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Comparative Effects of Local Coagulants on the Nutritive Value, *in vitro* Multienzyme Protein Digestibility and Sensory Properties of Wara Cheese

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ABSTRACT

Wara, a Nigerian soft cheese, was produced from cow milk using different crude coagulants obtained from (1) the juice of *Calotropis procera*, (2) an aqueous solution of calcium chloride, (3) an aqueous solution of alum and (4) steep waste water from pap production. The objective of this study was to evaluate the effect of these coagulants on the yield, proximate analysis, mineral composition, energy content, *in vitro* multienzyme protein digestibility and sensory evaluation of Wara. The result revealed no significant difference ($p \leq 0.05$) in the yield of the cheese (31.5-32.5%) from the different coagulants. The protein (25.56%), Mn (0.23), Zn (1.9) of *Calotropis procera* coagulated cheese was significantly higher ($p \leq 0.05$) than that of other coagulants. However, it had the least value in energy (6.5 cal g⁻¹), K (26.04), Ca (22.5) and Na (16.98). The steep water coagulated cheese gave a significantly higher ($p \leq 0.05$) fat (21.9%), Fe (1.7), Mg (54.3), K(56.5), Ca(43.6), Na (45.2), energy (8.1 cal g⁻¹) and *in vitro* multienzyme protein digestibility (86.3-92.6%) than the cheese produced by other coagulants. Calcium chloride coagulated cheese had the lowest content of protein (17.85%), Fe (0.993), Zn (0.785) and *in vitro* multienzyme protein digestibility (80.7-83.6%). Alum coagulated cheese had a high content of protein (23.64%), Fe (1.583), Ca (36.9) and Na (31.4). The results obtained from sensory evaluation showed that *Calotropis procera* coagulated cheese gave the best coagulum. The locally used coagulant in Nigeria for cheese production, steep waste water, appears to be promising because of its high content of minerals, though the sensory quality could be improved.

Key words: Cheese, milk, calotropis procera, steep water, alum, CaCl₂

INTRODUCTION

Cheese is one of the numerous products from the processing of milk (Scott, 1986). In many milk-producing countries, a large fraction of the milk produced is used for cheese making (Davies and Law, 1984). Cheese is used as a form of preserving essential nutrients in milk and is an excellent source of nutrients such as proteins, fat, minerals and vitamins. Cheese making in Africa is not properly documented and is largely dictated by tradition. However, the production of cheese in African countries has increased and about one-third of the total volume of milk is used for this purpose (FAO, 2002). The milk clotting properties are important both with regard to the quality and yield of cheese. It has been suggested that a firmer curd at cutting is positively

correlated to yield of cheese (Aleandri *et al.*, 1989; Martin *et al.*, 1997). Johnson *et al.* (2001) showed that a firmer curd at cutting resulted in reduced-fat Cheddar cheeses with higher moisture content. There is widespread traditional manufacture of soft cheese locally called wara in Nigeria. Wara is eaten in various forms such as raw cheese, flavored snack, sandwich filling or fried cake. In Nigeria, *tofu*, a coagulated soymilk, is produced at household level using coagulants such as $\text{CaCl}_2 \cdot 2\text{H}_2\text{O}$, alum and steep water (from pap produced from maize) (Oboh and Omotosho, 2005). The coagulant used in cheese making has a dual role. The primary function is to coagulate milk to produce cheese curd that is, converting the liquid milk to a gel form. This conversion is catalyzed by different proteases (Green, 1984). In addition, a small proportion of the coagulant is carried over into the cheese. This residual coagulant remains proteolytically active in most aged cheeses and plays an important role in the development of texture and flavor (Law, 1987).

Milk is currently graded according to the concentrations of total protein and milk fat. It would be of economic advantage to the dairy industry if a specific marker could be used to identify milk suitable for cheese making; that is, good milk clotting properties and high cheese yield (Wedholm *et al.*, 2008, 2006). In Nigeria, due to the lack of industrial manufacture of cheese, the nutritional benefits have not been fully derived. Nigerian cheese, wara, is usually manufactured from a coagulant obtained from the juice of *Calotropis procera* (Sodom Apple leaves: bomubomu in the Yoruba language). However, other potential sources of cheap coagulants suitable for the production of wara include steep water, $\text{CaCl}_2 \cdot 2\text{H}_2\text{O}$ and alum. The study will reveal the nutritional advantages and *in vitro* multienzyme digestibility of cheese produced from these local coagulants over that from *Calotropis procera* that is popularly used. A comparative analysis of the properties of cheese produced from these potential coagulants will provide a basis for their further development and use in the local production of wara.

MATERIALS AND METHODS

Milk collection and experimental design: Whole milk samples (5 L) were expressed from cows and *Calotropis procera* plants were obtained from the research farm of the Federal University of Technology, Akure, Nigeria in 2004. Three coagulants used in this work include calcium chloride, alum and steep water from pap production. The Calcium salt and Alum were industrial grade, while the steep water was collected from a local pap manufacturing industry. The milk was mixed to form one batch and prepared for coagulation studies in the laboratory.

Preparation of Wara: Four Individual milk samples of 1 L each were preheated to 50°C and each was inoculated with each of the coagulants (0.1 g L^{-1}) namely *Calotropis procera* juice (CP), Calcium chloride (CH), Alum (AW) and steep water from pap production (SW), stirred and heated in a pilot plate heating apparatus at 95°C until the gel was formed. The gel formed was moulded. The scheme for preparation of wara is shown in Fig. 1.

Determination of yield: The amount of cheese produced per litre of milk with the different coagulants was weighed and recorded as the yield.

Nutritional analysis: The nutrient composition (ash, fat, moisture, carbohydrate and crude fibre) of the cheese produced from the different coagulants was determined using the standard AOAC method (1990). The protein content was determined using the micro Kjeldal method. The mineral composition (K, Na, Mg, Fe, Zn and Ca) was determined using a Perkin-Elmer atomic absorption spectrophotometer (model 372) (Perkin-Elmer Inc., 1982).

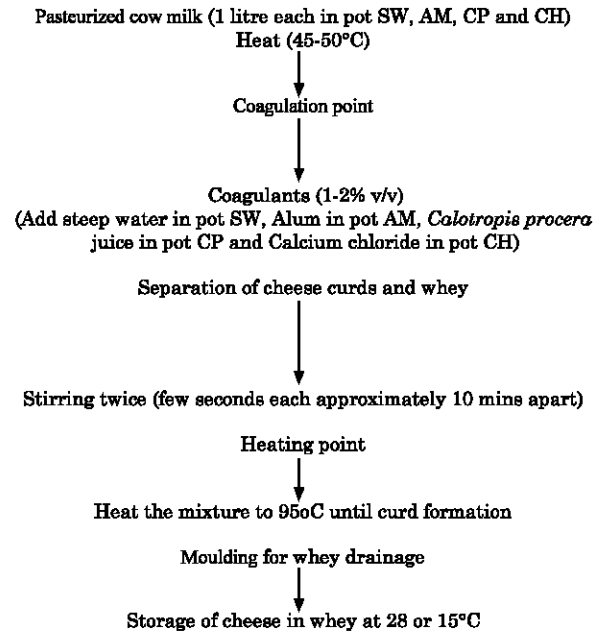


Fig. 1: Flow chart of the coagulation of cow milk for cheese (wara) manufacture

Determination of energy values: The 0.1 g of each cheese was ignited electrically in a Ballistic Bomb Calorimeter (Gallemkamp, CBB- 330-010 L) and burned in excess oxygen (25 atm). The rise in temperature obtained was compared with that of benzoic acid to determine the calorific value of the sample material.

In vitro multienzyme protein digestibility: The *in vitro* protein digestibility of each cheese was carried out using the method of Hsu *et al.* (1977). A suspension of the cheese produced from each coagulant was prepared by dissolving 1.75 g in 50 mL distilled water. The suspension was adjusted to pH 8.0 with 0.1 M NaOH, while stirring in a water bath at 37°C. A multienzyme solution consisting of 1.6 mg trypsin, 3.1 mg chymotrypsin and 1.3 mg peptidase mL⁻¹ was maintained in an ice bath and adjusted to pH 8.0 with 0.1 M HCl. 5 mL of the multienzyme solution was added to each cheese sample suspension with constant stirring at 37°C. The pH of the suspension was recorded 15 min after the addition of the multienzyme solution and the *in vitro* digestibility was calculated using the regression equation of Hsu *et al.* (1977).

$$Y = 210.46 - 18.10X$$

where, Y is the *in vitro* digestibility (%) and X is the pH of the sample suspension after 15 min digestion with the multienzyme solution.

Sensory analysis: The organoleptic properties of each cheese produced using the various coagulants were determined using the method of Potter, (1968). The products were assessed for aroma, taste, texture, colour and general acceptability on a seven-point Hedonic scale (7, excellent; 6, very good; 5, good; 4, average; 3, fair; 2, poor; 1, very poor) and the attribute mean score calculated.

Statistical analysis of data: The results were expressed as mean±standard deviation. Analysis of Variance (ANOVA) and Duncan's multiple range tests were used to determine significant differences between the mean values at $p \leq 0.05$.

RESULTS

Yield: Cheese yield produced from each of the coagulants was expressed as grams of cheese per one hundred grams of milk as shown in Fig. 2. The result revealed that there was no significant difference ($p \leq 0.05$) in the cheese yield of each of the coagulants. However, *Calotropis procera* gave the highest amount of cheese (32.75%) followed by alum (32.5%), while calcium chloride gave the least yield of cheese.

Nutritional analysis: The proximate composition of the coagulated cheese is shown in Table 1 and the mineral composition of cheese produced from each of the coagulants is shown in Table 2, there was a significant difference ($p \leq 0.05$) in the protein contents of cheese (17.85-25.56%). The protein content of *Calotropis procera* coagulated cheese (25.56%) was significantly higher ($p \leq 0.05$) than that of Calcium chloride (17.85%) and there was no significance difference ($p \leq 0.05$) in the cheese coagulated with alum (23.64%) and steep water (22.96%).

Furthermore, the fat content of steep water coagulated cheese (21.94%) was significantly higher than that of the *Calotropis procera* (20.01%) and alum (20.29%) coagulated cheese.

Energy values: The energy values determined for cheese produced from each of the coagulants are shown in Fig. 3, the energy content of the steep water (8.1 cal g^{-1}), calcium chloride (6.9 cal g^{-1}) and alum coagulated cheese were significantly higher ($p \leq 0.05$) than the energy content of *Calotropis procera* (6.5 cal g^{-1}).

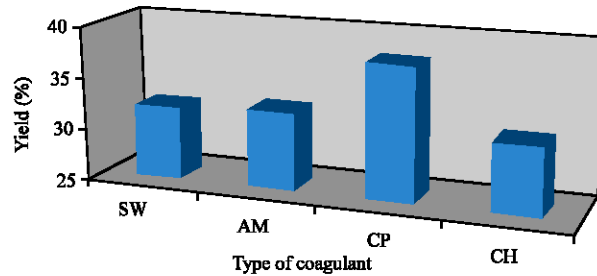


Fig. 2: The yield of cheese produced using locally sourced coagulants SW: Steep water coagulated cheese, AM: Alum coagulated Cheese, CP: *Calotropis procera* coagulated cheese, CH: Calcium chloride coagulated cheese

Table 1: Proximate composition of cheese (% wet weight)

Sample	SW	AM	CP	CH
Protein	23.0±0.3 ^b	23.8±0.3 ^c	25.6±0.1 ^d	17.9±0.6 ^a
Fat	21.6±0.6 ^b	20.1±0.3 ^a	19.9±0.1 ^a	21.7±0.4 ^b
Ash	1.2±0.1 ^a	1.6±0.04 ^b	1.8±0.1 ^c	1.9±0.1 ^c
Carbohydrate	4.2±0.9 ^b	4.1±0.2 ^b	2.5±0.005 ^a	7.8±0.9 ^c
Moisture	50.1±0.005 ^b	50.5±0.3 ^{ab}	50.1±0.1 ^a	51.0±0.6 ^b

Value represents mean of triplicate readings. Values with the same superscript along the same row are not significantly different. SW: Steep water coagulated cheese, AM: Alum coagulated cheese, CP: *Calotropis procera* coagulated cheese, CH: Calcium chloride coagulated cheese

Table 2: Mineral composition of cheese (ppm wet weight)

Sample	SW	AM	CP	CH
Fe	1.7±0.1 ^b	1.7±0.1 ^b	1.2±0.2 ^a	0.99±0.2 ^a
Zn	1.9±0.01 ^b	1.5±0.005 ^b	1.9±0.2 ^b	0.8±0.4 ^a
Mn	0.2±0.1 ^a	1.4±0.3 ^c	1.9±0.4 ^c	0.8±0.2 ^b
Mg	54.3±0.1 ^c	30.8±0.0 ^a	37.0±0.2 ^b	36.7±0.4 ^b
K	56.5±0.1 ^d	37.2±0.3 ^b	26.0±0.2 ^a	51.2±0.3 ^c
Ca	43.6±0.1 ^d	36.9±0.05 ^c	22.3±0.3 ^a	32.4±0.1 ^b
Na	45.1±0.05 ^d	31.2±0.4 ^c	17.0±0.2 ^a	25.5±0.6 ^b

Value represents mean of triplicate readings. Values with the same superscript along the same row are not significantly different. SW: Steep water coagulated cheese, AM: Alum coagulated cheese, CP: *Calotropis procera* coagulated cheese, CH: Calcium chloride coagulated cheese

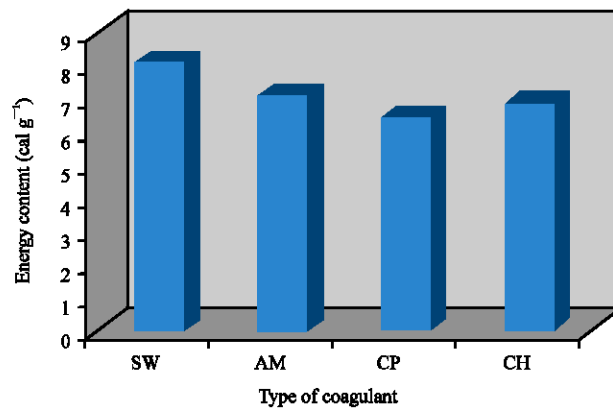


Fig. 3: The Energy Content of Cheese Produced Using Locally Sourced Coagulants: SW: Steep water coagulated cheese, AM: Alum coagulated Cheese, CP: *Calotropis procera* coagulated cheese, CH: Calcium chloride coagulated cheese

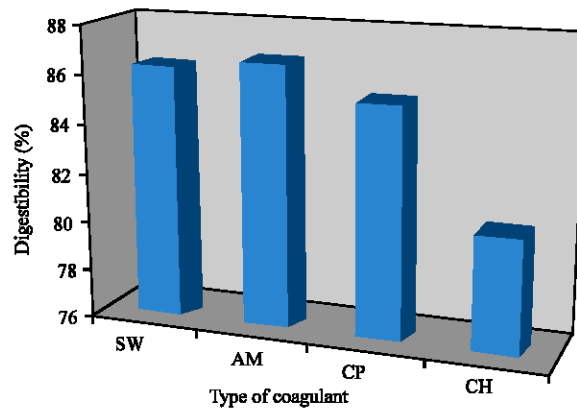


Fig. 4: *In vitro* Multienzyme Protein Digestibility of Cheese: SW: Steep water coagulated cheese, AM: Alum coagulated Cheese, CP: *Calotropis procera* coagulated cheese, CH: Calcium chloride coagulated cheese

Protein digestibility: The *in vitro* multienzyme protein digestibilities of the cheese produced using each of the various coagulants is shown in Fig. 4; the result revealed that there was a

Table 3: Sensory evaluation of cheese

Sample	SW	AM	CP	CH
Taste	1.2±0.1 ^a	1.6±0.3 ^b	1.7±0.3 ^b	0.99±0.2 ^a
Structure	1.9±0.1 ^c	1.4±0.3 ^b	1.9±0.3 ^{bc}	0.8±0.1 ^a
Colour	1.8±0.1 ^a	1.5±0.2 ^b	0.2±0.2 ^a	0.8±0.1 ^a
Odour	37.0±0.2 ^b	30.9±0.2 ^a	54.2±0.4 ^c	36.8±0.3 ^b
Texture	26.0±0.2 ^a	37.3±0.2 ^b	56.4±0.4 ^d	51.3±0.1 ^c
General acceptability	22.5±0.4 ^b	36.9±0.3 ^a	43.8±0.5 ^d	32.5±0.4 ^b

Value represents mean of triplicate readings. Values with the same superscript along the same row are not significantly different. SW: Steep water coagulated cheese, AM: Alum coagulated cheese, CP: *Calotropis procera* coagulated cheese, CH: Calcium chloride coagulated cheese

significant difference in the digestibility of the cheese produced using the various coagulants. Cheese coagulated using steep water had the highest protein digestibility (86.3%). The cheese coagulated with alum had no significant protein digestibility over *Calotropis procera* (85.4%) and calcium chloride (80.7%) coagulated cheese.

Sensory evaluation: The result of the sensory evaluation is shown in Table 3, the result revealed that steep water coagulated cheese had a significantly lower ($p \leq 0.05$) general acceptability than *Calotropis procera*, alum and calcium chloride coagulated wara as typified by the taste, structure, texture, odour and colour.

DISCUSSION

One of the important steps in cheese making is the induced coagulation of milk. The coagulation properties of milk may be used as indicators of processed and final cheese quality. The results from this investigation showed the effect of various coagulants on the proximate and mineral composition of cow milk clotting properties and yield of cheeses. The fact that there was no significant difference ($p \leq 0.05$) in the yield indicated that the various coagulants under consideration may not differ substantially in their coagulating ability. Any slight differences could arise from extraneous substances introduced by the coagulants.

The high protein content in the *Calotropis procera* and steep water could be attributed to the likelihood that the protein in the plant and pap effluent might have been transferred into the cheese, unlike calcium chloride which is a pure salt. Furthermore, it could also be speculated that the double salt in the alum created a better coagulating environment for the protein present in the cheese. However, the protein content in the *Calotropis procera*, alum and steep water gave a higher value than the alum coagulated *tofu*, a coagulated soymilk product manufactured locally (Oboh and Omotosho, 2005). Furthermore, the protein content of the calcium chloride coagulated cheese, which gave the least value, is higher than some commonly consumed tropical plant foods such as yam (4-10%), cassava products (4-12%) and some commonly consumed green leafy vegetables in Nigeria (Akindahunsi and Oboh, 1998, 1999, 2003; Akindahunsi *et al.*, 1999; Oboh *et al.*, 2002).

Likewise, the higher fat content of steep water could also be attributed to the fact that the coagulated protein in the milk might have trapped some of the fat in the heterogeneous solution of steep water. However, the fat content of the cheese were high compared to fat reported for steep water coagulated *tofu* as well as some commonly consumed plant foods in Nigeria (Akindahunsi and

Oboh, 1999; Akindahunsi *et al.*, 1998, 1999, 2003; Oboh and Omotosho, 2005; Oboh *et al.*, 2002). However, there was no significant difference ($p \leq 0.05$) in the moisture and ash content of the cheese using various types of coagulants.

The result of the mineral content (Fe, Zn, Mn, Mg, K, Ca and Na) of the cheese was generally high when compared with the tofu coagulated with various coagulants (Oboh and Omotosho, 2005) and with some consumed plant foods in Nigeria. However, steep water had the highest content of Fe, Mg, K, Ca and Na while *Calotropis procera* had the highest content of Zn and Mn. Conversely, Fe, K, Ca and Na were higher in alum coagulated tofu. (Oboh and Omotosho, 2005). Steep water coagulated tofu also had the highest content of Mn and Mg and alum coagulated tofu had the highest content of Fe, K, Ca and Na (Oboh and Omotosho, 2005). The minerals were higher than that of some commonly consumed plants foods in Nigeria such as edible wild seeds (Oboh and Ekperigin, 2004), cassava products (Akindahunsi and Oboh, 2003), cultivated and wild yams (Akindahunsi and Oboh, 1998) and green leafy vegetables (Akindahunsi and Oboh, 1999; Oboh *et al.*, 2005). The high mineral content in the cheese could be attributed to the high cheese yield, thereby trapping of the minerals in the protein matrix of the cheese.

The alum coagulated gave a higher energy content (7.1 cal g^{-1}) compared with the highest value of steep water and alum coagulated *tofu* (6.6 cal g^{-1}) (Oboh and Omotosho, 2005). Cheese is very rich in protein and fat, which are energy producing macromolecules (Prestamo *et al.*, 2002). This shows that the protein matrix of the cheese traps a lot of energy giving macromolecules.

The low general acceptability of steep water coagulated cheese compared to *Calotropis procera*, alum and calcium chloride coagulated cheese, could be attributed to the fact that the steep water as an heterogeneous mixture with characteristic taste, odour and colour might have imparted its taste, odour and colour on the cheese, which actually reduce the acceptability of the cheese produced from steep water despite its high nutrient content in minerals as highlighted earlier. Although, the acceptability of the texture and structure was lower than that produced by alum and Calcium chloride cheese, the difference was not significant ($p \leq 0.05$). The cheese produced by alum and Calcium chloride had a very good general acceptability while the general acceptability of steep water coagulated cheese was average (Potter, 1968).

The protein digestibility of steep water cheese was higher than that of the tofu coagulated steep water and palm wine yeast fermented cassava flour (79.1%) (Akindahunsi and Oboh, 2003; Oboh and Omotosho, 2005). It could therefore be concluded that tannin, trypsin inhibitor and chymotrypsin inhibitors can interact with proteins in the cheese or the digestive enzymes thereby reducing digestibility of the protein in the cheese and the amount of inhibitors coagulated may vary from one coagulant to another. However, the reduction in digestibility is more evident in Calcium chloride and *Calotropis procera* coagulated cheese than that of steep water.

In conclusion, cheese coagulated from the plant source *Calotropis procera* coagulant has the highest yield value. The locally sourced coagulant from steep water (effluent from pap production) which is considered as a waste appears to be richer in nutritious biochemical macromolecules and has a higher digestibility when compared to calcium chloride and *Calotropis procera*. However, the general acceptability of the cheese produced using steep water is low when compared to the other coagulants. Further study is required on how to improve the sensory quality of the cheese produced using steep water without compromising its nutritional quality.

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