European Scientific Journal September 2014 edition vol.10, No.27 ISSN: 1857 – 7881 (Print) e - ISSN 1857- 7431

EFFECTS OF DIFFERENT CONCENTRATIONS OF FRESH AND DRIED *CALOTROPIS PROCERA* (SODOM APPLE) EXTRACT ON COW MILK COAGULATING TIME, CHEESE YIELD AND ORGANOLEPTIC PROPERTIES OF WEST AFRICAN SOFT CHEESE (*WAGASHIE*)

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Abstract

This study was conducted to determine the effects of different concentrations of fresh and dried *Calotropis procera* extract (*CPE*) on cow milk coagulating time, yield, whey volume and organoleptic properties of West Africa soft cheese (WASC) (*wagashie*). Three different concentrations of fresh and dried CPE (F2, F5, and F7) and (D2, D5, and D7) were respectively obtained from 2 g, 5 g and 7 g (on dry matter basis) of fresh and dried *Calotropis procera*. Each of the extracts prepared was used as a sole coagulant of pasteurized cow milk and the effects on the milk coagulating time, WASC yield, whey volume, and organoleptic properties of WASC were determined. All the parameters assessed were significantly ($p < 0.05$) influenced by the type and concentration of CPE. The average milk coagulating time, cheese yield and whey volume ranged between 14 - 29 minutes, 134.9 – 237.4 (g/l) and 657.1 – 771.4 (ml/l) respectively. The coagulating time of the fresh *Calotropis procera* extract (*FCPE*) treatments were significantly shorter than that of the dried *Calotropis procera* extract

(*DCPE*) treatments. Generally, milk coagulating time decreased with increased concentration of CPE. The yield of WASC was significantly ($p < 0.05$) higher among DCPE treatments as compared to FCPE treatments. Cheese yield increased with increased concentration of CPE. There was strong negative correlation ($p < 0.05$) between milk coagulating time and WASC yield for both FCPE and DCPE treatments. The type and concentration of CPE added to the milk had significant ($P < 0.05$) effects on the organoleptic properties (taste, colour, texture and overall acceptability) of the WASC produced. Generally, extracts from 2 g of Calotropis had better performance in terms of cheese yield, and organoleptic properties of WASC (*wagashie*).

Keywords: *Calotropis procera*, cheese, coagulating time, concentration

Introduction

Cheese is the fresh or ripened solid or semi-solid product obtained from coagulating milk. Most cheese types are made by the use of rennet to coagulate the casein micelles in the milk and the addition of starter culture to produce lactic acid. The addition of rennet or coagulating agents has been greatly used in the coagulation of milk for the production of cheese. Rennet is a general term that describes a variety of enzymes of animals (especially calves), plant or microbial origins used to coagulate milk during cheese preparation (Craw, 1983). Acids such as acetic acid, citric acid, vinegar and lemon juice have also been used as milk coagulants (O' Connor, 1993).

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 to cheeses (Visser, 1977). Examples of plants with such properties are papaya (papain), pineapple (bromelain), castor seed oil, latex from fig tree and Sodom apple (*Calotropis procera)* plant (O'Connor, 1993).

The shortage in the world supply and high prices of calf rennet (Rogelj *et al*., 2001), refusal to accept cheese made from animal rennet in general and porcine rennet by vegetarians and Muslims respectively (Roseiro *et al*., 2003), necessitated the need to substitute animal rennet with easily available, cheap, and acceptable source of rennet for cheese preparation. For these reasons, the plant Sodom apple (*Calotropis procera*) has been given much attention since it has the potential to completely substitute animal and microbial rennet used for commercial cheese production.

Extracts from the succulent leaves and stems of *C. procera* has been reported to contain rennet enzymes called calotropin that coagulates milk (Belewu and Aina, 2000). The extracts from the Calotropis plant has been used traditionally as the sole coagulant of milk in a number of African countries for the production of a soft cheese called '*wagashie'* (Ibiama and Griffiths, 1987; O'Connor, 1993; Ashaye *et al*., 2006; Chikpah *et al*., 2014). However, a green colouration and bitter nature of the cheese produced are some problems that might be associated with the use of Calotropis plant as coagulant of soya milk (Chikpah *et al*., 2014).

However, due to the seasonal variations in the availability of *Calotropis procera*, and its scarcity in urban and peri-urban cities, there is the need to find suitable forms of processing the plant material for easy storage and transport. Also studies need to be conducted to reduce or completely eliminate the green colouration and bitter effects that *Calotropis procera* extracts (CPE) have on cheese products. Therefore, this study seeks to determine the effects of different concentrations of extracts from fresh and dried *Calotropis procera* succulent leaves and stems on cow milk coagulating time, cheese yield, and organoleptic properties of West Africa soft cheese (*wagashie*).

Materials and Methods

Study area

The Experiment was conducted in the Food Technology Unit of the Faculty of Agriculture, University for Development Studies (UDS), Ghana.

Calotropis procera plant samples collection and processing

The succulent leaves and the stems of *Calotropis procera* plant were harvested in March, 2014 from Nyankpala town in the Tolon District of the Northern region of Ghana. After harvesting, the leaves and stems were washed with clean water and sliced into smaller sizes. The plant samples were then divided into two (2) equal portions. Plant extract was obtained from one of the portions, whilst the other portion was air- dried for four (4) days. The dried leaves and stems of *Calotropis procera* were then ground using a domestic electric blender and extracted.

Extraction of *Calotropis procera* leaves and stems

Three different strengths of extracts were prepared from the powdered and fresh *Calotropis* leaves and stems. Approximately 2 g, 5 g, and 7 g of the powdered C. *procera* plant samples were measured and extracted using 100 ml of pasteurized cow milk according to the method described by Chikpah *et al*. (2014). Similarly, equivalent amounts of the fresh plant samples (18.7 g, 46.7 g and 65.4 g of fresh weight) representing 2 g, 5 g, and 7 g on dry matter basis were measured for extraction. A mixture of equal proportions of succulent leaves and stems of *Calotropis procera* contained 89.3 % moisture and 10.7 % DM. Each of the quantities of fresh Calotropis plant samples measured were ground separately using a laboratory mortar and pestle. The homogenized plant materials were also extracted according to the method described by Chikpah *et al*. (2014). The filtrates or extracts obtained from each of the three different quantities of fresh and dried Calotropis samples were used as sole coagulants of cow milk for the preparation of West African soft cheese (*wagashie)*. Each treatment was replicated three times.

Cow milk collection and pretreatment

Fresh cow milk was purchased from local herds men in Tamale and pasteurized at 70 ^{o}C for 15 minutes before use.

Preparation of West African soft cheese (*wagashie)*

Each of the fresh and dried Calotropis extracts prepared (approximately 100 ml) was added to 900 ml of pasteurized cow milk measured into a clean aluminium saucepan and stirred thoroughly. The mixture was then warmed with an electric plate to temperatures between 45 and 50 °C and carefully observed till coagulation commenced. Coagulation was allowed to continue for about 20 minutes when adequate coagulum was observed. The mixture of coagulum and water (whey) was allowed to cool and then filtered using clean plastic mesh to separate the whey from the raw curd. The curd was allowed to stand for about 30 minutes and pressed to drain excess whey and to make the curd firm. The curds were then molded into fine shapes. Each product preparation was repeated three times.

Data collection

The experimental parameters considered in the present study include, milk coagulating time, cheese yield, volume of whey discharged, and organoleptic properties of the raw cheese (*wagashie*).

Measurement of milk coagulating time

The coagulating time was the time taken for the first clot to form. The set up for cheese preparation was carefully observed and the time (minutes) taken for the first clot to form for each treatment was recorded. The average of the three replicates for each treatment was calculated and used as the mean coagulating time.

Determination of cheese yield (g/L) and volume of whey (ml/L)

This was obtained by weighing the raw curd using an electronic scale (Sartorius, TE 612). The average curd weight of the three replicates for each treatment was computed and used as the cheese yield of the treatment. The yield was expressed as weight of curd (g) per litre of milk used. Similarly, the amount of whey (ml/l) that was discharged at the end of the cheese preparation process was taken and the average of the three replicates was calculated for each treatment.

Evaluation of Cheese organoleptic properties

A five-point hedonic scale (Table 1) described by Sugri and Johnson (2009) with some modifications was used to assess the sensory characteristics of the milk curd samples. The sensory qualities considered include: taste, flavour, colour, texture and overall acceptability of the raw products. Fifteen panelists were randomly selected and trained for the sensory evaluation of the products.

Table 1: The five way hedonic scale used to assess the organoleptic properties of the cheese

Hedonic scale	1	2	3	4	5
Taste	No sweetness/bitter	Slightly sweet	Sweet	Very sweet	Extremely sweet
Flavor	Off flavor	Slightly strong	Strong	Very strong	Extremely strong
Colour	Almost white	Slightly green	Green	Very green	Extremely green
Texture	Very soft	Slightly soft	Hard	Very hard	Extremely hard
Overall acceptability	Dislike	Fair	Good	Very good	Excellent

Sugri and Johnson (2009)

Data analysis

The data obtained were subjected to analysis of variance (ANOVA) using GenStat statistical software (fourth edition) and where significant differences were observed, the means were compared at 5 % level of significance.

Results and Discussion

Effects of dried and fresh *Calotropis* extracts on milk coagulating time, cheese yield and whey volume

The average coagulating time, cheese yield and whey volume of the various treatments are shown in Table 1. The average coagulating time ranged between 14 - 29 minutes. The average coagulating time differed significantly ($p < 0.05$) between FCPE treatments and DCPE treatments. Generally, the FCPE recorded shorter coagulating time as compared to the DCPE treatments. The average coagulating time was shorter in treatment F7 (14 minutes) but longer in treatment D2 and D5 (29 minutes). Generally, the coagulating time reduced with increasing concentration of CPE.

Similarly, the average yield of West African soft cheese (WASC) was also significantly ($p < 0.05$) different between the FCPE and DCPE treatments (Table 1). The average WASC yield was highest in treatment D7 (237.4 g/l) whiles F5 recorded the lowest yield (134.9 g/l). The cheese yield of DCPE treatments were not significantly different ($p > 0.05$) but higher than the yield of FCPE treatments. Generally, WASC yield increased with increasing concentration of CPE.

The average whey volume also differed significantly ($p < 0.05$) among the FCPE and DCPE treatments. Conversely, the whey discharged was significantly lower in the DCPE treatments as compared with the FCPE. The average whey volume ranged between 657.1 – 771.4 ml/l. This indicates that about 65.7 - 77.1 % of the volume of milk used was expulsed as whey.

The fresh plant extracts recorded rapid coagulating activity as compared to the dried extracts. This suggests that the process of drying might have influenced the coagulation activities of the plant proteins or enzymes in the dried Calotropis plant materials. However, the fresh extracts produced lesser curd yield and higher whey volume. The findings from this study supports earlier findings by Akinloye and Adewumi (2014) that different local coagulants used for cheese production had different curd yields, clotting time and whey volumes. The higher volumes of whey obtained as compared to the cheese yield could be attributed to the fact that water is a major component of milk representing about 87 % of the total milk composition (Potter and Hotchkiss, 1995). Otchoun *et al*. (1991); Egountely *et al*. (1994) reported that about 5 litres of milk is required to produce one kilogram of cheese. This implies that, to produce larger quantities of cheese, more milk is needed irrespective of the type or quantity of coagulant used.

Table 1: Effects of varying concentrations of dried and fresh Calotropis procera extracts on coagulating time, cheese yield and whey volume.

Treatments	**Clotting time (minutes)**	**cheese yield (g /l)**	**Whey volume** (ml/l)
D2	29.0^{a}	203.1^{a}	707.1^{a}
D5	29.0^{a}	214.3^{a}	678.6^{a}
D7	27.5^{a}	237.4^{a}	657.1^{a}
F2	16.0^{b}	138.1^{b}	771.4^{b}
F5	15.0^{b}	134.9^{b}	771.4^{b}
F7	14.0^{b}	153.6^{b}	771.4^{b}
P-value	**0 .001**	**0.005**	**0.001**

Mean values were computed from three replicates. Means within a column having different superscripts are significantly different ($p < 0.05$). Treatments (D2, D5, and D7) respectively represent extracts from 2 g, 5 g, and 7 g of dried Calotropis leaves and stems whereas (F2, F5 and F7) represent extracts from 2 g, 5 g, and 7 g (on dry matter basis) of fresh Calotropis leaves and stems.

Correlation between cow milk coagulating time and cheese yield

There was strong negative correlation ($p < 0.05$) between milk coagulating time and WASC yield for DCPE and FCPE treatments with correlation coefficient (r) values of -0.947 and -0.775 respectively (Figure 1). The strong negative correlation observed between the CPE treatments is an indication that cheese yield would increase when coagulating time decreased perhaps as a result of increased concentration of the coagulant. Therefore the shorter the coagulating time the higher the cheese yield. Similar, result was observed when CPEs at different concentrations were used as coagulants of soymilk for soy cheese preparation (Chikpah *et al*., 2014).

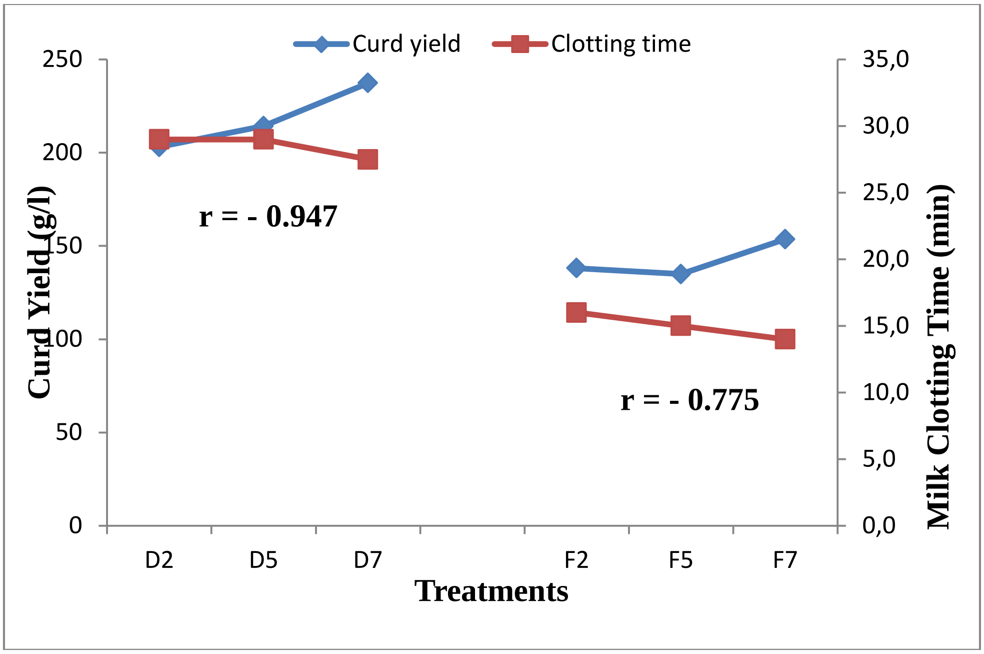

Figure 1: shows the relationship between curd yield (g/l) and milk coagulating time (minutes). Treatments (D2, D5, and D7) respectively represent extracts from 2 g, 5 g, and 7 g of dried Calotropis leaves and stems whereas (F2, F5 and F7) represent extracts from 2 g, 5 g, and 7 g (on dry matter basis) of fresh Calotropis leaves and stems.

Effects of different concentrations of fresh and dried extracts of Calotropis on the organoleptic properties of West African soft cheese (*wagashie*)

The organoleptic properties of the cheese produced from the different concentrations FCPE and DCPE treatments are shown in Table 2. Taste, colour, texture and overall acceptability of all the cheese products were significantly ($p < 0.05$) affected by the coagulants. The tastes of WASC for all the treatments were scored between 1.60 - 2.68. This indicates that the taste of WASC products varied between slightly sweet to sweet. Generally, the taste of the WASC changed towards no sweetness with increasing concentration of FCPE and DCPE. The significant difference in the taste of the various products is an indication that the concentration of CPE as coagulant had significant influence on the taste of cheese products.

The colour of WASC products were strongly affected by the concentration of CPE ($p < 0.05$). The colour of cheese products of DCPE treatments changed from slightly green (1.97) to green (2.80) whereas cheese obtained from FCPE treatments had colour range of slightly green (1.60) to very green (3.63) as the amount of plant extract increased from 2 g to 7 g (DM). This suggests that the green colour of Calotropis leaves influenced the colour of WASC produced. This supports the finding of Chikpah *et al.* (2014) when CPEs were used to coagulate soy milk.

The texture of WASC of treatments D2, D5, D7, F5 and F7 did not differ significantly ($p > 0.05$) but varied slightly from cheese products of treatment F2 (Table 2). The textures of all WASC products were slightly soft. The general soft nature of WASC observed in the study supports the earlier reports that *Calotropis procera* extracts (*CPEs*) as coagulants are not suitable for hard cheese production (O'Connor and Tripathi, 1992).

The flavour of the cheese products were however not influenced ($p > 0.05$) by the CPE. The flavours of all cheese products were slightly strong.

The overall acceptability of WASC products were scored between fair and good. Cheese of treatments D2, F2, and F5 were much preferred to the rest of the cheese products. Generally, the acceptability of the products reduced when the concentration of CPE used increased. The significant influence of the CPE concentrations on the taste, texture, and colour of WASC might have affected the overall acceptability of the various WASC products. Generally, treatment D2 had better taste, texture and colour as well as high overall acceptability.

Table 2: Effects of Coagulant type and concentration on the organoleptic properties of West African Soft cheese (WASC)

Treatments	**Taste**	**Flavor**	**Colour**	**Texture**	**Overall acceptability**
D2	2.33^{bc}	2.28^{ab}	1.97^{a}	2.33^{a}	2.90^{c}
D5	1.83^{a}	2.13^{a}	2.30^{b}	2.27^{a}	2.37^{a}
D7	1.90^{ab}	2.60^{b}	2.80^{c}	2.33^{a}	2.13^{a}
F2	2.68^{c}	2.00^{a}	1.60^{d}	1.53^{b}	2.67^{bC}
F5	2.33^{bc}	2.27^{ab}	2.63^{e}	2.27^{a}	2.60^{bC}
F7	1.60^{a}	2.13^{a}	3.63^{f}	2.57^{a}	2.23^{ab}
P-value	**0.001**	**0.091**	**0.001**	**0.001**	**0.017**

Mean values were calculated from three replicates. Means within a column having different superscripts are significantly different ($p < 0.05$). Treatments (D2, D5, and D7) respectively represent extracts from 2 g, 5 g, and 7 g of dried Calotropis leaves and stems whereas (F2, F5 and F7) represent extracts from 2 g, 5 g, and 7 g (on dry matter basis) of fresh Calotropis leaves and stems.

Conclusion and Recommendations

The concentrations of FCPE and DCPE significantly affected the cow milk coagulating time and yield of WASC produced. The milk coagulating time was lower when FCPEs were used as coagulant as compared to DCPEs. However, the yield of WASC was significantly higher in DCPE treatments than the FCPE. Generally, with increased concentration of CPE, milk coagulating time decreased whiles cheese yield increased. Milk coagulating time and cheese yield were strong and negatively correlated. The colour, taste, texture and overall acceptability of cheese were significantly influenced by the concentrations of CPE. Generally, Treatment D2 recorded better performance in terms of cheese yield, and organoleptic properties. Therefore DCPE can be used as cow milk coagulants. Further studies should be conducted to improve the texture, taste, and colour of West African cheese (*wagashie*) when CPE are used as coagulants of cow milk.

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