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A COMPARATIVE ASSESSMENT OF THE NUTRITIONAL CONTENTS OF 'WARA' A WEST AFRICAN SOFT CHEESE USING *CALOTROPIS PROCERA* AND *CYMBOPOGON CITRATUS* AS COAGULANTS

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ABSTRACT

The processing line of West African soft cheese varieties (processed with *Calotropis procera* (Sodom apple) and *Cymbopogon citratus* (lemon grass) leaf extracts was assessed for nutrient compositions (nitrogen, crude protein, fat, lactose, moisture content), pH, total aerobic plate count and trace elements (Fe, Zn, Cu, Mn, Na, Ca, Mg and K). The percentage of nutrient composition, pH and the total aerobic plate count of microbes were: milk (nitrogen (2.05), protein (2.78), fat (5.33), lactose (1.86), moisture contents (88.75), pH (3.91) and total aerobic plate count (7.3logcfu/ml); *Calotropis procera* processed cheese (nitrogen (2.00), protein (2.56), fat (4.43), lactose (1.72), moisture contents (62.89), pH (3.58) and total aerobic plate count (7.34logcfu/ml); *Cymbopogon citratus* processed cheese (nitrogen (2.01), protein (2.53), fat (4.33), lactose (1.68), moisture contents (63.56), pH (3.56) and total aerobic plate count (7.43 logcfu/ml). There were significant differences between the parameters measured in the raw milk and processed cheese at 95% confidence limit. However, the parameters measured varied slightly in the two cheese varieties (*Calotropis procera and Cymbopogon citratus).* The total aerobic plate counts in milk and cheese were higher than international standards set by Codex alimentarius. Addition of leave extracts *(Calotropis procera* and *Cymbopogon citratus*) increased the total aerobic plate counts but the counts dropped at the curdling point during processing. *Cymbopogon citratus* cheese had a higher total aerobic plate count than the *Calotropis procera* cheese although not at a significant level. There was an increase in Fe, Zn, Cu and Na along the processing line, but a decrease ensued in Mn, Ca, Mg and K. There was no significant difference in the Zn, Cu, Mn, Ca, Mg, K, N, protein, fat and lactose contents of the two cheese types although all trace elements and nutrients assayed were higher in the *Calotropis procera* processed cheese with the exception of Fe content. The study suggests the use of *Cymbopogon citratus* leaf extract as a local milk coagulant due to reports of probable health hazard from the use of *C. procera*. However, further work on improving the yield of cheese when *Cymbopogon citratus* is used as coagulant is still necessary.

Key words: Cheese, *Calotropis procera, Cymbopogon citratus*, nutritional, aerobic

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INTRODUCTION

Cheese is made in almost every country in the world with the existence of more than 2,000 varieties [1]. It provides an ideal medium for preservation of valuable nutrients in milk and is an excellent source of protein, fat, minerals, vitamins, and essential amino acids. The ingredients of cheese processing are milk, starter, and coagulants [2, 3]. The general steps of cheese manufacturing involve heat treatment of milk, addition of starter or coagulant, and removal of whey. Slight changes are, however, made from time to time depending on the variety of the cheese [1, 4].

In Nigeria, milk production is mainly done by the Fulani nomadic people who are pastoralists involved in the rearing of cattle moving from one location to another in search of green pasture. Due to lack of refrigeration facilities, the Fulani women process the surplus fresh milk into a soft, unripened cheese called "warankasi" or "wara" in short term. The wara cheese is widely consumed at home and sold on the market in southwestern Nigeria. A typical block of wara cheese weighs approximately 55-60 g, and is consumed without the addition of a coloring agent [1]. The cheese is usually stored in a mixture of whey at room temperature (28^{o}C). Under these storage conditions, wara is highly perishable and has a shelf life of 2-3 days. The coagulants used for wara processing include the leaves and stem extracts of Sodom apple (*Calotoropis procera*) or pawpaw (*Carica papaya*). The *Calotropis procera* extracts are preferred over the extracts of pawpaw because *Calotropis procera* processed cheese has a sweeter flavor compared to the cheese processed with pawpaw leaf extracts [1].

One of the modern technologies employed in the curdling of milk is the use of commercial preparations of lactic acid bacterial cultures and milk coagulants, which are usually imported. Production of lactic acid by the starter flora during manufacture of cheese results in a decrease in the pH of milk and this, in combination with cooking and stirring, promotes syneresis of the curd and expulsion of whey [2]. While all acid coagulated cheeses are consumed fresh, most rennet coagulated cheese undergoes a period of ripening which can range from about three weeks for Mozzarella to two years or more for Parmesan and extra-mature Cheddar [3].

Because of inaccessibility to these substances and financial constraints confronting the local cheese processors, alternative methods of processing cheese have been devised. One such approach is the production of wara cheese in West Africa using the juice extract of the plant *Sodom apple (Calotropis procera)* as the milk coagulant [1]. However, soft cheese produced by local processors has high microbial load and pathogenic isolates because of the unhygienic methods of processing and lack of standards in the processing techniques. Adegoke *et al.* [4] reported the introduction of naturally associated microorganisms of the plant into the milk used in the processing of cheese although Adetunji *et al.* [5] used lemon juice as a coagulant, which suppressed the enterobacterial count. In this study [5] the lemon juice used as coagulant was standardized for volume and all equipment for processing were sterilized before use.

The amount of vegetable juice (leaf extract) or rennet required for a given quantity of milk is not known in the traditional method of soft cheese processing [6]. Thus the finished product lacks consistency in quality characteristics compared with conventional processes [7]. There is, therefore, the need to increase knowledge on the mechanism governing the quality of the final product to develop production processes yielding consistent results [8]. Belewu [8] evaluated the nutritional and rheological qualities of West African soft cheese made from *C. procera* during storage. These qualities may vary due to the differences in the composition in milk and maturity of the cheese [9, 10, 11]. These changes may also be related to the changes in pH, moisture, and salt content [8].

The thrust of this work is to evaluate the nutritional and microbial qualities of the cheese processed from the extract of the two indigenous plants *Calotropis procera (Sodom apple)* and *Cymbopogon citratus* (lemon grass).

MATERIALS AND METHODS

Wara cheese processing

One liter of milk was poured into each pot (A and B). The milk in the pots was heated to approximately 50^{o}C in about 30 - 40 min. The leaf extracts of *Calotropis procera* and *Cymbopogon citratus* (100 ml) were then added separately to the warmed milk. The extract had been prepared by finely chopping 60 g leaves of *C. procera or Cymbopogon citratus* (Amazon Herbs, Wonglaan, 10, Paramaribo, Suriname). The chopped leaves were then macerated in 100 ml of warmed milk. This mixture was then sieved into one liter of the warmed milk according to previous methods Adetunji and Chen, 2011[12]. The mixture was heated slowly with intermittent stirring until it reached the boiling point. The milk with added leaf extract was kept at boiling point (95^{o}C) until it coagulated and there was visible separation of curd and whey (in 10-15minutes). The pot was then removed from the heating source and the curds and whey were ladled or poured into sterile 8 mm diameter egg separators, which facilitated whey drainage and also gave the cheese its characteristic shape and size. All equipment used for processing was sterile.

Organoleptic Evaluation

Organoleptic evaluation of the cheese samples was done by 2 panelists who had wara as part of their diet. The two cheese types were coded randomly with 3 digit numbers (371; 432). Coded samples were evaluated at room temperatures. Evaluation was done for overall quality (texture, taste). The scoring was done on a scale of I- III. I (Fair – 50 – 59%); II (good – 60 – 70%); III (Very good – 71 – 90%).

Microbiological sampling

Ten millilitres of milk or 10 g of cheese were sampled aseptically at different phases of wara West African soft Cheese processing (Figure 1) using two indigenous plants *Calotropis procera* and *Cymbopogon citratus* as coagulants of the raw milk used in this study. For each sampling, 10 ml of milk or 10 g of cheese samples were aseptically weighed and the surface pH of the cheese was taken using a Van Waters and Rogers (VWR) scientific model 8,000 electrode pH meter. The pH meter was

calibrated using commercial buffers. The cheese samples were then homogenized in 9 ml sterile 0.1% peptone water in sterile stomacher bags (Seward Stomacher Lab system). Milk samples and samples of the homogenate were then serial diluted in 0.1% peptone water and aliquots from appropriate dilutions (10^{-6}) were surface plated on plate count agar for enumeration of total aerobic plate count (TAPC). All the plates were incubated aerobically at 37^{o}C for 24 h. Bacteria colonies were counted using an automatic colony counter (Leica Quebec Dark Field, Model 3325). The number of colonies per ml /gm of samples was determined by multiplying the colonies on the media by the dilution factor of the sample and reported as Colony forming units per gm/ml (cfu/ml/gm) of tested sample. This was then converted to $\log_{10}$cfu/ml/g. The experiment was performed in 2 replicates.

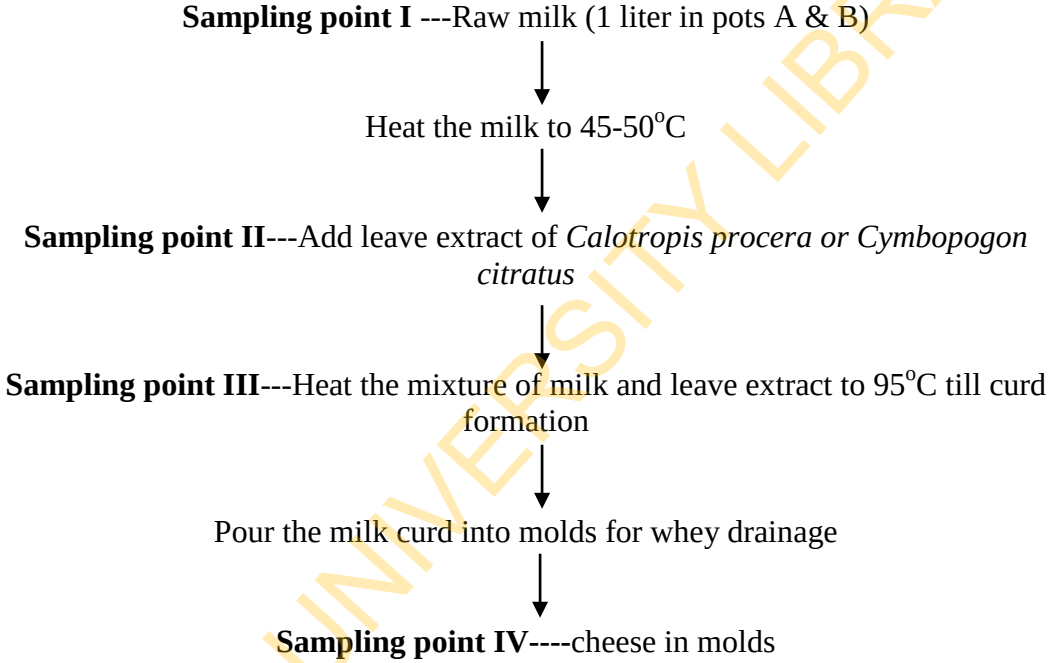

Figure 1: Flow chart of wara cheese processing

Nutritional analysis

Fat content was determined by the Babcock-fat test described by Bartels *et al.* [13]. Nitrogen, crude protein, lactose, starch and moisture content were measured as described by Association of Official Analytical Chemists [14]. Trace and major elements Fe, Zn, Cu, Mn, Na, Ca, Mg, K, Mn, and Zn were determined using an atomic absorption spectrophotometer (AAS). The analysis was performed in a Varian AA200 atomic absorption spectrophotometer equipped with a graphite furnace (GFAAS).

A microwave system was used for acid digestion of all the samples. All the samples were dried at 70°C in a hot air oven until dry weight. To 0.3g finely crushed dry sample, 6.0 ml of HNO_3 (65% conc.) and 1.0 ml of H_2O_2 (30%conc.) were added. The solution was filtered, deionised water (50 ml) was added and the sample was then stored in sterile bottles.

Data analysis

The study was performed in two replicates. Data for nutritional contents were subjected to analysis of variance (ANOVA) to test for significant differences at $P<0.05$. All microbiological data were transformed into Log_{10} CFU/ml or Log_{10} CFU/g before comparison of means. Analysis of microbial data was accomplished using the Fisher's least significant difference of means of bacterial populations calculated with the General Linear Model (GLM) procedure of SAS [15] based on a 95% confidence level.

RESULTS

Panelist -1 for the organoleptic test scored the tw**o** cheese types as very good (71-90%), while the panelist-2 scored *C. procera cheese as* very good, but the *C. citratus* cheese as good (60-70%).

The pH of raw milk decreased from 3.91 to 3.86 when *C. procera* extract was introduced and was reduced further to 3.58 in the *C. procera* cheese. A similar reduction trend was observed in milk processed with *Cymbopogon citratus* extract as pH reduced from 3.91 to 3.79 in raw milk following the addition of *Cymbopogon citratus* extract and further reduced to 3.56 in the *Cymbopogon citratus* cheese.

The total microbial counts increased from $7.30\log_{10}$ cfu/ml to $7.38\log_{10}$ cfu/ml in *C. procera* processed milk and 7.30 to 7.37 $\log_{10}$ cfu/ml in *Cymbopogon citratus* processed milk following the addition of the two leaf extracts to the raw milk. At the curdling point (when temperature was raised to 95°C), the microbial count reduced from $7.38\log_{10}$ cfu/ml (in *Calotropis procera* processed milk) to 6.28 $\log_{10}$ cfu/ml. A similar reduction pattern was observed in *Cymbopogon citratus* processed milk. An increase in the total microbial count from curdling point to the finished product was observed in the two cheese types (Table1).

Moisture content increased from 58.75 % (in raw milk) to 60.33% and 61.48% with the addition of *C.procera* and *Cymbopogon citratus* extracts to milk, respectively. Further increases in moisture content to 62.89% and 63.56% were also obtained in *C. procera* and *Cymbopogon citratus* cheese, respectively.

The percentage concentrations of nitrogen, protein, fat and lactose in *C. procera* extract processed milk were 0.01 to 0.6, slightly higher than the *Cymbopogon citratus* extract processed milk (Table 1). A similar trend was obtained in *C. procera* processed cheese and *Cymbopogon citratus* cheese. The observed nitrogen values were milk (2.05%), milk with added extracts (2.02 and 2.01%, respectively) for *C. procera* and *Cymbopogon citrates.* The values obtained were slightly lower (2.00 and 2.01% respectively) in the *C. citratus* cheese and the *C. procera* cheese.

Protein values were 2.56 in *C. procera* cheese and 2.53 in *Cymbopogon citratus* cheese. A similar trend was obtained for percentage fat (Table 1). The trace elements varied significantly ($p<0.05$) along the processing line of the two cheese varieties.

There was an increase in Fe, Zn, Cu, and Na along the processing line, but a decrease was obtained in Mn, Ca, Mg and K (Table 2). There was no significant difference in the Zn, Cu, Mn, Ca, Mg, K, N, protein, fat and lactose contents of the two cheese types although all trace elements and nutrients assayed were higher in the *Calotropis procera* processed cheese with the exception of Fe content (Table 2).

DISCUSSION

The scores of the organoleptic test, which were close, showed that the two cheese types were comparable. The decrease in pH along the processing line for the two plant exracts agrees with the findings of Adegoke *et al*. [4] and Adetunji *et al*. [5] who also reported a decrease in pH of dairy products following the addition of *C. procera* and *Cymbopogon citratus* extracts, respectively. The pH of *C. procera* cheese (3.58) and the *Cymbopogon citratus* (3.56) in this work is lower than the pH range of 5.10 – 5.36 of the one-day-old cheese obtained from the local market in Ilorin, Kwara state, Nigeria reported by Belewu [8] and 5.70 – 6.90 pH values reported by Adegoke *et al*. [4] in one-day-old *C. procera* processed cheese. Beresford *et al*. [3] reported that the optimum pH for the growth of most common bacteria is around neutral and growth is often poor at pH values < 5.0. The real inhibitor is thought to be the undissociated form of the organic acid [16].

The rise in the microbial counts with the addition of the two plants extracts is probably due to contamination of milk by the plants naturally associated microorganisms [4]. A similar increase in microbial counts after addition of plant extract was reported by Adetunji *et al*. [17]. The reduction at curdling point in microbial load was due to the extremely high temperature that inactivates the microorganisms [18, 19] similar reports of reduction in microbial load were made by [4, 5, 6]. The subsequent increase in the total microbial count from 6.28 (at curdling point) to 7.34 $\log_{10}$ cfu/ml (in *C. procera* cheese) and 6.30 (at curdling point) to 7.43 $\log_{10}$ cfu/ml (in *Cymbopogon citratus* cheese) was as a result of the increase in the moisture content and drop in temperature of the two varieties of cheese, which provided an ideal environment for the microorganisms. Concentration of the protein cheese curd after coagulation could also be a factor since microbes will also be drawn together. However, most foodborne pathogens will multiply below the temperature of 60^{o}C. Therefore, food must be kept above this temperature (>60^{o}C) or below 4^{o}C to reduce the risk of microbial spoilage of food and possibility of food poisoning.

In this study, moisture content was highest proportion followed by fat and protein. Alalade and Adeneye [6] reported similar results. The values for moisture content were lower than the 70.75 ± 0.48% moisture content values reported by Alalade and Adeneye [6] but higher than 61.7% of soft cheese obtained from Oyo in Nigeria as reported by Adegoke *et al*. [4]. This difference may be due to the lack of standardized methods in the processing of wara cheese. The slight differences in nutritional contents of the two cheese types suggest that *Cymbopogon citratus* can be used as an alternative coagulant in cheese processing. The lower nitrogen (2.00 and 2.01%) in the cheese types in this study were lower than 2.20% reported by Belewu [8]. While the protein values of 12.56 and 12.53 were greater than the values 8.30 and 13.3 –

14.2 reported by Belewu *et al*. [20] and Adegoke *et al*. [4], respectively. This variation is probably because of lack of standardized techniques in the processing of wara cheese. The result obtained from percentage of fat also followed a similar trend. The reduction in nutrient composition (especially in protein) observed in a different processing phase in the two varieties of cheese may be due to the rise in temperature the mixtures were subjected to during the processing. High environmental temperature denatures the total protein content of milk and may decrease the firmness of curd produced from such milk [21]. A report of a decrease in trace elements was made by Coni *et al*. [22] similar to some of the trace elements in this study, but the increase in some of the elements may be due to the contribution of the processing equipment in to the cheese. Some elements from the equipments used for the processing may have migrated into the cheese during processing. A negative correlation between the pH of cheese and the populations of total aerobic bacteria was observed by Yazici [23]. Similar reports were made by other researchers [24, 25, 26]. The slight drop in pH, however, was not inimical to the increase in microbial flora. Other factors like the moisture contents may have contributed to this microbial picture. The microbial load in cheese was high (>7.0log10 cfu/gm), above international standard of 4.0 log10 cfu/gm by Codex Alimentarius [27]. One of the major sources of extraneous contamination of processed food is the equipment used for processing [28]. All equipments used in this study were sterile, and the increase in microbial counts during the storage of wara cheese is primarily due to the multiplication of the initial microbial populations in the milk used to process the wara cheese. Ensuring proper decontamination of cooking or processing equipment is essential to improve the safety of food for human consumption.

CONCLUSION

The comparative study on the nutrient composition of the two varieties of cheese processed from the leaf extract of the two plants suggested the *Cymbopogon citratus leaf* extract to be an alternative local coagulant in wara cheese processing since the nutrient composition of the two cheeses varied only slightly. Further work on yield enhancement for *Cymbopogon citratus* use as coagulant in wara cheese processing is still necessary.

Table 1: Means of nutrients and microbial load changes along the line of processing of *C. procera* cheese and *Cymbopogon citratus* cheese

Processing Stages	N%	Protein	Fat (%)	lactose %	Starch %	M.C%	pH	TPC(cfu/ml)	Log_{10} cfu/ml
Raw Milk	2.05a	2.78a	5.33a	1.86a	0.0	88.75e	3.91a	20×10^6	7.30e
Calotropis extract + milk	2.02b	2.63b	4.78b	1.80b	0.0	60.33d	3.86b	24×10^6	7.38b
Cymbopogon citratus extract+ milk	2.01c*	2.58c	4.60c	1.74c	0.0	61.48c *	3.79c	23.5×10^6	7.37c
Curdling point lemon grass (took a longer time)	NT	NT	NT	NT	NT	NT	NT	20×10^6	7.30f
Curdling point Calotropis	NT	NT	NT	NT	NT	NT	NT	19×10^6	7.28g
Cymbopogon citratus cheese	2.00d	12.53e	14.33d	1.68e	0.0	63.56a *	3.56e	27×10^6	7.43d
Calotropis procera cheese	2.01c	12.56d	14.43d	1.72d	0.0	62.89b	3.58d	22×10^6	7.34d

N=nitrogen, MC=moisture content, TPC=total plate count, NT=not tested

Values are means

Means with the same letters within the same group are not significantly different at P<0.05

Means with different letters within the same group are significantly different at P<0.05

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Table 2: Trace elements and pH along the line of processing of *C. procera* cheese and *Cymbopogon citratus* cheese

Samples								
	Fe ppm	Zn ppm	Cu Ppm	Mn ppm	Na Ppm	Ca %	Mg %	K %
Raw Milk	10.31a	6.26a	1.23c	2.64b	13.11b	0.39e	0.34d	0.161d
Calotropis extract + milk	9.64e	5.89d	1.41a	2.52e	12.84e	0.77d	0.34d	0.155e
Cymbopogon citratus extract+ milk	10.11b*	5.71e	1.36b*	2.57d	13.09c	0.81c	0.36b*	0.166a*
Cymbopogon citratus curdled cheese	10.03c*	6.01c	0.98e	2.61c	13.17a*	0.83b	0.35c	0.163c
Calotropis procera curdled cheese	9.69d	6.13b	1.03d	2.65a	12.98d	0.85a	0.37a	0.165b

Values are means

Means with different letters within the same group are significantly different at P<0.05

Means with the same letters within the same group are not significantly different at P<0.05

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