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ORIGINAL PAPER

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CHARACTERISATION AND STANDARDISATION OF WANGASHI CHEESE PRODUCTION STEPS

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ABSTRACT

This research aims to assess the effect of *Calotropis procera* plant stems as a coagulant treatment on the Wangashi cheese in order to characterize and standardize its production. Crude extract obtained from the *Calotropis* plant stems added to milk powder were used as a solution dissolved at various pH (4–8) and temperature (35–80°C) in order to examine the effect of pH and temperature on milk clotting and proteolytic activities. The pH 5.5 and temperature of 70°C were recorded as optimum pH and temperature. After that the concentration of the crude extract enzyme was assayed to purify it using ammonium sulfate precipitation at various percentage of saturation (20–80%) at determined optimum pH and temperature, whereby the saturation of 70% was detected to be the best because of its high specific activity, yield and purification fold. Two types of Wangashi cheese were produced in laboratory, one using directly the crude extract and the other the purified crude extract from *Calotropis procera* at optimum condition. Their chemical, textural and color properties were determined using standard methods. A significant difference between parameters tested was observed ($p < 0.05$). A decrease in moisture content, increase in protein content and also an improvement of color and textural parameters were recorded for the cheese obtained using purified crude extract *Calotropis procera* stems.

Keywords: Wangashi cheese, *Calotropis procera*, milk clotting activity, proteolytic activity, standardization

INTRODUCTION

The use of the enzyme is the main step to milk clotting in cheese production. In general, ruminant stomachs are the main source for the most common coagulant agents used for cheese production. On the other hand, enzymes from plants and microbial sources are also used as coagulants in cheese production Harboe et al. (2010), Jacob et al. (2011). The use of microbial and plant enzymes has been recognized as an alternative solution, in the case when the animal source encounters some problems. The problems encountered range from the limited availability of ruminant stomachs (Jacob et al., 2011), the high price of rennet, some religious concerns (for example, Islam and Judaism), diet (vegetarianism) or the ban on recombinant calf yeast (in France, Germany and the Netherlands). Microbial enzymes (such as those from mushroom origins, *Rhizomucor pusillus*, *Cryphonectria parasitica* and *R. miehei* proteases), due to their proteolytic activity, these enzymes are defined as milk coagulants and therefore less suitable. Excessive proteolysis causes reduced ripening time. For excessive proteolysis control, coagulants with higher heat stability capacity

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than calf rennet should be avoided so that there would be no difference in coagulation temperature (Sousa and Malcata, 2002). The main area of innovation in cheese making is related to the development of the sensory and textural properties of the products (García et al., 2012). Many plant coagulants are used for cheese processing. Plant-based enzymes coagulants are widely used in cheese making all around the world (West Africa, Mediterranean and Southern Europe). Nowadays, plant-based enzymes are recognized as an alternative that can replace the coagulants from animal source and microbial enzymes in cheese making. Almost every part of the plant (leaf, root, latex, etc.) can be used as milk coagulants (O'Connor, 1993). In most cases, the use of the plant as a coagulant leads to obtaining the desired final product and contributes to the nutrition of those who have been banned from animal yeast for some reason (Gupta and Eskin, 1997). Coagulation activity of many plant preparations has been described in several scientific articles such as: Aworh et al. (1987), Edwards and Kosikowski (1983), Padmanabhan et al. (1993), Pozsar et al. (1969), Tamer (1993). However, due to the excessive proteolytic activities observed in these plants, cheese yield may decrease and cheese can have undesirable flavors (Lo Piero et al., 2002). For example, some traditional Portuguese and Spanish cheese have been made using cardoon extract for centuries. In some African countries (Benin, Ghana, Togo, Burkina Faso, Chad, Nigeria), the traditional use of extracts from the *Calotropis* plant as milk coagulant was identified as an effective coagulant (Ashaye et al., 2006; Chikpah et al., 2014). *Calotropis procera* is a plant known particularly because of its medical use. It is included in traditional medicine by traditional doctors in West Africa and Asia due to its antibiotic properties and other medical properties to relieve diarrhea, leprosy, fever, ringworm, cough and asthma. It is also frequently recommended to cure digestive problems, colds and to increase appetite (Mainasara et al., 2011). It is used worldwide by Fulani farmers, especially in West Africa (Benin, Nigeria, Ghana, etc.); many articles showed that *Calotropis procera* has some clotting properties and is used as a basic material for milk coagulation in cheese production (Dossou and Adote, 2006). In Benin, fresh leaves and stems of *Calotropis procera* are traditionally used for milk clotting, which is an important step in peulh cheese processing (Tossou, 2018). The study carried out by Capo-chichi (2004) revealed that almost all parts of calotropis procera: leaves, stems, fruits and latex had a coagulating effect on milk casein at different coagulation rate. It is stated that fruits are less effective but leaves and stalk have similar clotting activity (Mahami et al., 2012). Haig (2008) showed that the milky sap of this plant contains three toxic glycosides: calotropin, uscharin and calotoxin, as well as steroidal heart poisons known as cardiac aglycones. Contact with *Calotropis* latex has also been reported in some accidental cases by de Lima et al. (2011). The risk of toxicity should be taken into account after long consumption of traditional Fulani cheese, but no food poisoning related to this cheese intake has been reported so far (Egounléty et al., 1994). In addition, other plant or vegetable extracts are used as cheese coagulants for instance lemon juice (Adetunji and Babalobi, 2007), as well as the leaves and sap of Carica papaya as a coagulant (Martínez-Ruiz et al., 2013). Wangashi contains a lot of animal protein. It is a highly nutritious food with a considerable source of protein, fat, vitamins and minerals such as calcium, iron and phosphorus. It is used instead of meat or fish, or in combination with them in miscellaneous food recipes especially for people with low incomes. This cheese could be a solution to proteins deficiency in the diets of African people (Aboudoulaye et al., 2018). It is assigned to the category of soft unripened cheese therefore it has a high moisture content ranged about 50–60% which makes it highly perishable. When the Wangashi cheese is left at ambient temperature, it undergoes considerable chemical changes. For example, the shelf life does not exceed three days. After the second day of storage, the change in the composition is squired by changes in the sensory quality of the product (Ashaye et al., 2006). Traditional Wangashi is known to have a bland taste as well as a bitter after taste. The bitter taste might be the result of the high non-specific proteolytic activity of the *Calotropis procera* known also under the appellation of Sodom apple used as coagulant in Wangashi cheese production. Unfortunately, the use of the Sodom apple also affects the yield of Wangashi and causes the production of excessive acid, bitter flavors and green coloration in the product (Mahami et al., 2012). However some products have bland taste mainly

because the production process has not been standardized. In most cases, the use of the plant as a coagulant produces the desired final product. However, due to the excessive proteolytic activities observed in these plants, the cheese yield may decrease and the cheese obtained can have undesirable flavors (Lo Piero et al., 2002). Another negative aspect is that the shelf life of the product may be affected and it also makes the product unsafe for the consumer. Aworh (1985) and Kees (1996) reported the processing technology of Wangashi and its stabilization by heat treatment, using chemical additives such as propionic acid and sorbates. In addition, Kèkè et al. (2008) reported a method of preservation of Wangashi using strains of *Lactobacillus plantarum*. Nevertheless, the preservation of Wangashi by chemical method has a negative effect on the sensorial quality of the product. Analyzing the enzyme that was extracted from *Calotropis procera* plant used for coagulation of milk to obtain the cheese and standardization of the cheese production, it was noted that steps could be implemented to improve the yield of Wangashi cheese in order to formulate the recipe and carry it into industrial scale for its commercialization. The Wangashi production mainly benefits milk sellers in the peri-urban milk producing areas where it provides employment to women and increases the income of fresh cow milk sellers. A higher demand for traditional soft cheese (Wangashi) has increased the income of milk sellers in Benin.

MATERIALS AND METHODS

The chemical analysis such as: pH, protein, fat content, solid non-fat, lactic acidity, moisture content values of raw milk and cheeses were obtained using the method of Kaya and Oner (1996) and AOAC (2012). The protein content in raw milk was determined by formal titration method (AOAC, 2006), Gerber method was described by Kirk (1991) while Free Fatty Acid (FFA) was determined by neutralized 40 mL of ethanol prepared in presence of three drops of phenolphthalein as an indicator and 0.1N NaOH solutions. To this solution, 5.00 g of each cheese samples cut into small part were added and mixed. The blend was bubbled on a hot plate and titrated with 0.1N NaOH. The titers were collected and the FFA content was determined as amount of oleic acid. All of the experiments were done in triplet; all data were collected and analyzed using a statistical program SPSS V. 22. ANOVA test was performed to observe the difference between the cheese treatments at p 5% and the results were presented within respective tables.

Extraction of enzyme

Calotropis procera stems were collected from Botanic garden of University of Abomey Calavi in Republic of Benin and were brought to Gaziantep within 24 hours with care to protect them. These stems were washed, cut into small portions and crushed using blender. An amount of 40 g of *Calotropis procera* stems were mixed with 100 mL of triple distilled water. The obtained mixing was also filtered using Whatman one phase separator (1 ps) filter paper/185 mm filter paper and centrifuged 3000 rpm at room temperature, for 15 min. The final solution was decanted and the clean separated liquid was stored at refrigerator (4 ±1°C) and used until all experiments had been completed.

Milk clotting activity

Milk clotting activity (MCA) of the enzyme at various pH and process temperature were determined using 0.25 g of powdered skim milk obtained from a Turkish local market that measured into a clean test tube accompanied by 0.75 mL of 0.05 M sodium acetate buffer pH 5.5. The test tube was shaken until the milk dissolved and was heated at 50°C using water bath for 15 min. Later the tubes were collected and 1 mL of crude extract or purified enzyme was added and the coagulation time was detected to estimate the enzyme's clotting activity.

Effect of pH on the milk clotting activity

As described above the same amount of powdered milk was taken into a clean tube test followed by 0.75 mL of 0.05 M sodium acetate buffer that the pH concentration was adjusted from 5.0 to 8.0. The test tubes were shaken until the milk powder was dissolved, after which tubes were undergo the heating process for 15 min at 50°C. In order to determine the milk clotting activity, one mL of crude extract or purified extract from *Calotropis procera* was added and the time milk was taken to be clotted by enzyme was recorded as coagulation time value.

Effect of temperature on the milk clotting activity

Precisely 0.25 g of powdered milk was weighted into clean test tubes containing 0.05 M sodium acetate buffer (pH = 5.5), these test tubes were placed in water bath and shaken for 15 min at different temperature ranging between 35–80°C until the dissolution of milk completely. After that, one mL of crude extract or precipitate enzyme extract from *Calotropis procera* was added into each test tube and the coagulation time was recorded as enzyme milk clotting activity.

Proteolytic enzyme activity

Protease activity (PA) was assayed using crude enzyme and casein as a substrate through the modified method of Ladd and Butler (1972). The casein (1%) solution was prepared by heating up to 100°C in water bath one gram of casein in 99 mL of 0.1 M of potassium phosphate buffer at pH 5.5 for 12 min without boiling the solution. Once the casein dissolved into the buffer, the solution was cooled and used substrate. 5 mL of 1% of casein solution was carried into tube to which one mL of crude enzyme or precipitated fraction was added, well mixed by swirling and let equilibrate in incubator for 10 min at 50°C. After incubation, 5 mL of 5% of trichloroacetic acid (TCA) was added to stop the reaction and tube was again incubated for 30 min. Before measuring the absorbance at 660 nm, the mixture were centrifuged at 3000 rpm for 30 min using eppendorf centrifuge 5810 R and to this mixture were added 5 mL and one mL respectively of 0.05 M of sodium carbonate solution and Folin's reagent. The activity of enzyme (units/mL enzyme) was obtained in term of µmoL tyrosine liberated time total volume of assay according to volume of enzyme assay, time and volume used in colorimetric determination.

Effect of pH on the proteolytic enzyme activity

Two different types of buffer solutions used to arrange the pH of the substrate were adjusted at around pH 5 and 8. Citrate and potassium phosphate buffers were used respectively for pH ranging between 5–6 and 7–8. The reaction mixture contains 1% of casein solution, 1 mL of crude enzyme and other reagents based on the process described above. To the tubes, 0.005 M sodium carbonate was added to regulate any pH drop created by the addiction of Folin's reagent, the mixing into test tubes were thoroughly mixed and incubated at 50°C. After which the proteolytic activities were recorded as µmoL tyrosine liberated and converted in term of (units/mL) enzyme.

Effect of temperature on the proteolytic enzyme activity

The reaction mixture contains 1% of casein solution, 1 mL of crude enzyme and other reagents such 5 mL of 0.05 M sodium carbonate solution and 1 mL of Folin's reagent based on the method described above. This solution was assayed at various temperatures (35, 40, 50, 60, 70 and 80°C) and constant pH of 5.5. The test tubes contained the substrate at constant pH were incubated at different temperatures and the absorbance values read were inserted in the standard curve in order to get the enzyme activity in term of proteolytic which was calculated according to enzyme concentration with total volume solution assayed by time of incubation and volume of enzyme assayed and expressed in units/mL.

Ammonium sulfate precipitation (SAP)

Ammonium sulfate (powder) obtained from Sigma-Aldrich was prepared at various percentage of saturation, from 20% to 80% by adding the amount of gram necessary for each percentage of saturation. 3 mL of the extract of crude enzyme was carried into 6 plastics centrifuge tubes and tubes were labeled from number 1 to 6. Each amount of ammonium sulfate previously weighted according to the saturation was added to the tube 2 through 6, while to the tube 1, same amount of crude extract enzyme was added instead of ammonium sulfate and used as a reference. It was stored at 4°C for subsequent assays. Once the salt was added, tubes were slowly and gently stirred to dissolve rapidly them in order to avoid forming the solutions and get desired saturation. After dissolving all salts, tubes were allowed to stand in ice for 15 min to obtain a best precipitation and then the solutions were centrifuged at 10 000×g for 15 min too. Each precipitate fraction was decanted and dissolved in 3 mL of 0.1 M potassium phosphate buffer, pH 7.4. Purified enzyme's and reference solutions obtained were assayed for protein content determination which allowed us to calculate the enzyme activities (milk clotting, proteolytic and specific activities) using the optimum pH and temperature

that are respectively 5.5 and 70°C in order to identify the best saturation.

Production steps of Wangashi cheese

Two types of the cheese were produced from cow's milk supplied from local farm. Milk was heated up to temperature 70°C then 40 mL of crude enzyme (cheese 1) or approximately 1/7 of crude enzyme value (6 mL) of purified extract enzyme (cheese 2) was added and cooked at about 100°C for 15 min.

After cooking the curd and whey mixture was poured into specific baskets and left to drain the whey about 160 min. Samples for analyses were cut into pieces, covered using aluminum paper and stored at different temperature (4°C and 25°C) in order to perform the texture analyses during two weeks. The flows charts given in Figure 1 describes the step of processing.

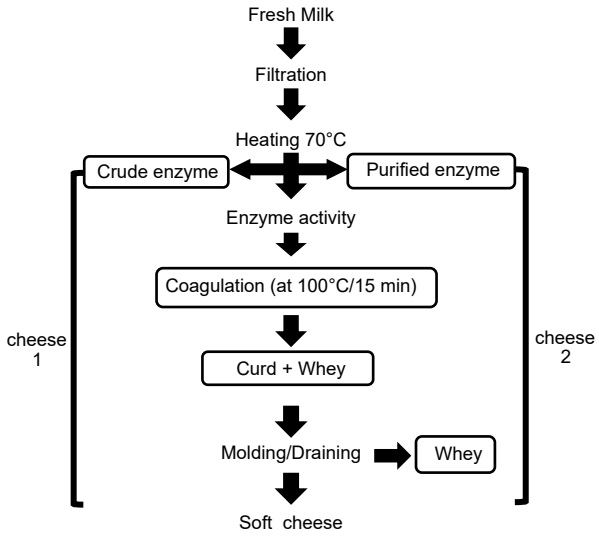

Fig. 1. Modified Wangashi processing

Texture profile analyses and color measurement

The color of Wangashi cheese was estimated by utilizing the *L*, *a*, *b* and YI color documentation framework. It esteems were recorded as *L** = darkness/lightness (0 = dark, 100 = white), *a** (*–a** = greenness and *+a** = redness), and *b**(*–b** = blueness, *+b** = yellowness) using Hunter Lab Color Flex, Hunter Associates Laboratory Inc., Reston, VA, USA while Texture properties of Wangashi cheese samples were checked by a Texture Analyzer TA-XT2 (Stable Micro Systems Ltd., Surrey, UK). The samples were molded consistently with a rectangle probe. Three estimations were taken on each Wangashi cheese sample. TPA parameter estimated was hardness. Test conditions were a pressure strain 25%, with pre-test speed of 3 mm/s, test speed 1 mm/s, post-test speed 1 mm/s, remove 15 mm, time 5 s and a contact power of 5.0 g. The samples were consistently molded with a size of 1 cm width, length and height.

RESULTS AND DISCUSSION

In this study, the stems of *Calotropis procera* were dissolved in different proportions (15, 20 and 25 g) with 100 mL of water and a preliminary study was made based on understanding the effect of its concentration. These three groups of extracts were used to coagulate the cow milk, and three types of Wangashi cheese were produced. The chemical properties of these cheeses were checked. It was observed that the chemical properties of cheese samples made using 20 g of SAP in terms of protein, fat, moisture and pH content were similar to these of Wangashi cheese prepared by Kora (2005). This rate was used in later studies to obtain crude extract. The effect of temperature and pH was examined to define the enzyme activity. The ideal temperature value was determined. Citrate or phosphate buffers were used when 0.1N NaOH or 0.1N HCl were also used to adjust pH values of substrates, respectively, for milk clotting and proteolytic activities.

Optimum temperature and pH

The results are shown in the graphs showing the values of proteolytic and milk coagulation activities at different pH and temperatures (Fig. 2A and 2B). Firstly, keeping the temperature constant, worked at different pH, milk coagulation feature was studied and the ideal pH was determined (pH: 5.5). Then, keeping the pH constant at 5.5, it was worked at different temperature values for optimum process temperature, milk clotting and proteolytic activities. According to the results obtained, *Calotropis procera* stems had proteolytic activity ranging between 18–40%, while milk clotting activity ranged between 14–53%. However, there was

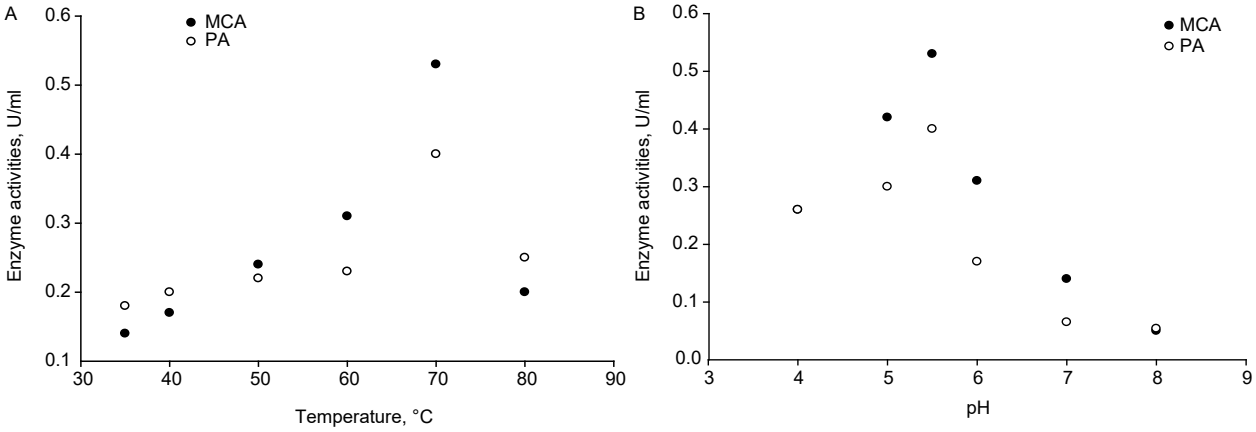

Fig. 2. Effect of temperature (A) and pH (B) on milk clotting and proteolytic activities

a complete loss of milk coagulation activity at pH 8, while proteolytic activity was observed at almost all pH values, but the pH decreased from 7 to 8. The optimum temperature for proteolytic and milk coagulation activities was determined as 70°C, but it was observed that the activity still continued at 80°C.

For *Calotropis procera* latex and papain, proteolytic activity was evaluated in previous studies using albumin as the substrate, and the optimum temperature was specified as 70°C and optimum pH 7.5. Although the temperature values are the same, the reason for the difference in pH values may be the substrate used. Optimum pH and temperature 5.5 and 70°C were used as process temperature and pH in the future studies. A portion of the crude extract was then fractionated using the ammonium sulfate precipitation method (Table 1). The pellet resulting from each precipitation step was re-dissolved in phosphate buffer pH 7.4 and analyzed for protein concentration and protease activity. At this step, the majority of the enzyme activity was found in the pellet of the precipitation of 70% saturation of ammonium sulfate, which gave not only the highest specific activity (thus the purification layer) but also the highest yield among all fractions (Table 1). This finding is consistent with that reported by Purwanto (2016), who assessed the role and efficiency

Table 1. Monitoring of fractionation using ammonium sulfate

Step	Activity µmoL/min/mL	Specific activity µmoL/min/mL/µg	Purification fold	Yield %
Crude extract	0.40	0.005	1	100
20	0.01	0.0009	0.18	3.00
30	0.02	0.0008	0.16	4.50
40	0.05	0.002	0.40	7.40
50	0.30	0.019	3.80	12.25
60	0.77	0.025	5.00	19.20
70	**0.99**	**0.043**	**8.60**	**25**
80	0.88	0.027	5.40	22

of ammonium sulfate precipitation in the purification process of Papain crude extract. The results showed that enzyme proteolytic activities increased with an increase in ammonium sulfate concentration from 20% to 70%, but decreased by 80% ammonium sulfate.

Wangashi cheese production

To see the effect of the purified of crude extract (purified enzyme) on the cheese structure, the crude extract and purified enzyme were used in cheeses production as shown in Figure 1 for the samples expressed as cheese 1 and cheese 2, and the samples were stored at room temperature conditions of 4–25°C. Produced cheeses were evaluated for comparison in terms of yield and coagulation time and other parameters. The cheese yield level was determined as the percentage of cheese weight (kg) by dividing it by the weight of milk (kg). Some of the produced samples were wrapped in aluminum and stored in the refrigerator (+4°C) and in a temperature-controlled incubator (25°C) to ensure normal storage conditions of cheese, since cheese is traditionally produced and consumed within a week at room temperature of 15–25°C.

The chemical, thermal and textural properties of the samples were analyzed and compared. A statistically significant difference was found between the moisture content of both cheese samples ($p < 0.05$; Table 2). The cheese obtained using raw enzyme (cheese 1) had the highest moisture content (59.70%), while lower moisture content was obtained in cheese produced using purified enzyme (cheese 2). This difference observed in moisture content showed a variation in the percentage yield of the cheese samples. The highest yield value was recorded with cheese 1. It could be explained by the fact that the coagulant type affected the cheese yield to the point where it expanded the cheese through to the small amount of whey proteins drained (Abdel Razig, 1996; Ustunol et al., 1985). This could be explained by the fact that cheese 1 was produced using the crude extract, which was not undergo any purification process during which some activity may be occurred to break down enzyme activity. Likewise, Doosh and Abdul-Rahman (2014) stated that high water content in cheese directly affects the yield rate because moisture was considered as one of the most important parameters affecting the expansion or the reduction in yield rate. There was a significant difference between the cheese types in terms of protein, fat, pH, FFA, ash and lactic acid ($p < 0.05$).

Table 2. Some chemical parameters of Wangashi cheeses

Parameter	Cheese	
	1	2
pH	5.01 ±0.04[b]	5.49 ±0.06[a]
Moisture, %	59.70 ±2.14[a]	46.67 ±3.05[b]
Protein, %	20.58 ±0.95[b]	26.01 ±1.81[a]
Fat, %	13.23 ±1.69[b]	15.28 ±0.07[a]
Lactic acid, %	0.44 ±0.06[b]	0.55 ±0.01[a]
FFA, %	7.05 ±0.08[a]	1.42 ±0.01[b]
Ash, %	4.22 ±0.01[b]	5.02 ±0.02[a]

FFA – free fatty acid.
Different capital letters indicate a statistical difference at a 0.05 level in each row.

Cheese 1 had a lower protein content (20.58%) compared to cheese 2, which was obtained using enzymes purified with 26.01% dry basis (d.b.) protein percentage. The lower protein recorded in cheese 1 might be due to the conversion of the protein to soluble nitrogen and then the loss of water-soluble nitrogen from the impaired protein. As for the rest of the parameters listed above, the highest values were observed with the cheese produced using purified enzyme 5.49. It was 15.25%; 5.02%; 0.55% for the percentage of pH, fat, ash and lactic acidity respectively. The percentage of lactic acidity versus the low pH value obtained from cheese 2 might be attributed to the increase in proteolytic activity observed in the purified enzyme compared to the crude enzyme. In the analysis performed, it was observed that the highest FFA content (7.03%) was observed with cheese prepared using raw enzyme. The moisture content (60.21%) obtained for cheese 1 samples was similar to the results from previous studies in various breeds of cow's milk. Indeed, the highest moisture content (60.21%) obtained during this study was compared to what recorded by Kora (2005). The lowest moisture content (51.25%) obtained in the cheese 2 sample was similar to that obtained with Sacramento (2008). The cheese with low water content has a high shelf life due to the limitation

of microbial growth. It could be summarized that the cheese obtained from the purified enzyme can be preserved for a longer period due to the relatively low water content compared to the cheese obtained using the raw extract enzyme (cheese 1). The result of the ash content (4.22% to 5.02%); Kora (2005), Sacramento (2008) and Aïsso et al. (2013) found 1.85%, 2.08% and 1.59% for the ash content in cheese, the ash content was considered as the mineral content of cheese, so that the cheese could be concluded cheese 2 had a higher mineral content. Purified enzyme coagulated cheese 2 obtained from the extract enzyme had the highest protein content of 26.01% (d.b.), what confirmed the finding of Sacramento (2008) that recorded lower protein content (12.56%) using non purified crude extract from *Calotropis procera*. However in the result found by Aïsso et al. (2013), it was recorded a similar protein content compared to what presented in this study, which may be related to the origin of used milk as raw material. Increased protein speed observed might be associated with enzyme treatment using the precipitation technique that allows the transfer of the precipitated protein to the cheese, as well as raw milk used for cheese preparation. It has been observed that the protein content in milk was lower than the protein content in both cheese samples and could be attributed to concentration during coagulation. Better yield in cheese depends on the content of casein in milk, Abakar (2012). The weight observed in cheese 1 yield was higher in the run but for the cheese 2 made using purified enzyme, the better coagulation created to offer high yield propellant cheese protein was higher than the results obtained in previous studies. High protein cheese can be useful for the population living in certain areas where it is very difficult to access food with a significant amount of protein. There was no significant difference between the fat content, but cheese 2 produced using the purified extract enzyme and the highest fat content (15.28%) was observed. While the fat value was higher than the results found in studies of various breeds such as Ayrshire milk (13.67%) and Friesian milk (12.81%), the fat content of cheese obtained from the raw extract enzyme (12.93%) was similar to the value obtained by Mazou (2011). It was important to emphasize that cheeses were produced using *Calotropis procera* latex, which had high proteolytic and milk coagulation activity in previous studies and that stems were used in this study. The approximate analysis results revealed that the cheese produced with the raw enzyme had the highest FFA content (7.05%), and the cheese recorded in cheese 2 was 1.42%. The high FFA rate could be caused by natural fermentation due to possible contamination through the action of lipolysis or due to the presence of some lipolytic enzymes in the untreated extract. pH values were evaluated in relation to storage time, temperature and cheese types. Table 3 shows the change of pH by temperature, storage time, cheese type and their interactions. It is important to emphasis that a small sensorial analysis was proceed using ten students from West Africa studying at Gaziantep University who know with Wangashi cheese. It was observed that all of them had mentioned an improvement in bitter taste in cheese produced using purified crude extract when compared to cheese obtained using non-purified crude extract and an improvement in color as well. The whiteness of cheese 2 was carried out when compared to cheese 1. According to the data, pH values of cheese samples differed according to cheese type, storage temperature and time ($P < 0.05$). For both cheese types, there was a significant difference between the pH values of the samples stored at +4°C; cheese 2 pH values were decreased while an increase was observed in cheese 1, however stored samples at 25°C, after the 7th day, the pH value of both kinds changed statistically. Furthermore, increasing

Table 3. pH of Wangashi cheeses at different storage time and temperature

T °C	Storage time days	Cheese	
		1	2
4	0	5.01 ±0.42^{b}	5.49 ±0.06^{a}
	7	6.40 ±0.10^{b}	5.24 ±0.01^{a}
	14	6.65 ±0.01^{b}	5.06 ±0.01^{a}
25	0	5.01 ±0.42^{b}	5.49 ±0.06^{a}
	7	6.41 ±0.01^{b}	5.57 ±0.01^{a}
	14	6.72 ±0.01^{a}	5.55 ±0.01^{b}

T is the storage temperature.
Different capital letters indicate a statistical difference at a 0.05 level in each row.

pH value might be the reason for the bitterness attributed to cheese 1. However, no significant effect was observed in terms of temperature-time interaction ($p > 0.05$).

Effect of enzyme purity on cheese color

The color determination results for both cheese samples showed that the color of the Wangashi cheese was basically white due to its high *L** values (Table 4). However, there was a significant difference between the *L**, *a** and *b** values of the samples ($p < 0.05$). Cheese 2 whiteness was higher than cheese 1, which could be explained by purification differences of the enzymes used. For cheese 2, the crude extract was purified, which cleans the coagulant from its original color. Further, cheese 1 had a higher *a** negative value (–1.10) compared to cheese 2 (Table 4). This increase in *a** value could be expressed by direct transfer of the high green color characteristic that the raw enzyme is responsible for, since this cheese was made directly using the raw enzyme, cheese 2 was produced with purified crude extract, which reduces the green color of the cheese sample with the purification method used.

Table 4. Color parameters of Wangashie cheeses

Parameter	Cheese	
	1	2
Color *L**	74.85 ±0.30^b	78.87 ±0.40^a
Color *a**	–1.10 ±0.60^a	–1.46 ±0.05^b
Color *b**	14.99 ±0.22^b	15.96 ±0.26^a

Different capital letters indicate a statistical difference at a 0.05 level in each row.

This basic decontamination method (ammonium sulfate precipitation) did not only ensure the strong removal of semi-proteases and shaded materials in the coarse concentrate (Barros et al., 2001), however, it also focused protein regulation on a serviceable volume that could be used for enzyme characterization and cheese making. However, for cheese 2, the *b** value (15.96) is slightly higher than that observed with cheese 1. This was somewhat different, explained by the fact that salt was used for enzyme precipitation, the colors of the precipitated protein could be transferred to the cheese.

The effect of enzyme purity on the textural properties of cheese

The data of the hardness of both cheese samples are shown in Table 5. Hardness was expressed as the maximum peak force during the initial compression in TPA. This was the force required to compress to achieve the first deformation. It was observed that storage temperature had a significant effect on cheese hardness ($p < 0.05$).

Change of storage temperature could be interpreted as both types of cheese 1 and 2 affected. On the contrary, it was observed that cheese types, storage times and their interactions on cheese hardness did not reveal a significant effect ($p > 0.05$). However, it was noted that when the duration and the temperature of the storage were increased, the hardness values of all cheese samples increased, but the hardness of cheese 1 decreased at 25°C on day 14. Cheese 2 had higher hardness, which may be justified by the high protein and low moisture values recorded in cheese 2 when compared to these found in cheese 1. On another hand it may be related to the high amount of water that was left in the curd of cheese 2 and some microbial growth during preservation period that may be investigated in future studies. Yuanrong et al. (2016) had reported that

Table 5. Hardness of Wangashi cheeses at different storage time and temperature

T °C	Storage time days	Cheese	
		1	2
4	0	271.32 ±11.84^b	635.84 ±106.26^a
	7	392.65 ±21.91^b	706.37 ±25.21^a
	14	486.71 ±318.93^b	916.95 ±88.64^a
25	0	271.32 ±11.84^b	635.84 ±106.26^a
	7	34 440.22 ±995.67^a	32 985.88 ±6056.38^b
	14	27 454.84 ±5072.96^b	34 132.56 ±1050.26^a

T is the storage temperature.
Different capital letters indicate a statistical difference at a 0.05 level in each row.

the low hardness values observed might be due to the higher protein breakdown rate in cheese. It could also be seen that when the temperature rises from the initial day to 14 days under 4°C and 25°C storage temperature, average hardness values increase by about 98%.

CONCLUSION

The results obtained from the studies carried out to determine the proteolytic properties of the enzyme used in Wangashi cheese by extraction and to determine its effect on cheese production were summarized below.

1. *Calotropis procera* is a plant traditionally involved in cheese production especially in West African countries due to its strong coagulation capacity. It was found that the extract of *Calotropis procera* had a more effective proteolytic effect than the stems used directly.

2. A statistical difference was observed in between the moisture content of cheeses produced using *Calotropis procera* directly or purified extract. Low moisture content (43%) was recorded in cheese made with purified *Calotropis procera* enzyme, while high value was observed in cheese produced with *Calotropis procera*.

3. A higher protein content was recorded in cheese produced using the extract of *Calotropis procera*. A higher FFA value was observed in cheese prepared with crude extract from *Calotropis procera*.

4. The cheese obtained by using *Calotropis procera* extract recorded higher *L** (lightness) and lower *a** (greens). Differences according to the textural parameters tested in both types of Wangashi cheeses were observed when various time and different storage temperatures were applied. These results once again showed that adjusting the production stage and using the processed raw extract of *Calotropis procera* improved the textural parameters of Wangashi cheese. High proteolytic activity can cause bitter taste in cheese. However, a higher proteolytic activity recorded in the purified enzyme may depend on the solvent used and also the purification technique. The results showed that in order to prevent the proteolytic effect and the negative changes in the formed cheese or to understand the interactions more clearly, a new purification technique should be developed.

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