

Full Length Research Paper

# Milk-clotting activity of berries extracts from nine *Solanum* plants

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**Nine *Solanum* species (*Solanum aculeastrum*, *Solanum aethiopicum*, *Solanum anomalum*, *Solanum cerasiferum*, *Solanum dasyphyllum*, *Solanum indicum*, *Solanum nigrum*, *Solanum nodiflorum* and *Solanum terminale*) berries were studied in order to find a new source of milk-clotting enzymes for artisanal use by Cameroonian farmers. The results showed that milk-clotting activity was obtained by soaking fresh and dried berries in either distilled water or 5% NaCl solution. Maximum milk-clotting activity was obtained by soaking dried berries in 5% NaCl solution at optimum time of extraction for each species. Dried berries released active enzymes more easily than fresh ones, and addition of salt facilitated the extraction process. Extract from *S. aethiopicum* berries had the highest milk-clotting activity. All extracts had proteolytic activity. High milk-clotting and proteolytic activities were found in extract from *S. cerasiferum* obtained after 24 h of soaking dried berries in 5% NaCl solution. High milk-clotting and low proteolytic activities were found in extract from *S. aethiopicum* obtained under same conditions.**

**Key words:** *Solanum*, extraction, milk-clotting, berries extract.

## INTRODUCTION

The transformation of raw milk to cheese is very important for developing countries such as Cameroon. The promotion of this technology beside local farmers could surely improve their incomes (FAO, 1995). Cheese-making starts with coagulation of milk, which is widely achieved by rennin, extracted from calf's abomasums before weaning.

The worldwide increase in cheese production has reduced the availability of rennin, which became short in supply and expensive for local farmers. It could be produced locally; however, the legislation in Cameroon forbids slaughtering of calf and other small ruminants (Decree N°62/22/COR, 1961). The reduced supply of rennin has led to the search for coagulant substitutes. Numbers of proteolytic enzymes from various sources have been used: bovine, porcine and chicken pepsins, fish chymotrypsins as well as proteases of fungi and transgenic microorganisms (Lopez et al., 1998). The use of these coagulants gave rise to unwanted final products, and led to ethic (been against genetically engineered foods), religious (Judaism and Islam), diet (vegetarianism)

and public health problems (bird flu, bovine spongiform encephalopathy, H1N1 virus and microbial toxins) (Roseiro et al., 2003).

Recent publications on new proteases from vegetable origin for milk coagulation indicated that they are subject with growing interest for dairy technology (Low et al., 2006; Egito et al., 2007; Duarte et al., 2009). Plant coagulants have been used in cheese-making since fifty centuries before our era. Since the renewal of interest in 1960, vegetable coagulants have been used the more in dairy technology; especially at the artisanal scale (FAO, 1995; Silva and Malcata, 2005). Cheese production with plant coagulants contributes significantly to the socio-economic development of a locality, region and hence whole country where it is produced. Protein input is improved for those populations to whom restrictions are imposed by the use of animal and microbial coagulants. The technology used by farmers is easy and straightforward (Roseiro et al., 2003). Such know-how can be interesting to Cameroonian farmers.

Several plant preparations are known for cheesemaking. Species from *Cynara* genus are used successfully as source of milk-clotting enzymes in the Iberian Peninsula and Latin America. Tropical plants such as: *Carica*

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**Table 1.** Scientific names of *Solanum* plants, colour, form, diameter, availability and usage of fruits from nine *Solanum* plants.

S/N	Species <sup>1,3</sup>	Colour <sup>1,2</sup>	Form <sup>1</sup>	Diameter (cm)	availability <sup>1</sup>	Usage <sup>1,2,3</sup>
1	<i>S. aculeastrum</i>	Yellow	Spherical to oval	5 ± 0.1	Low	Medicinal
2	<i>S. aethiopicum</i>	Red	Round	1.5 ± 0.2	High	Food
3	<i>S. anomalum</i>	Red	Globular	2 ± 0.2	Low	Medicinal
4	<i>S. cerasiferum</i>	yellow	Round	3 ± 0.7	Very low	Medicinal
5	<i>S. dasyphyllum</i>	Yellow	Oval	4.5 ± 0.2	Low	Medicinal
6	<i>S. indicum</i>	Orange	Globular	2.3 ± 0.1	Low	Medicinal
7	<i>S. nigrum</i>	black	Round	1 ± 0.02	High	Medicinal
8	<i>S. nodiflorum</i>	Black	Spherical	1.2 ± 0.01	Average	Medicinal
9	<i>S. terminale</i>	Red	Spherical to oval	0.8 ± 0.02	average	Medicinal

<sup>1</sup> Institute of Agronomic research for development (Cameroon National Herbarium).

<sup>2</sup> Dupriez and De leener, 1987.

<sup>3</sup> Lester and Hawkes, 2001.

*papaya*, *Ananas comocus*, *Ficus glabra*, *Calotropis procera* and *Lactuca sativa* are also sources of milk-clotting enzymes (Uchikoba and Kaneda, 1996; Asakura et al., 1997; Lo Piero et al., 2002). Unfortunately, most of these coagulants have been found to be unsuitable in cheese-making, because they produced bitter cheese (Ahmed et al., 2009).

Aqueous fruit extracts from *Solanum dobium* are used traditionally as coagulant in the making of white and soft cheese in Sudan (Youssif et al., 1996). Other species of *Solanum* genus such as: *Solanum incanum*, *Solanum esculentum*, *Solanum macrocarpon* and *Solanum melongena* have shown the ability to clot milk (Suleiman et al., 1988; Guiama et al., 2010).

Nine other species from same genus are available in Cameroon. These plants belong to the Solanaceae family. They are woody herbs with solid erect stems, green in color and about 30 cm high. Their leaves are alternate, long petiole, simple, ovate, acuminate or obtuse at the apex and pale green in color, while their rootlets are brown and their roots about 5 mm thick and 15 cm long. Their flowers are hermaphrodite. Their unripe fruits are green, while the ripe ones are yellow, black or red (Table 1). Their fruits are edible or medicinal berries (Dupriez and De Leener, 1987; Bukenya and Bonsu, 2004). The fruits of these plants have not yet been experimented as a source of milk-clotting enzymes, which can substitute rennin.

The objective of the present work is to find alternative sources of milk-clotting enzymes from the nine species of *Solanum* genus found in Cameroon; with the aim to select the best among them for the promotion of cheese-making in Cameroon. This preliminary study describes the milk-clotting activity of each fruit extract from each species, the efficiency of enzyme extraction from fresh and dried matter with distilled water and 5% sodium chloride as extracting medium. Besides, enzymatic activity of each fruit extract was determined.

## MATERIALS AND METHODS

### Vegetal materials

Fruits of individual species of nine *Solanaceae* collected in August 2009, were used for this study. These species were identified by Cameroon National Herbarium: *Solanum aculeastrum*, *Solanum aethiopicum*, *Solanum anomalum*, *Solanum cerasiferum*, *Solanum dasyphyllum*, *Solanum indicum*, *Solanum nigrum*, *Solanum nodiflorum* and *Solanum terminale*. Colour, diameter, form, availability and usage of ripe fruits from each species of *Solanum* plants are shown in Table 1. These fruits were chosen, because they are available during rainy season, which corresponds to maximum milk production (Libouga et al., 2001). These fruits are well known by farmers who use them for food or medicinal reasons. Recent publications on plant preparations from *Solanum* plants for milk-clotting revealed that only these fruits are used as source of milk-coagulating enzymes (Suleiman et al., 1988; Yousif et al., 1996; Ahmed et al., 2009; Guiama et al., 2010). Sampling of plant organs such as root, leaf, bark and sap, can lead (in average or long term) to ecological disaster (Brnic et al., 2007).

### Coagulation substrate

Raw milk of zebu (*Bos indicus*) was used as coagulation substrate. Authors have shown that coagulation tests are better with skim raw milk than reconstituted skim milk powder (Martin et al., 2008).

### Preparation of coagulation substrate

Zebu raw milk was obtained from dairy farm situated in the Dang area, near the University of Ngaoundere (Adamawa Region, Northern Cameroon) and stored at 4°C for 48 h before use. The zebu raw milk was skimmed by centrifugation at 3000 g and 30°C for 30 min. This raw skim milk was stored at 4°C with sodium azide (0.2 g/l) added as preservative. To ensure complete equilibration of the caseins and salts, the raw skim milk was held at 30°C for 1 h before addition of coagulant (Martin et al., 2008). This raw skim milk was also used for extraction of zebu whole casein.

### Preparation of crude enzyme solution

Fresh biological material was washed several times with distilled

water and disinfected with sodium hypochloride (10% v/v) (Duarte et al., 2009). The berries of each species were fragmented into very small pieces, which were either maintained fresh or dried at 40°C for 24 h and then both were ground. The extracts were prepared by immersing 10 g of ground fresh or dried berries of individual species of the nine *Solanum* in 100 ml of distilled water or 5% (w/v) NaCl solution as extractant medium. The mixtures were stirred at room temperature (22°C). The extraction procedure was continued for 1, 3, 6, 9, 15, 24 and 36 h at 4°C. Then, mixtures were filtered through a filter paper and maintained at 4°C. This solution was used for the determination of pH, dry weight, protein content, milk-clotting and proteolytic activities.

### Milk-clotting activity determination

The milk-clotting activity of each fruit extract was determined following the procedure described by IDF (1992). Extracts were added at a proportion of 1 ml per 10 ml of zebu raw skim milk. The coagulation point was determined by periodic manual rotation of the test tube, at very short time intervals. The clotting time was recorded when discrete particles were discernible. One milk-coagulating unit (U) was defined as the amount of protein that coagulates 10 ml of zebu raw skim milk at 30°C in 100 s (Silva and Malcata, 2005). The milk-clotting activity of each extract was measured, with the assumption that all the soluble proteins from the extract were enzymes which coagulate milk at 30°C.

$$\text{MCA (U/ml)} = (100/\text{CT}) \times \text{S/E}$$

MCA: the milk-clotting activity (U/ml); CT: the clotting time (s); S: the zebu raw skim milk (ml); E: the enzyme volume (ml).

### Proteolytic activity determination

#### Preparation of zebu whole casein

Whole casein was prepared following the procedure described by Egito et al. (2007), with slight modification. Zebu raw milk was precipitated at pH 4.6 with 1 M HCl. The precipitate was washed three times with distilled water. It was solubilized at pH 7.0 with 1 M NaOH and dialyzed against distilled water at 4°C for 24 h. This zebu whole casein was used as substrate to determine proteolytic activity.

#### Hydrolysis of zebu whole casein

Proteolytic activity was determined according to Silva and Malcata (2005), with some modification. Zebu whole casein 1% (w/v) was subjected to hydrolysis at 30°C in 100 mM phosphate buffer (pH 6.7). The hydrolysis was initiated by addition of 1 ml of each extract to 10 ml of zebu whole casein solution. The reaction was stopped after 30 min by heating at 100°C for 5 min. The proteolytic activity was quantified by evaluating the soluble peptides in 6% (w/v) trichloroacetic acid (TCA). 1 ml of each sample was treated with 5% (w/v) TCA at a volumetric ratio of 1:2; the mixture was allowed to settle for 10 min, and then centrifuged at 7,500 g for 30 min. The absorbance of supernatant was measured at 280 nm. An appropriate control was prepared in which the TCA was added before the extract. One unit of proteolytic activity (U) was arbitrarily defined as the amount of enzyme required to cause an increase of 0.1 in absorbance at 280 nm, under the assay conditions. Proteolytic activity was as follows: P.A. (U/ml) =  $(\Delta\text{Abs}_{280\text{nm}} \times 10 \times \text{dilution factor}) / (\text{E} \times \text{t})$ , where  $\Delta\text{Abs}_{280\text{nm}}$  is the variation of absorbance between assay and control, E is the volume of crude enzyme solution and t is the time of reaction.

### Statistical analysis

The experiments were conducted in triplicate and the results are the means and standard deviations of these three independent trials. For the extraction procedure, a randomized split-plot design was used, with berries of each species as the main factor; extraction time and sodium chloride concentration were used as the secondary factors. Statistical analysis was carried out using Statgraphic 3.0 Software, Excel 2007 (Microsoft Office 2007 Professional) and SigmaPlot (SPW11).

## RESULTS AND DISCUSSION

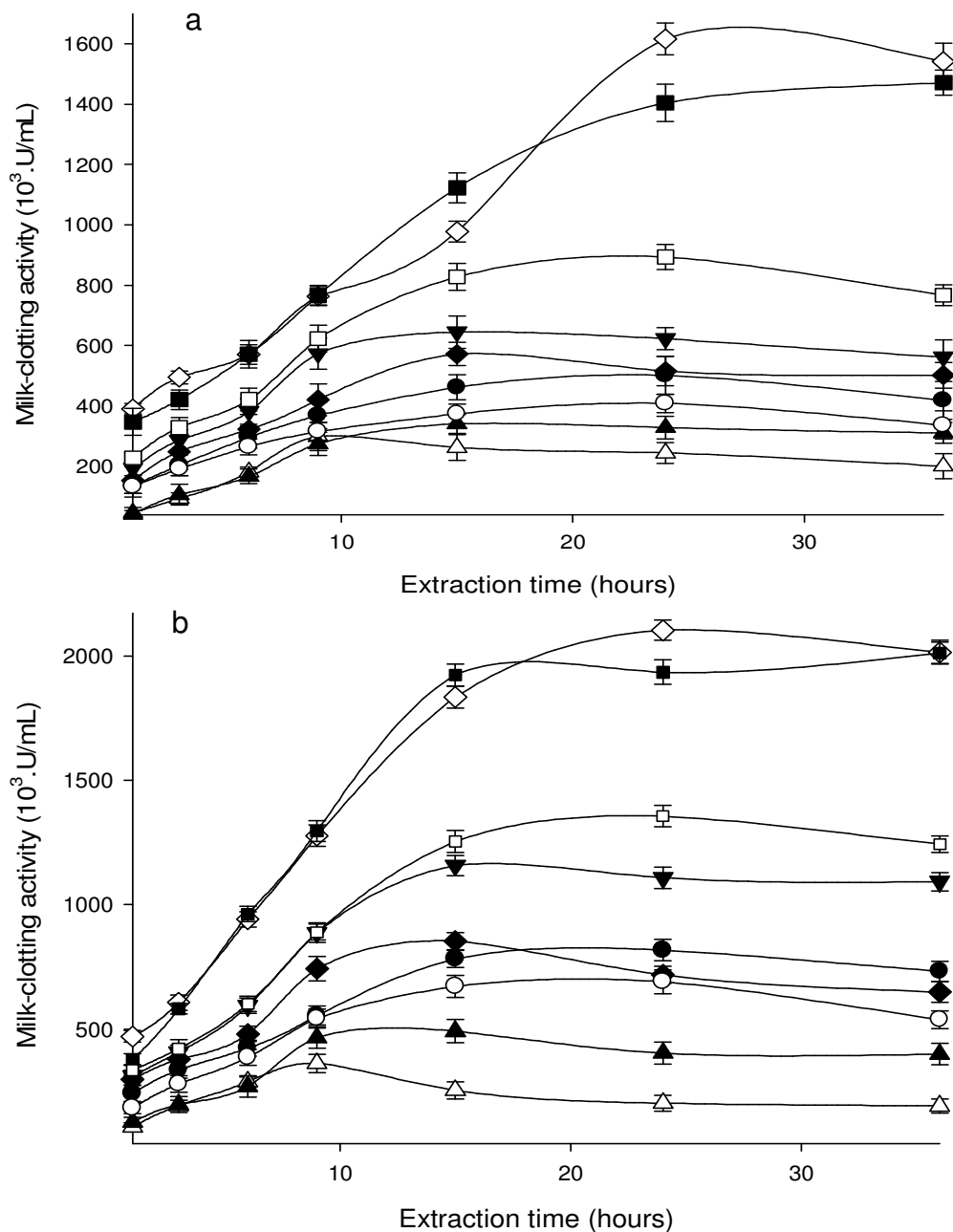
### Effect of soaking fresh berries in distilled water

Milk-clotting activity of coagulants prepared by soaking fresh berries from individual species in distilled water at various extraction times is shown in Figure 1a. In general, milk-clotting activity of extract was varied with extraction time. Enzyme extraction from *S. aculeastrum* fresh berries was initially faster until the ninth hour of soaking; however milk-clotting activity of this extract which was the lowest ( $0.3 \pm 0.047$  U/ml) diminished gradually after this period. The milk-clotting activity of extracts from *S. anomalum*, *S. dasyphyllum*, *S. indicum* and *S. nigrum* fresh berries increased during the first fifteen hours of soaking, and decreased after this time. Enzyme extraction from *S. eathiopticum*, *S. nodiflorum* and *S. terminale* fresh berries increased during 1 day of soaking, but decreased after 24 h of soaking. Milk-clotting from *S. cerasiferum* fresh berries increased significantly with soaking time until 24 h. Milk-clotting activities of extracts from *S. eathiopticum* ( $1.6 \pm 0.053$  U/ml) and *S. cerasiferum* ( $1.5 \pm 0.041$  U/ml) fresh berries are greater than the extracts from *S. terminale* ( $0.9 \pm 0.041$  U/ml), *S. nigrum* ( $0.64 \pm 0.054$  U/ml), *S. dasyphyllum* ( $0.57 \pm 0.039$  U/ml), *S. indicum* ( $0.5 \pm 0.062$  U/ml), *S. nodiflorum* ( $0.4 \pm 0.031$  U/ml), *S. anomalum* ( $0.33 \pm 0.032$  U/ml) and *S. aculeastrum* ( $0.3 \pm 0.047$  U/ml) berries.

The milk-clotting activity was not significantly different between extracts from *S. eathiopticum* and *S. cerasiferum* berries ( $P = 0.07$ ); however, there was a significant difference between the other extracts ( $P < 0.02$ ). Milk-clotting enzymes were successfully extracted from fresh berries of individual *Solanum* species in distilled water as extractant.

### Effect of soaking fresh berries in 5% (w/v) NaCl solution

When fresh berries were soaked in 5% (w/v) NaCl solution, milk-clotting activity was higher than that obtained with distilled water (Figures 1b and Figure 1a). The activities of the extracts from *S. eathiopticum* and *S. cerasiferum* fresh berries were the highest and increased up to 15 and 24 h for *S. cerasiferum* and *S. eathiopticum*, respectively; and then remained constant. However, the activity of the other extracts from fresh berries increased



**Figure 1.** Mean and standard deviation of milk-clotting activity of coagulants prepared by soaking vegetal material from *S. aculeastrum* ( $\Delta$ ), *S. aethiopicum* ( $\diamond$ ), *S. anomalum* ( $\blacktriangle$ ), *S. cerasiferum* ( $\blacksquare$ ), *S. dasyphyllum* ( $\blacklozenge$ ), *S. indicum* ( $\bullet$ ), *S. nigrum* ( $\blacktriangledown$ ), *S. nodiflorum* ( $\circ$ ) and *S. terminale* ( $\square$ ). (a) fresh berries in distilled water; (b) fresh berries in 5% NaCl solution; (c) dried berries in distilled water; (d) dried berries in 5% NaCl solution.

with extraction time and decreased after 24 h (*S. nodiflorum* and *S. terminale*), 15 h (*S. anomalum*, *S. indicum*, *S. nigrum* and *S. dasyphyllum*) and 9 h (*S. aculeastrum*) of extraction time.

Enzyme extraction from those solanum fresh berries was faster up to the first nine hours. There was no significant difference between *S. cerasiferum* and *S. aethiopicum* fresh berries during extraction procedure.

Using salt solution as extractant was as effective as using the distilled water. This was so for all fresh berries from individual species of *Solanum* plants. These observations are consistent with those of Yousif et al. (1996) who extracted a milk-clotting enzyme from similar plant species, *Solanum dodium*, using 5% (w/v) NaCl solution as extractant. Presence of sodium chloride increased the ionic strength, and hence improved the solubility of the

milk-clotting enzyme. Recent publications indicated that milk-clotting enzymes from plants were extracted by soaking fresh biological material in salt solution at varied concentrations (Duarte et al., 2009).

### Effect of soaking dried berries in distilled water

Figure 1c shows the variation of milk-clotting activity of extracts from *Solanum* plants, when dried berries were soaked in distilled water for a period of 1 to 36 h. Enzyme extraction increased with extraction time up to optimum variable by the extracts. After that, milk-clotting activity decreased (*S. aethiopicum*, *S. cerasiferum*, *S. dasyphyllum*, *S. nigrum* and *S. terminale*) or remained constant (*S. aculeastrum*, *S. anomalum*, *S. indicum*, *S. nodiflorum*). Thus, the maximum milk-clotting activity was obtained during 15 h of extraction for *S. aculeastrum*, *S. nigrum* and *S. terminale* and 24 h for *S. aethiopicum*, *S. anomalum*, *S. cerasiferum*, *S. dasyphyllum*, *S. indicum* and *S. nodiflorum*.

The milk-clotting activity was higher than that obtained by soaking fresh berries in distilled water (Figures 1c and Figure 1a). However, enzyme extraction was greater when fresh berries were soaked in 5% NaCl solution (Figures 1c and Figure 1b). The milk-clotting activity was not significantly different between extracts from *S. aethiopicum* and *S. cerasiferum* berries ( $P = 0.09$ ), which had the greatest activity after 24 h of soaking. Other authors had used successful dried vegetal material to prepare milk-clotting extract (Yousif et al., 1996; Lopez et al., 1998; Egito et al., 2007; Nouani et al., 2009; Guiama et al., 2010). Dried berries released most milk-clotting enzymes in the distilled water than the fresh ones. It seems that drying process may have weakened a cellulosic layer around the membrane, thus, making milk-clotting enzymes more available.

### Effect of soaking dried berries in 5% NaCl solution

Figure 1d shows variation of milk-clotting activity of different coagulants prepared by soaking dried berries of each plant in 5% NaCl solution. Globally, enzyme extraction increases with the time of soaking until maximum, which depends on the extracts. The highest milk-clotting activity was achieved for each extract after a specific time of extraction, and then, it remained constant or decreased gradually. The highest milk-clotting activity was found in extracts from *S. aethiopicum* and *S. cerasiferum*, the lowest was found in extracts from *S. aculeastrum*. There was no significant difference between extracts from *S. aethiopicum* and *S. cerasiferum* according to the comparison of means with Duncan's multiple range test. Milk-clotting activity of extract was more achieved during 15 h of extraction for *S. aculeastrum*, *S. dasyphyllum*, *S. indicum* and *S. terminale* and 24 h of

extraction for *S. anomalum*, *S. aethiopicum*, *S. cerasiferum*, *S. nigrum* and *S. nodiflorum*.

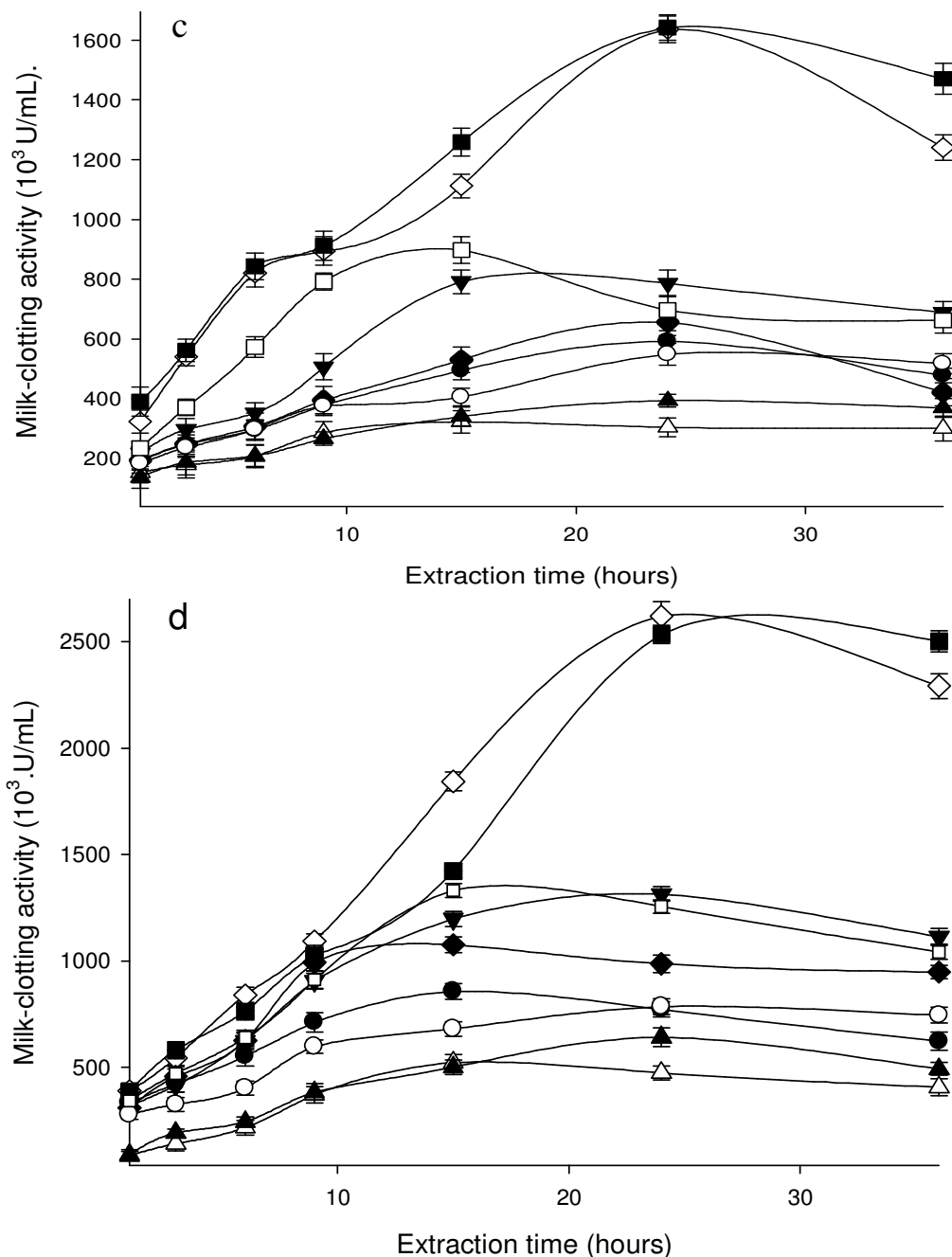
The milk-clotting activity of these extracts was higher than coagulants from fresh berries in salt solution (Figures 1d and Figure 1b), dried berries in distilled water (Figures 1d and Figure 1c) and fresh berries in distilled water (Figures 1d and Figure 1a). Milk-clotting enzymes were released more from dried berries soaked in the salt solution. Some publications showed that milk-clotting enzymes from plants were extracted by soaking dried biological material in salt solution at varied concentrations (Yousif et al., 1996; Libouga et al., 2006; Egito et al., 2007; Guiama et al., 2010). Drying of the biological material may increase the availability of milk-clotting enzymes. The use of salt solution as an extraction medium gives better extraction of milk-clotting enzymes. It is obvious that enzyme extraction was more effective when dried berries were soaked in the salt solution.

Dried berries released active enzymes more easily than fresh ones, and addition of salt facilitated the extraction process. These findings are particularly important in terms of long term storage of feedstock plant material for rennet.

### Classification of milk-clotting extracts

Figure 2 shows the classification of milk-clotting extract from each *Solanum* plant on the scale from 0.0 to 3.0. Maximum milk-clotting activity varied from 0.5 to 2.6 U/ml for crude enzyme solution prepared by soaking dried vegetal material in the 5% (w/v) NaCl. Figure 2 shows also that extract from *S. aethiopicum* had the greatest milk-clotting activity (2.62 U/ml) followed by extracts from *S. cerasiferum* (2.53 U/ml), *S. terminale* (1.33 U/ml), *S. nigrum* (1.31 U/ml), *S. dasyphyllum* (1.1 U/ml), *S. indicum* (0.86 U/ml), *S. nodiflorum* (0.79 U/ml), *S. anomalum* (0.64 U/ml) and *S. aculeastrum* (0.56 U/ml). The milk-clotting activity of extract from *S. aethiopicum* berries was equivalent to extract from *S. dobium* seed, and higher than extract from *S. dobium* whole berries, prepared by soaking vegetal material in 5% NaCl solution for 7 days (Yousif et al., 1996).

These results suggest that crude enzyme solution from *S. aethiopicum* may be an interesting source of milk-clotting enzyme useful in cheese-making by Cameroonian farmers. Besides, extract from *S. aethiopicum* berries could be a reliable ingredient in cheesemaking for farmers, because these berries are edible and available (Dupriez and De Leener, 1987; Huetz De Lemps, 2001). Low proteolytic activity of extract from *S. aethiopicum* berries could reinforce the interest of this extract in dairy technology. Further analyses are being conducted presently to extract milk-clotting enzymes from coat and seed of *S. aethiopicum* berries, with the aim to determine the part of berry which releases maximum milk-clotting enzymes. Cheese-making experiments are also carried out



**Figure 1.** Continued. (c) dried berries in distilled water; (d) dried berries in 5% (w/v) NaCl.

with *Solanum aethiopicum* berries extract as coagulant to determine the acceptability of cheese obtained.

#### Enzymatic activity

Table 2 shows the pH, dry weight, protein content, milk-clotting activity, proteolytic activity and the R (milk-clotting activity / proteolytic activity) of extracts prepared by

soaking dried berries in 5% NaCl solution for optimum time of extraction. PH varied from 4.8 (*S. nigrum*) to 5.3 (*S. aethiopicum*). These values were higher than the isoelectric precipitation pH 4.6. Thus, milk-clotting activity observed was due to enzymatic activity. The milk-clotting by proteolytic enzymes is very important in dairy technology in general and especially in cheese-making (Lopez et al., 1998). The enzymatic coagulation of milk involves a specific hydrolysis of the Phe-Met bond kappa-

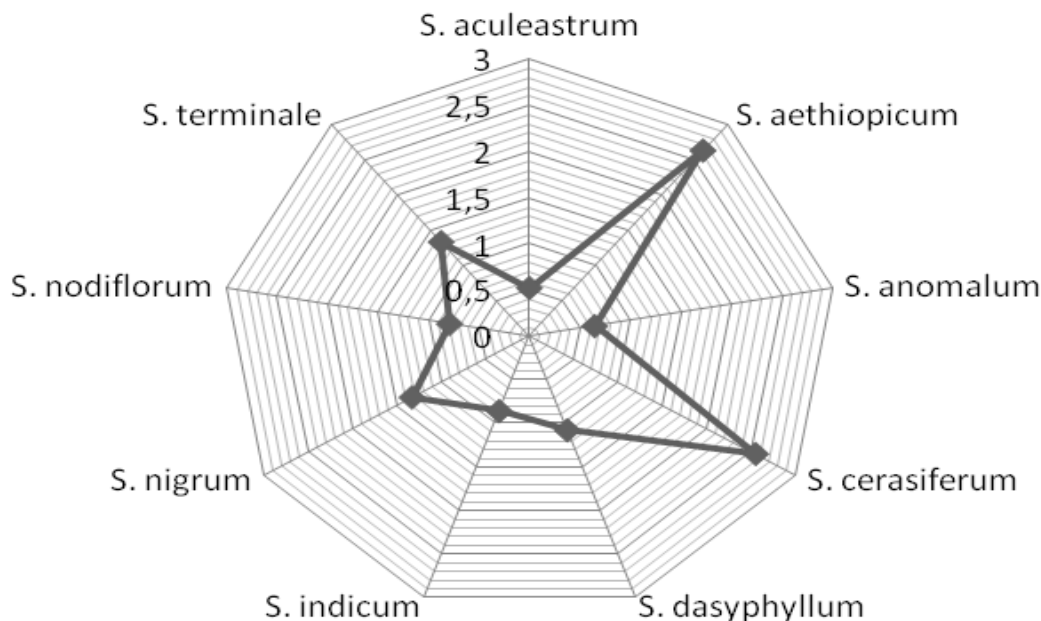


Figure 2. Radar classifying of milk-clotting activity (10<sup>-1</sup>U/ml) of extracts on the scale from 0.0 to 3.0.

Table 2. pH, dry weight, protein content, milk-clotting activity, proteolytic activity and R of different types of coagulant.

Types of coagulant	pH	Dry weight (mg/ml)	Protein content (mg/ml)	Milk-clotting activity (U/ml)	Proteolytic activity (U/ml)	R*
<i>S. aculeastrum</i>	4.98	10±0.4	0.64±0.01	0.56±0.01	0.18±0.02	2.9
<i>S. aethiopicum</i>	5.3	10±0.0	0.88±0.02	2.62±0.1	0.27±0.02	9.7
<i>S. anomalum</i>	5.1	10.5±0.5	0.49±0.01	0.64±0.01	0.15±0.01	4.3
<i>S. cerasiferum</i>	5.04	11.67±0.6	0.78±0.02	2.5±0.14	0.35±0.01	7.2
<i>S. dasyphyllum</i>	4.82	12.34±0.6	0.69±0.06	1.1±0.06	0.22±0.01	4.9
<i>S. indicum</i>	5.17	9.9±0.2	0.52±0.02	0.86±0.01	0.33±0.01	2.6
<i>S. nigrum</i>	4.8	10.34±0.6	0.64±0.04	1.31±0.0	0.30±0.02	4.4
<i>S. nodiflorum</i>	4.99	11±1.0	0.57±0.03	0.79±0.01	0.17±0.02	4.6
<i>S. terminale</i>	4.89	11±1.0	0.47±0.02	1.33±0.02	0.32±0.01	4.2

\* R = Milk-clotting activity / proteolytic activity.

casein covering the protein micelles (Law, 1999). Extracts from *Solanum* plants had proteolytic activities. Extract from *S. cerasiferum* had the highest proteolytic activity which can discourage its use as milk coagulant. All proteolytic enzymes can clot milk; however the main one for this action is rennin. Rennin or a rennin-like enzyme adequate for cheese production is characterized by having high milk-clotting activity and low proteolytic activity. Ratio between milk-clotting and proteolytic activities (R) of extract from *Solanum* plants was higher than 1.0, meaning that milk-clotting activity was higher than proteolytic, and hence extract from *S. aethiopicum* had the highest R value. R can be used as an index to justify the adequacy of enzymatic extract to be used as calf rennet substitute (Hashem, 1999). These results

show that crude enzyme solution from *S. aethiopicum* berries can be successfully used as milk coagulant in local dairy technology.

### Conclusion

The results of this study show the presence of milk-clotting and proteolytic activities in the extracts from *Solanum* berries, collected in the North of Cameroon. Milk-clotting enzymes from *Solanum* were successfully extracted by soaking both fresh and dried berries in distilled water and 5% NaCl solution. However, milk-clotting enzymes extraction was more effective by soaking *Solanum* dried berries in 5% NaCl solution for optimum time of extrac-

tion. High milk-clotting and low proteolytic activities were found in the coagulant from *S. aethiopicum* obtained after 24 h of soaking dried berries in 5% NaCl solution. Extract from *S. cerasiferum* berries shows high milk-clotting and proteolytic activities. Besides, *S. cerasiferum* berries are not eaten, that constrains their use as source of milk-clotting enzymes in cheese-making. For the reasons mentioned above, we can suggest further and deeper studies on *S. aethiopicum* berries, in order to face this plant as a new source of milk coagulant for local dairy products and particularly, cheese in Cameroon.

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