactis*. The bacteria isolated from the cheese produced using different coagulants are *L. acidophilus, L. plantarum, Bacillus badius, B. larvae, B. alvei, Aeromonas hydrophili,* and *Pseudomonas aeruginosa*. The sensory scores revealed various significant differences in all the parameters evaluated on tigernut milk and cheese. Highest taste and texture were recorded for all the cheese produced while commercial cheese had the lowest taste and texture. This study revealed that tigernut milk and cheese produced from it possess low microbial load and good sensory quality. Tigernut milk may therefore serve as a good alternative to cow milk for the production of cheese.

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1. INTRODUCTION

Tigernut (*Cyperus esculentus* Lativum) belongs to the Division-Magnoliophyta, Class-Liliopsida, Order-Cyperales and Family-Cyperaceae. It is a cosmopolitan, perennial crop of the same genus as the papyrus plant [1]. The tubers are about the size of peanuts, grow freely and is consumed widely in Nigeria, other parts of west Africa, east Africa, parts of Europe particularly Spain as well as in the Arabian Peninsula [2]. Previously, it was cultivated in the ancient Mesopotamia between the rivers Tigris and Euphrates [3]. It has many other names like Zulu nut, yellow nutgrass, ground almond, chufa, edible rush and rush nut. In Nigeria, the Hausas call it “Aya”, Yorubas “imumu”, the Igbos “aki Hausa”, “ofio” in southern Nigeria [4].

The tigernut milk compared with any other soft drink, it is not just a refreshing drink but also very healthy. It contributes to the reduction in the cholesterol by diminishing the ‘bad’ cholesterol (low density Lipoprotein (LDL), and increasing the ‘good’ cholesterol (high density Lipoprotein (HDL) [5]. Tigernuts are rich in Vitamin E which is a reference to a group of fat-soluble compound with distinctive antioxidant qualities ideal for coronary heart disease [6]. It was also reported to help in preventing heart thrombosis and activates blood circulation, responsible for preventing, treating urinary tract and bacterial infection, assist in reducing the risk of colon cancer [7]. Tigernut is thought to be beneficial to diabetics and those seeking to reduce cholesterol or lose weight, the very high fibre content combined with a delicious taste make them ideal for healthy eating [8]. Tigernut milk has been found to be good for preventing arteriosclerosis, since its consumption can help prevent heart problems and thrombosis and activate blood circulation [6]. Tigernut without sugar can be used for diabetes for the carbohydrate content with best of sucrose and starch (without glucose) and due to its high content of arginine, which liberates the hormone insulin.

The increase in cheese production, coupled to a diminishing supply of natural animal rennet, is responsible for increases in the demand for alternative milk-coagulating sources. Due to this and a variety of factors (vegetarian, religious beliefs, etc.), attention is being turned to the use of microbial coagulants and coagulants extracted from plants [9]. As regards plant coagulants, although a wide variety of plant-derived proteases is available for milk coagulation, their excessive proteolytic nature reduces cheese yield and increases the risk of bitter tastes, making its use more difficult for cheese production [10]. The advantages of using plant proteases is that such natural enzymes can be eaten by vegetarians and also may be certified as Kosher and Halal [11]. Juice extracts from fruits and other plant parts have long been used as milk coagulating agents. The proteases present in the plant extracts and those naturally present in milk are believed to play a role in the impartation of aroma and texture of cheeses [12]. Examples of plants with such properties are papaya (papain), pineapple (bromelin), castor seed oil, latex from fig tree and Sodom apple (*Calotropis procera)* plant [13].

The high cost of milk in developing countries has led to the development of alternative source of milk from plant minerals. An inexpensive substitute in the form of a milk or beverage made from locally available plant foods, high in protein, with satisfactory quality milk could play an important role in reducing protein malnutrition [14]. Most researches have centered on the use of soybean as alternative source of milk analogue or substitute with little attention on the use of other under-utilized crops such as bambara nut, tiger nut, melon, etc. The yield and quality of cheese is said to be determined by the quality of the milk used and the type of coagulants. The present study is aimed at assessing the effect of different coagulants on the yield and microbial quality of soft cheese (*wara*) produced from tigernut milk.

2. METHODOLOGY

2.1 Collection of Samples

Fresh tigernuts were purchased at Oba market, Akure, Ondo state. The samples were taken to the laboratory of Department of Microbiology Federal University of Technology, Akure for further processing and analysis.

2.2 Preparation of Tiger Nuts Milk

The method of Udeozor [15] was adopted in the production of tigernut milk. About 500 g of fresh tiger nuts were sorted, washed with water, decontaminated with 1% hypochlorite solution and thereafter rinsed with sterile water. The nuts were ground in clean blender with the addition of 1000 mL of sterile water. The milk was extracted by using clean muslin cloth, filtered and then divided into five portions (200 mL per portion). Pasteurization was carried out on one portion of the milk by heating at 77.7°C for 15 to 25 seconds, cooled immediately and bottled separately. Fig. 1 depicts the flow chart of the processes involved in the production of tigernut milk.

2.3 Preparation of Coagulants

The three coagulants used were: *Carica papaya* (Pawpaw) leaf juice, *Calotropis procera* (Bombom leaf) leaf juice and *Citrus limon* (lemon) fruit juice. The coagulants were washed separately with water, decontaminated with 1% hypochlorite solution and thereafter rinsed with sterile water. The leaves were cut with knife, squeezed, filtered and kept separately in sterile container. While the lemon fruit was cut with knife and the water was squeezed out and kept inside a sterilized container.

2.4 Preparation of Cheese

About 200 mL of tigernuts milk was measured in three cleaned stainless cups, 2 drops of coagulant from *Carica papaya, Calotropis procera* and lemon juices were added to the three stainless cups. The cups were heated with constant stirring up to 70°C for 15 to 20 minutes using Bursen burner with tripod stand and wire gauze. The milk became thickened during heating and was transferred to another clean container and cooled to take the shape of the new container.

2.5 Yield of Cheese

The yield of cheese produced with different coagulants was calculated by measuring the volume of milk used and the weight of the cheese after production.

$$\text{Yield} = \frac{Cheese\ obtained\ \times\ 100}{Volume\ of\ milk\ used}$$

2.6 Isolation of Bacteria and Mould from Cheese Samples

Nine (9) mL of buffer peptone water was measured in 10 test tubes, the tubes were plugged with cotton wool wrapped in aluminium foil and sterilized in an autoclave at 121°C for 15minutes. One milliliter (1 mL) of milk and 2g of cheese were taken aseptically and 2 g each for the cheese (cheese made with lemon fruit, *Calotropis procera* and *Carica papaya* leaf juice) were separately weighed and macerated in a sterile mortar with pestle. Aliquots (1 ml) was obtained and serially diluted with sterile peptone water up to 10^{-10}. The same procedure as stated above was carried out for the other cheese samples. The tubes were incubated at 37°C for 24 hours.

Fresh Tigernuts
↓
Sorting and washing
↓
Grinding/ milling
↓
Extracting/ sieving
↓
Pasteurizing
↓
Homogenizing
↓
Bottling
↓
Cooling
↓
Tigernut milk

Fig. 1. Flow chart for Tigernut milk production
Source: Udeozor (2012) [15]

From the incubated tubes, 1 mL each was taken from 10^{-1} dilution and inoculated into six (6) sterile Petri-dishes (triplicate), 1 ml was transferred from 10^{-2} dilution into another six (6) Petri-dishes and 1 ml from 10^{-4} dilution into another 6 Petri-dishes for each of the replicates. Nutrient agar and Potato dextrose agar were prepared and sterilized at 121°C for 15minutes. After cooling, it was poured inside the plates containing the replicate for the growth of bacteria and fungi with appropriate labeling on the Petri-dishes. The plates were incubated appropriately at 37°C for 24 hours and 28°C for 72 hours respectively for bacteria and mould [16]; [17]. Also, cultural, morphological and biochemical characterization were used for the identification of bacterial isolates [17] while cultural and microscopic features of fungi were used for their identification [16].

2.7 Sensory Evaluation of Milk and Cheese

The cheese and milk samples produced were subjected to organoleptic analysis with commercial cheese and fresh cow milk serving as control. Total number of 26 people consisting 10 females and 16 males, within the age group of 22 to 35 years who were familiar with cheese were drawn from students of the Federal University of Technology, Akure. The cheese were cut, coded and served in clean white plates with spoon while the milk were bottled, coded and served in clean disposable cups. The exercise was conducted in a clean, well ventilated class room with good lighting to the panellist. The panellist drank the milk and ate the cheese and rated the products for overall acceptability and sensory attributes such as colour, texture and taste for each sample using a 9-points Hedonic scale [18] where 1 = dislike extremely and 9 = like extremely.

2.8 Statistical Analysis

Data gathered in the course of this research were subjected to Analysis of Variance (ANOVA) and Turkey"s test was used for comparison of means. Statistical significance was accepted at $p<0.05$.

3. RESULTS

3.1 Comparative Effects of Coagulants on Yield of Cheese Made from Tigernut Milk

The yield of Cheese produced from different coagulants is shown in Table 1 yield of Cheese from lemon fruit juice was the highest (97%) while the cheese from *Carica papaya* leaf was the lowest (94%).

3.2 Bacterial Load of Tigernut Milk and Cheese Samples Made from Different Coagulants

The bacterial load of tigernut milk at day one (1.5×10^1cfu/mL) was lower than that of day fourteen (1.8×10^1cfu/mL) and the bacterial load of cheese produced from lemon fruit at day one (9.7cfu/g) was lower than cheese produced from *Carica papaya* leaf and *Calotropis procera* leaf (1.2×10^1cfu/g). The bacterial load of cheese produced from lemon fruit at day seven (1.2×10^1cfu/g) was higher than cheese produced from *C. papaya* leaf and *C. procera* (1.0×10^1cfu/g) while the bacterial load of cheese produced from lemon fruit at day fourteen (1.1×10^1cfu/g) was higher than cheese produced from *C. papaya* leaf and *C. procera* leaf juices (1.02×10^1cfu/g) (Fig. 2). Bacteria isolated from fresh tigernut milk at day one of preparation were identified as *Lactobacillus casei*, *L. plantarum* and *Streptococcus lactis*.

Table 1. Comparative effects of coagulants on yield of cheese made from tigernut milk

Samples	Volume of milk used (mL)	Weight of cheese obtained (g)	Yield of cheese (%)
Cheese produced using lemon fruit juice as coagulant	100.00	97.00	97.00
Cheese produced using *Carica papaya* leaf as coagulant	100.00	94.00	94.00
Cheese produced using *Calotropis procera* leaf as coagulant	100.00	96.00	96.00

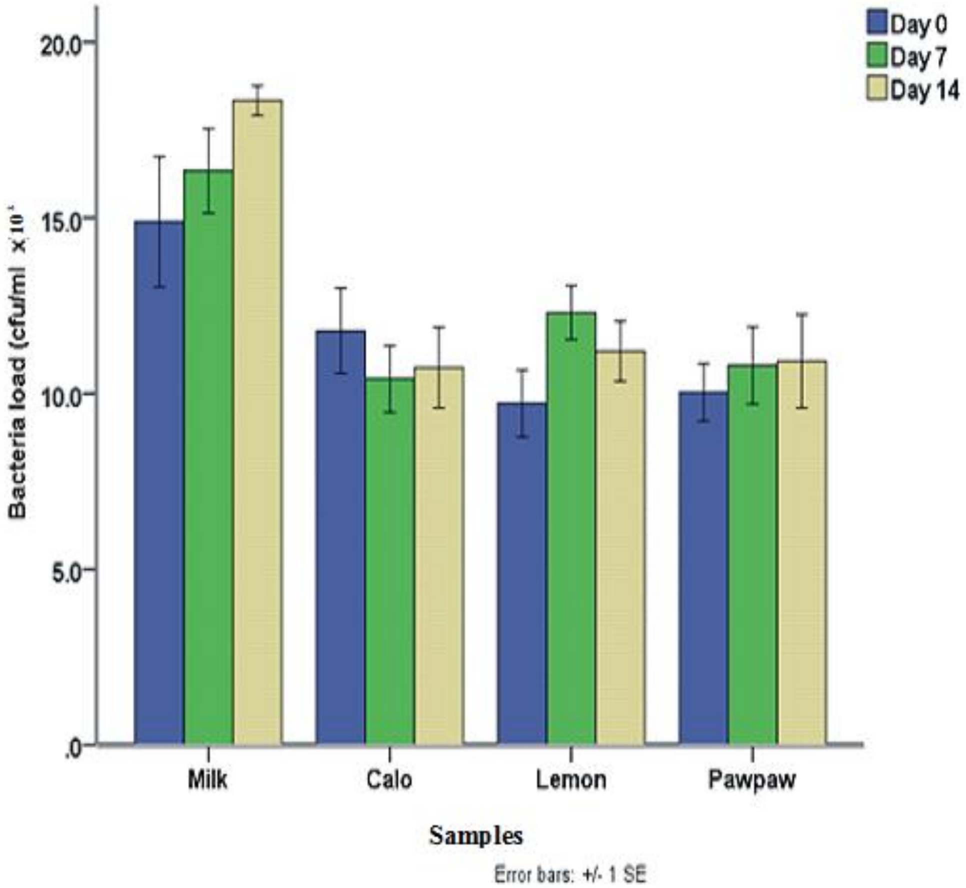

Fig. 2. Bacterial load of tigernut milk and cheese before and after storage

Legend: Milk: Tigernut milk; Calo: Cheese made with Calotropis procera leaf; Lemon: Cheese made with Lemon fruit juice; Pawpaw: Cheese made with Carica papaya leaf

3.3 Bacteria Isolated from Cheese Samples during Storage

Effect of storage period on microbiological quality of cheese made from tigernut milk is shown in Table 2. It was observed that *Lactobacillus plantarum* was present in the cheese sample at day 1, 7 and 14 in the cheese made with *Calotropis procera* as coagulant. Also, at day one the following bacterial were isolated *Bacillus badius, Lactobacillus plantarum* and *B. larvae* compared with day 14 that have *Aeromonas hydrophilia*, *L. plantarum* and *Pseudomonas aeruginosa.* In the cheese sample produced with lemon fruit as coagulant, it was noted that at day 1 *Bacillus alvei* and *L. plantarum* were present while at day 7, *Aeromonas hydrophilia*, *Bacillus alvei* and *L. plantarum* and *P. aeruginosa* were present compared to day 14 which have *B. cereus, B. subtilis, L. plantarum* and *P. aeruginosa.* Furthermore, cheese produced using *Carica papaya* leaf as coagulant has *B. larvae* and *L. plantarum* at day 1, with highest bacterial diversities (*A. hydrophilia, B. cereus, L. plantarum, P. aeruginosa,* and *L. acidophilus*) at day 7 and *B. cereus, P. aeruginosa, L. plantarum* and *B. subtilis* were isolated at day 14. Generally, *L. plantarum* was the most dominant bacteria in all the samples at different day of storage.

3.4 Sensory Attributes of Milk and Cheese Produced from Tigernut

The sensory scores revealed various significant differences in all the parameters evaluated on tigernut milk and cheese. The taste and texture value was high in tigernut milk (A) while fresh cow milk has higher value (B) in colour (Table 3). For the cheese, highest taste value was recorded for (A) and (B) while (C) had the lowest taste value (Table 4).

Table 2. Effect of storage period on microbiological type in cheese made from tigernut milk

Cheese samples	**Bacterial isolated during the storage**		
	Day 1	**Day 7**	**Day 14**
Cheese produced using *Calotropis procera* as coagulant	*Bacillus badius* *Lactobacillus plantarum* *Bacillus larvae*	*B. larvae* *Pseudomomas aeruginosa* *L. plantarum*	*A. hydrophilia* *L. plantarum* *P. aeruginosa*
Cheese produced using Lemon fruit as coagulant	*Bacillus alvei* *L. plantarum*	*Aeromonas hydrophilia* *L. plantarum* *P. aeruginosa* *Bacillus alvei*	*B. subtilis* *B. cereus* *L. plantarum* *P. aeruginosa*
Cheese produced using *Carica papaya* leaf as coagulant	*B. larvae* *L. plantarum*	*A. hydrophilia* *B. cereus* *L. plantarum* *P. aeruginosa* *L. acidophilus*	*B. cereus* *P. aeruginosa* *L. plantarum* *B. subtilis*

Table 3. Sensory properties of milk

Samples	Texture	Colour	Taste	Overall Acceptability
A	8.14 ± 0.13^b	6.71 ± 0.22^a	7.64 ± 0.21^b	6.98 ± 0.12^a
B	6.36 ± 0.28^c	8.07 ± 0.12^b	5.92 ± 0.25^a	7.40 ± 0.12^a

Legend: A: Tigernut milk; B: Fresh cow milk

Table 4. Sensory properties of cheese produced from tigernut milk

Samples	Texture	Colour	Taste	Overall Acceptability
A	7.36 ± 0.17^a	7.64 ± 0.19^a	8.00 ± 0.19^b	7.47 ± 0.09^a
B	7.93 ± 0.15^b	7.42 ± 0.19^a	8.00 ± 0.14^b	8.14 ± 0.14^a
C	7.29 ± 0.27^a	7.14 ± 0.12^a	6.71 ± 0.22^a	6.98 ± 0.12^a
D	8.00 ± 0.12^b	8.29 ± 0.15^b	7.86 ± 0.14^b	8.96 ± 1.91^a

Legend: A: Cheese produced from Lemon fruit juice; B: Cheese produced from Carica papaya leaf; C: Cheese produced commercially; D: Cheese produced from Calotropis procera leaf

4. DISCUSSIONS

The results from this study shows that there was significant difference ($p\leq0.05$) in the microbial load of tigernut milk during storage meaning that the microorganisms were able to derive needed nutrients from the tigernut milk for their growth. Milk is an excellent source of nutrients such as vitamins, amino acids, fats, minerals, proteins and sugar, making it an excellent medium for microbial proliferation [19].

The bacterial load of cheese produced from tigernut milk treated with different coagulants shows that cheese produced from lemon fruit juice has lower microbial load than cheese produced from *Carica papaya* leaf and *Calotropis procera* leaf at day one. This may be due to the lower pH of the lemon fruit juice. However, the bacterial load of cheese produced from C. *papaya* leaf and C. *procera* leaf were lower than cheese produced from lemon fruit juice at day seven and at day fourteen. Bacteria strife well in higher pH than lower pH [20]. The *Calotropis procera* plant had attracted much attention due to the following biological activities; anticancer, antifungal and insecticidal activities [21]. The flowers of the plant exhibit hepato protective activity, anti-inflammatory, antipyretic, analgesic, and antimicrobial effects and larvicidal activity [21]. This might have contributed to the decrease in the microbial load during storage. There were differences in the bacterial load of these cheese with different coagulants during storage but the bacterial load obtained from this study were still regarded as safe for consumption because proper hygiene and good microbiological quality was observed. However, this is not in line with the work of Abou Dawood et al. [22] who obtained high counts for aerobic bacteria on hawked cheese from different location in Egypt.

Different Gram positive microorganisms were also isolated from the tigernut milk such as

Lactobacillus casei, Streptococcus lactis and *Lactobacillus plantarum*. This shows that Lactic acid bacteria are dominant. Other bacteria isolated from cheese samples during storage were *Bacillus badius, Bacillus larvae, Bacillus alvei, Lactobacillus plantarum, Aeromonas hydrophilia, B. cereus, L. acidophilus, Bacillus* sp., *B. subtilis* and *Pseudomonas aeruginosa*. This is in line with the work of Oladipo and Jadesimi [23] and Sangoyomi et al. [24] in their studies had earlier isolated some members of *Enterobacteriaceae* including *E. coli*, and yeast from soft cheese.

The high water content and a neutral pH of milk favours the growth and survival of these bacteria. The lactic acid bacteria (LAB) were the only bacteria that occurred throughout the days of storage. LAB ferment sugar into lactic acid and can grow at low pH (5.0), most LAB have probiotic characteristics [25].

The presence of *Bacillus* species in this work may be as a result of contamination from the environment. *Bacillus cereus* and *Bacillus subtilis* were absent at day zero of this work but occurred at latter days. Some strains of *Bacillus cereus* are harmful to humans and cause food borne illness while other strains can be beneficial as probiotics for animals [26]. *Bacillus subtilis* bacteria are non-pathogenic, they can contaminate food however they seldom result in food poisoning.

The sensory scores revealed various significant differences ($P \leq 0.05$) in all the parameters evaluated on tigernut milk with fresh cow milk and cheese. Higher value was recorded on taste and texture for tigernut milk but fresh cow milk has higher value in colour. There were no significant difference ($P \leq 0.05$) on overall acceptability.

The cheese produced from *C. procera* leaf had the highest value in terms of texture (8.00), colour (8.29), and overall acceptability (8.96); cheese produced from lemon fruit juice and *C. papaya* leaf shows highest values based on taste but cheese produced from *C. procera* leaf shows lesser value based on taste though there were no significant differences ($P \leq 0.05$). Cheese produced commercially had the lowest value based on taste, colour and texture. There were no significant difference ($p \leq 0.05$) on overall acceptability of all the cheese samples.

5. CONCLUSION

This study revealed that tigernut milk can be used for the production of Nigerian soft cheese (*wara*) which is very nutritious, tasty, cost effective, alternative protein source and shown minimum microbial contamination. The non-conventional coagulants (lemon juice and *Carica papaya* leaf juice) also shows promise as good coagulants agents for production of cheese. The sensory evaluation shows that tigernut milk and cheese produced from it were acceptable and preferred to fresh cow milk and cheese produced from it.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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