Comparative Evaluation of Nutritional Quality of Soft Cheese Made from Four Plant Sources

G.A. Ibhaze[1,*]

[1] Department of Animal Production and Health, Federal University of Technology, Akure, Nigeria

* Corresponding author Email: begladalways@yahoo.com

Received: 30 August 2017 Accepted: 25 August 2018

ABSTRACT

A laboratory study was conducted to comparatively assess the nutritional profile of cheese precipitated from 20% concentration of four plants coagulants (*Calotropis procera* leaf, unripe *Carica papaya* pulp, unripe *Citrus limon* juice and *Annona muricata* pulp) in a completely randomized design. The moisture, protein, fat, ash, free fatty acids, acid value, pH, peroxide value, percentage yield and mineral (include Calcium, Manganese, Magnesium, Zinc, Iron, Sodium and Potassium) compositions were determined. The proximate composition revealed that moisture (55.47%) was significantly ($P < 0.05$) higher in unripe *Carica papaya* precipitated cheese and least (50.03%) in *Citrus limon* cheese. The protein concentration (17.17%) was significantly ($P < 0.05$) lower in *Annona muricata* cheese compared with 25.65% obtained in *Citrus limon* precipitated cheese. There was no significant difference ($P > 0.05$) in the ash content which ranged from 1.30−2.55%. *Annona muricata* precipitated cheese showed significantly ($P < 0.05$) higher value of 59.91% fat compared to 45.54% obtained in *Calotropis procera* cheese. Free fatty acid values showed significant ($P < 0.05$) differences ranging from 0.32−0.64%, with *Carica papaya* cheese having the highest value. Acid value was significantly ($P < 0.05$) higher in *Carica papaya* cheese with values ranging from 12.40−22.51 mgKOH/kg. Peroxide value (3.07−9.62 mEq/kg) was significantly ($P < 0.05$) lower in *Carica papaya* cheese. The pH values ranged from 4.08−6.41 and the percentage yield was highest (14.79%) in *Citrus limon* precipitated cheese. The mineral (mg/kg) profile showed that *Annona muricata* precipitated cheese has higher contents of Calcium (138.50) Manganese (0.07), Magnesium (2.59) and Potassium (11.50). However, Iron significantly ($P < 0.05$) increased in *Calotropis procera* cheese (0.60). It was concluded that unripe *Carica papaya* pulp, *Annona muricata* pulp and *Citrus limon* juice have potential of being used as coagulants in cheese production as they compete favourably with the commonly used *Calotropis procera* leaf extract.

Keywords: *Annona muricata*, *Calotropis procera*, *Carica papaya*, *Citrus limon*, milk

Thai J. Agric. Sci. (2018) Vol. 51(3): 118−124

INTRODUCTION

Cheese production has been found to be one of the methods used in preserving and conserving the inherent nutrients in milk. Cheese manufacturing is essentially a dehydration process in which the fat and casein of milk are concentrated 6−10− fold (Omotosho *et al.,* 2011).The West African soft cheese '*wara*', is an important component of the diet among the nomadic Fulani of Nigeria and a much cherished delicacy among the urban and peri−urban dwellers of Southwestern Nigeria (Olorunnisomo and Adewumi, 2016) as well as the Northern part of the country. Since long, the animal rennin has been used in cheese production (Raghunath and Gunjan, 2014). Roseiro *et al*. (2003) reported that milk coagulants of plant origin have over−ridden the use of animal rennet. This is because animal rennet may be limited for religious reasons (Judaism and Islam), diet (vegetarianism) or being genetically engineer food, of which the Germans and the Dutch forbid the use of recombinant calf rennet (Roseiro *et al*., 2003). In recent times, alternatives to animal rennet as coagulants have been found in plant extracts and this has given room to choice cheese production. Among such are *Carica papaya*, Moringa seed, lime, lemon, mango fruit, pineapple juice, latex of some Euphorbiaceae family (Olorunnisomo and Ibhaze, 2010; Oladapo and Jadesiimi, 2013; Raghunath and Gunjan, 2014; Adewumi and Akinloye, 2015; Ibhaze *et al.,* 2017). In Nigeria, *Calotropis procera* known as Dead Sea Apple has been found suitable as alternative to animal rennet in cheese production. Inspire of its popularity and usefulness, it is not cultivated commercially (Adetunji and Salawu, 2008). This stimulates the fear of its extinction in years to come hence, the need to search for its suitable substitutes. This study therefore aimed at comparatively evaluating the nutritional quality of soft cheese precipitated with *Calotropis procera* leaf extract, *Carica papaya* pulp extract, *Annona muricata* pulp extract and *Citrus limon* juice from fresh cow milk

MATERIALS AND METHODS

Study Site

The study was conducted in the Nutrition Laboratory of the Department of Animal Production and Health, Federal University of Technology, Akure, Ondo State, Nigeria.

Collection of Milk

Fresh whole cow milk was obtained by hand milking from lactating White Fulani cows between the ages 3−4 years at Ipinsa cattle ranch in Akure, Ondo State, Nigeria. The raw milk was clarified using a cheese cloth and refrigerated to prevent any possible change that might occur while preparing the extracts.

Preparation of Extracts

Calotropis procera leaf (CLE), *Carica papaya* fruits (UPE), were obtained from a backyard farm, while the *Citrus limon* (ULE) and *Annona muricata* (SSE*)* fruits were purchased from a fruit stall. All materials were washed. The *Calotropis procera* leaves (100 g) was weighed and crushed using a mortar and pestle. The crushed leaves were soaked with distilled water at ratio 1:1 (w/v). After five minutes, the mixture was sieved to collect the extract. The washed unripe *Carica papaya* was peeled to obtain 100g of the pulp which were mixed with distilled water at ratio 1:1(w/v), blended with a juice blender and the juice extract was obtained using a muslin cloth. Same procedure was carried out for *Annona muricata* and *Citrus limon* fruits. The extracts which formed the treatments were in the following proportions; 20% CLE, 20% UPE, 20% ULE and 20% SSE.

Preparation of Cheese

The clarified milk (1 L each) was poured into four stainless steel pots and heated to a temperature of 50°C on a low−intensity burner and stirred intermittently. The extracts were added at the stipulated concentrations and heating of the milk continued with intermittent stirring until the milk formed in to curds. The curds were poured into a sieve of 0.2 mm to drain out the whey. The curd formed was placed inside the muslin cloth and a weight of 500 g was placed on it for 30 mins in order to expel more whey. This was done in triplicate.

Chemical Analysis and pH Determination

Milk used and the cheese samples produced were analysed for chemical compositions. In determining the pH of the cheese samples, 10 g cheese sample was homogenized in 20 ml distilled water using in a Phillip blender (PHILIPS NL 9206AD−4 Drachten). The pH of the homogenate was determined using pH meter (Hanna instruments) Peroxide value was determined by titrimetric method of Pearson (1981). 1 g of the sample was weighed into a clean dry boiling tube to which 1 g of powdered potassium iodide and 20 ml mixture of glacial acetic acid and chloroform in the ratio of 2:1 were added. The tube was held in boiling water for 30 sec after which the contents were transferred into a 250 ml conical flask containing 20 ml of 5% potassium iodide solution. This was titrated against 0.002 M sodium thiosulphate solution using 1ml of starch as indicator. A blank titration (without any sample) was also made and the results were reported as the number of 0.002 M sodium thiosulphate per gram of sample.

The free fatty acids of the samples were determined according to the AOAC (2005) methods. Five grams of cheese was weighed into a conical flask, 25 ml diethyl ether, 25 ml alcohol (ethanol) and 1 ml of phenolphthalein solution (1 percent) were added and neutralized with 0.1 M sodium hydroxide solution shaking constantly until a pink colour was obtained. The acid value was calculated from the following equation:

$$\text{Acid value} = \frac{\text{Titration (ml)} \times 5.61}{W_t \text{ (g) of sample used}}$$

Cheese Yield Determination

The yield of cheese was calculated using the formula: Yield = $(W_1 \times 100) / (W_2 + W_3)$ as described by (Raghunath and Gunjan, 2014). Where, W_1 was the weight of the cheese prepared, W_2 was the weight of the milk and W_3 was the weight of the coagulant used

Experimental Design

The experimental design was completely randomized design. Data generated were subjected to one way analysis of variance (ANOVA), using SAS 2012 (version 9.2). Where there were significant differences, treatment means were compared using new Duncan's multiple range test of the same statistical package

RESULTS AND DISCUSSION

The proximate composition of cheese produced using *Calotropis procera* leaf (CLE), *Carica papaya* fruits (UPE), *Citrus limon* (ULE) and *Annona muricata* (SSE) fruits is shown in Figure 1. The proximate composition showed significant difference ($P < 0.05$) in the moisture, protein and fat contents. The UPE processed cheese had the highest moisture content (55.47%) and least (50.03%) in *Citrus limon* cheese. The ULE cheese had the highest protein content (25.65%) while the least (17.17%) was obtained for SSE cheese. The fat content was highest (59.91%) in SSE cheese and least (45.54%) in CLE cheese. There was no significant difference ($P > 0.05$) in the ash content which ranged from 1.30−2.55%. In Table 1, free fatty acid values showed significant ($P < 0.05$) difference ranging from 0.32−0.64%, with UPE cheese having the highest value. Acid value significantly ($P < 0.05$) increased in UPE cheese with values ranging from 12.40−22.51 mg KOH/kg. Peroxide value (3.09−9.62 m Eq/kg) was significantly ($P < 0.05$) lower in UPE cheese. The pH values ranged from 4.08−6.41and the percentage yield was highest (14.79%) in ULE precipitated cheese. The mineral profile (mg/kg) as presented in Table 2 showed that SSE cheese precipitated has higher contents of Calcium (138.50) Manganese (0.7), Magnesium (2.59) and Potassium (11.50). Iron was significantly ($P < 0.05$) higher in CLE cheese (0.60).

Table 1 Physico−chemical evaluation of cheese precipitated from four plant sources

Concentrate (%)	PV (MEq/kg)	AV (mgKOH/kg)	FFA (g)	pH	%Cheese yield
20CLE	8.52 ^{a}	12.40^{b}	0.32^{c}	6.41^{a}	5.42^{c}
20UPE	3.07^{c}	22.51^{a}	0.64^{a}	6.14^{a}	9.10^{b}
20ULE	8.44^{b}	14.71^{b}	0.38^{c}	4.80^{b}	14.79^{a}
20SSE	9.62^{a}	21.82^{a}	0.59^{b}	4.08^{b}	6.21c
SEM	0.47	1.52	0.03	0.11	0.75

Note: ^{a,b,c} Means with the same superscripts along the same column are not significantly different (P > 0.05)
Calotropis procera leaf (CLE), Unripe Carica papaya fruits (UPE), Unripe Citrus limon (ULE) and Annona muricata (SSE) fruits extracts, PV−peroxide value, AV−acid value, FFA− Free fatty acid

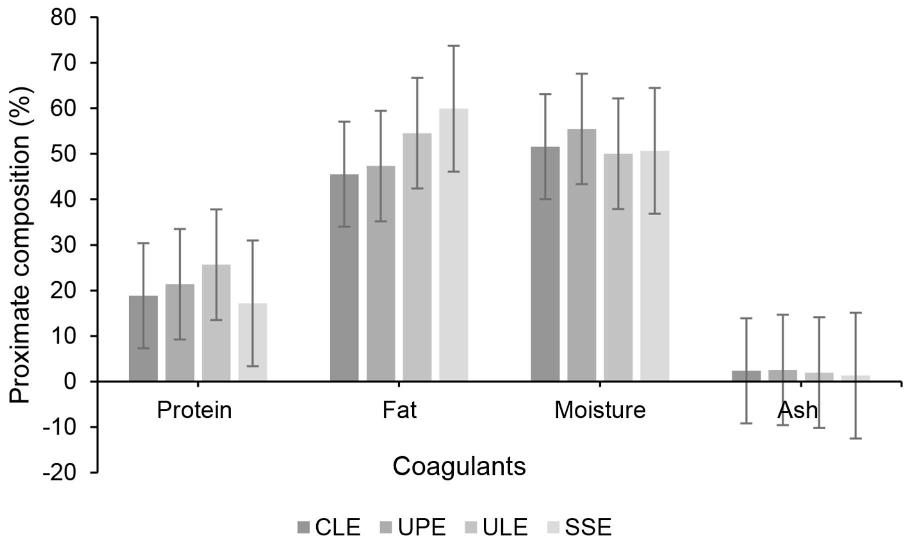

Figure 1 Proximate composition of cheese precipitated from four plant sources Calotropis procera leaf (CLE), Unripe Carica papaya fruits (UPE), Unripe Citrus limon (ULE) and Annona muricata (SSE) fruits extracts

Table 2 Minaral (mg/kg) assessment of cheese precipitated from from four plant sources

Concentration(%)	Calcium	Manganese	Mangnesium	Zinc	Iron	Sodium	Potassium
20 CLE	105.02[b]	0.06	2.52	0.54[b]	0.60[a]	3.56[a]	4.18[c]
20 UCE	113.50[b]	0.03	2.57	0.73[a]	0.34[c]	3.68[a]	8.15[b]
20 ULE	133.00[a]	0.05	2.54	0.82[a]	2.73[b]	2.73[a]	4.80[c]
20 SSE	138.50[a]	0.07	2.59	0.78[a]	2.05[b]	2.05[b]	11.50[a]
SEM	9.13	0.06	0.02	0.06	0.27	0.27	1.32

Note: [a,b,c] Means with the same superscripts along the same column art not significantly different ($P > 0.005$)

Cheeses produced in this study can be classified as soft cheese as their moisture content were within the range of 50−80% for soft cheese. The ULE cheese had the least moisture content suggesting that ULE had the highest coagulating strength than other coagulants. Similar observation was also reported by Adewumi and Akinloye (2015). The higher protein content obtained for ULE cheese indicated that ULE precipitated more casein from the milk which was incorporated into the cheese while the SSE incorporated more fat into the cheese. The protein values obtained in this study were higher than 3.3% for *Calotropis procera* cheese, 2.72% for Carica papaya cheese and 2.23% for lemon juice cheese reported by Adewumi and Akinloye (2015). This variation could be attributed to the difference in the concentration of the extracts used in this study, while the supernatant of the extracts was used by the other researcher. The pH of cheese observed could be attributed to initial pH of the coagulants used suggesting that cheese pH is correlated with pH of the coagulant used. Fatty acids originate from the hydrolysis of the fat in milk. Milk fats contain remarkable amount of short chain fatty acids ranging from C4 to C10 (Stadhouders *et al.,* 1983). The presence of large amounts of free fatty acids (FFA) can facilitate the rate of lipid oxidation (Wassim *et al.,* 2011). In this study, the UPE cheese showed the highest concentration of free fatty acids and the least in CLE cheese. This indicates that UPE cheese would be more susceptible to lipolysis. The higher FFA in UPE could be attributed to disruption of the fat which exposed the fat to lipase enzyme thereby increasing the chance of lipolysis by microbial lipases activities during processing and storage (Wassim *et al*., 2011). The least FFA obtained in CLE cheese may be as a result of the lower fat content in the cheese sample. Reports also have shown that lipolysis of milk and dairy products can occur by three different sources: namely; induced lipolysis, spontaneous lipolysis and microbial lipolysis (Deeth and Fitz−Gerald, 1976; Park, 2001). Induced lipolysis is influenced by several factors, such as processing factors including agitation, separation, pumping, mixing, foaming, presence of air, homogenization, activation by temperature changes, freezing and thawing, storage and processing. Spontaneous lipolysis can occur through two main factors, such as milk processing factors and milking animal factors including stage of lactation, feed, season, breed, mastitis, milk and fat yield and physiological factors. Microbial lipolysis is caused by many microorganisms that contaminate dairy products. These organisms produce lipase which develop rancid flavour. The psychrotrophic bacteria are most common sources of these lipases (Park, 2001). Bacterial lipases are different from milk lipases, are not inactivated by pasteurization and can attack the intact fat globules in milk (Hickey *et al*., 2007; Velez *et al*., 2010; Evers, 2004). The acid value is defined as the weight of KOH in mg needed to neutralize the organic acids present in 1 g of fat and it is a measure of the free fatty acids (FFA) present in the fat or oil. The least peroxide value observed in UPE sample suggest a lower oxidation of fatty acids. The amount of FFA accumulated during ripening may be an overall measure of lipolysis and is quite variable depending on the type of cheese, lactic and secondary starters, rennet type used, ripening time and manufacturing methods and other factors (McSweeney and Sousa, 2000; Collins *et al*., 2003; Velez *et al.,* 2010). The highest percentage yield observed in ULE cheese could be related to the ability of unripe *Citrus limon* to precipitate more protein from the milk. Protein and fat content of milk play a very significant role in yield of cheese (Law and Tamime, 2010). The higher values of calcium, manganese, magnesium and potassium in SSE cheese suggest that *Anonna muricata* is rich in these minerals and the matrix formed in this cheese may be tighter to have bound these minerals. The highest Iron value (0.60 mg/kg) obtained in cheese made from *Calotropis procera* may be attributed to high concentration of Iron in the extract as plants (leaves) are known to be a rich source of iron.

CONCLUSION

This study revealed that unripe Citrus limon, unripe Carica papaya and *Annona muricata* compete favourably with *Calotropis procera*, hence could be good alternatives to *Calotroipis procera* in cheese production. From the percentage yield result, ULE showed superiority to other coagulants. However, calcium, manganese and potassium were higher in *Annona muricata* as a result cheese, it can therefore be used as food supplement in nutritional deficiency of these elements.

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