



Clotting activity of camel milk using crude extracts of ginger (*Zingiber officinale*) rhizome

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Abstract

This study was conducted to assess the clotting activity of camel milk using ginger rhizome (*Zingiber officinale*) crude extracts (GCE) and identify the optimum pH, temperature and concentration of GCE that would result in strong coagulation of camel milk. The result revealed that temperature, pH and concentration of GCE had significant ($P < 0.05$) effect on the clotting activity of camel milk. The highest camel MCA was observed at pH of 5.0, temperature of 65°C and crude extract concentration of 10% by volume of milk, while the lowest value was recorded at pH of 4.5, temperature 55°C and GCE concentration of 40% by volume of camel milk. Cow milk was considered for comparison of MCA and had the highest clotting activity at pH of 5.5, temperature of 60°C and GCE concentration of 10% by volume of milk. An increase in camel MCA was observed with a decrease in milk pH from 5.5 to 5.0; however, camel MCA decreased when milk pH reduced to 4.5. Camel MCA increased with increasing temperature; however, it decreased with increase in GCE concentration. Hence, GCE can be used to coagulate camel by adjusting the temperature and pH of the milk.

Keywords: Camel milk, coagulation, clotting activity and ginger crude extract.

INTRODUCTION

Dromedary camels (*Camelus dromedarius*) produce more milk of high nutritional quality for a longer period of time in hostile environments (Khan and Iqbal, 2001). Camel milk is consumed fresh in traditional pastoral systems in several countries. However, processing of camel milk into more shelf stable value added milk products is not yet well developed thus camel milk products are not common place (Farah and Bachmann, 1987; Mehaia, 2006). Unlike milk of cow and small ruminants, camel milk doesn't readily coagulate by rennet due to its inherent properties. However, possibilities of cheese-making from camel milk was reported (Khan et al., 2004; Mehaia, 2006; Ahmed and ElZubeir, 2011) using coagulants of animal origin.

In Ethiopia, although cheese-making from cow milk and consumption have been part of the culture of a substantial proportion of the society, cheese is not traditionally made from camel milk. Recently, camel chymosin was developed using recombinant DNA

technology by Danish scientists (Kappeler et al., 2006). However, it is not easily available; when available it is not affordable particularly by pastoralists. Different scholars also indicated that availability of calf rennet for cheese-making is curtailed by factors such as high cost and limited availability of coagulants (Jacob et al., 2010; Hashim et al., 2011a).

Globally, demand for milk coagulating enzymes started to exceed the supply since over 50 years and in 2009 only 20 - 30% of the demand could be met by calf rennet (Jacob et al., 2010). Searching for substituting coagulants from easily and locally available resources such as plant extracts is, thus, not only feasible but also essential in order to meet the demand for milk coagulants for manufacturing of processed camel milk products mainly cheese by pastoralists. Several researchers tested the coagulation potential of bovine milk using different plant extracts (Abdalla et al., 2011; Garcia et al., 2011; Hashim et al., 2011a). Crude extracts of ginger was

used to coagulate cow milk (Llorente et al., 1997). The specific property of proteases in ginger rhizome extract that coagulates casein micelles makes it an appropriate and potential candidate that can be used for clotting camel milk. Ginger extract had higher proteolysis activity (Huang et al., 2011; Hashimet al., 2011a; Hashim et al., 2011b). Ginger extracted enzyme had high specificity for α -casein followed by β -casein and κ -casein and exhibited a similar affinity for κ -casein, α - and β casein Hashim et al.(2011a) and higher specificity for κ -casein with increasing temperature (Huang et al.,2011). As indicated by Hashim et al. (2011a), the clotting activity of ginger extract is higher than that of calf rennet and papain for cow milk; however, it is lower than mucor rennet.

In Ethiopia, there is very limited work conducted that targeted to improve the utilization of camel milk commonly produced by pastoralists. The current study was, therefore, conducted to assess the clotting activity of camel milk using ginger crude extract and identify the optimum pH, temperature and concentration of the crude extract of ginger rhizome that results in strong coagulation of camel milk.

MATERIALS AND METHODS

Milk samples were obtained from pastorally managed camels from Erer valley of Eastern Ethiopia and transported in an icebox to the dairy technology laboratory of Haramaya University. For comparison, cow milk produced at Haramaya University dairy farm was used. Five litre of milk was sampled at a time from two pastoralist household and from two lactating cow. The milk was collected by directly milking into sterile bottles. The pH of milk samples was adjusted to 4.5, 5.0 and 5.5 as described by Farah and Bachman (1987). Temperature was adjusted to 55°C, 60°C and 65°C using a thermostatically controlled water bath (Model 25, Shaking Water Bath, Illinois-60647, Chicago) and a thermometer was used to check the temperature. The extraction of ginger crude extract was performed according to the procedure described by Hashim et al.(2011a) with some modification as follows. Briefly, fresh ginger rhizomes were peeled, chopped, washed with de-ionized water and frozen at -23°C, then homogenized using a blender (Model 38BL40 Blender 8010E, Christiano Scientific Equipment, USA) with five parts of cold acetone (w/v) (-23°C) and kept at 4°C for about 15-20 min. The homogenate was filtered through cotton cloth and the precipitate was further washed with cold acetone followed by air drying. The air dried material was made into powder using a food grinding mill (Model M20, KIKA® WERKW, Germany). The powder was then homogenized in 20 mM phosphate buffer (pH 7.0) for 2.4 minutes (Hashim et al., 2011b) and the extract was filtered using a muslin cloth. The filtrate was centrifuged at 12,000 g for 20 min (Model 1020, D Centrifuge,

Centrurion Scientific LTD) and the supernatant was considered as crude extract. The milk containing 0.15g of CaCl_2 per litre of milk was prepared as suggested by FAO (2001). The milk clotting activity (MCA) was determined according to the method described by Soledad et al.(2007). Briefly, 10, 20, 30 and 40% by volume of the crude extract was added into 10ml of the milk samples and MCA was determined by rotating the test tube at regular intervals and checking for visible clot (fine curd) formation on the wall of the test tubes. The formula suggested by Guiama et al.(2010a) was used to calculate the MCA expressed in U/ml.

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

Where:

MCA = Milk Clotting Activity (u/ml)

CT = Clotting time (s)

S = Substrate (milk) volume (ml)

E = Enzyme volume (ml)

The result was expressed as MCU/ml. Only 10% (by volume) inclusion of GCE was considered for the curd firmness study with camel chymosin being used as control. Firmness of the curd samples was measured using Texture Analyzer (Model TA-Plus Lloyd, UK) according to Salvador and Fiszman (2004). Each experiment was executed in triplicate. Completely Randomized Design (CRD) with 3*3*4 (three levels of temperature and pH, and four levels of GCE concentration) factorial arrangement was used to analyze the MCA data. MCA treatment means were compared using Duncan's Multiple Range Test, while means of curd firmness were separated using Least Significant Difference method.

RESULTS

Milk Clotting Activity

The different levels of temperature, pH and concentration of crude extract of ginger rhizome used in the current study had marked ($P < 0.05$) difference on camel milk clotting activity (Table 1). The highest camel MCA (MCU/ml) was observed at a temperature of 65°C, pH of 5.0 and crude extract concentration of 10% by volume with the lowest observed at temperature, pH and crude extract concentration of 55°C, 4.5 and 40% by volume, respectively (Table 1). The crude extract of ginger had higher thermal stability showing its higher clotting units at higher temperature (65°C) for camel milk (Table 1).

A significant difference ($P < 0.05$) was also observed in the clotting activity of cow milk among the different levels of temperature, pH and concentration of ginger crude extract (Table 2). The clotting activity (MCU/ml) of cow milk was higher at a temperature of 60°C, pH of 5.5 and crude extract concentration of 10% by volume (Table 2).

Table 1. Clotting activity (MCU/ml) of camel milk using crude extracts of ginger.

Concentration of crude extract (% inclusion by volume) in 10ml of camel milk	Temperature (°C)								
	55 (°C)			60 (°C)			65 (°C)		
	pH 4.5	5.0	5.5	pH 4.5	5.0	5.5	pH 4.5	5.0	5.5
10%	7.49 ^{lv}	16.37 ^{im n}	24.87 ^{gn}	21.94 ^{ln}	34.51 ^c	30.68 ^{oe}	45.05 ^d	59.26 ^a	26.01 ^g
20%	4.17 ^{ww}	11.37 ^{pqrst}	14.74 ^{imnop}	15.09 ^{imnp}	20.06 ^{ijk}	18.09 ^{ku}	25.88 ^g	33.43 ^{oe}	17.68 ^{ki}
30%	2.85 ^w	9.48 ^{stu}	11.24 ^{qrst}	12.09 ^{opqrst}	16.96 ^{kim}	13.38 ^{no pqr}	21.30 ^j	28.92 ^{ef}	13.53 ^{mno pqr}
40%	2.32 ^w	12.76 ^{opqrs}	10.94 ^{qrst}	10.14 ^{rstu}	14.00 ^{mno pq}	9.27 ^{tu}	20.08 ^{jk}	23.48 ^{gm}	10.58 ^{qstu}

Means with the same superscript letter in the table are not significantly different ($P > 0.05$). Values in the Table are means of three replications; Milk clotting activity was expressed as MCU/ml.

Table 2. Clotting activity (MCU/ml) of cow milk using crude extracts of ginger.

Concentration of crude extract (% inclusion by volume) in 10ml of cow milk	Temperature (°C)								
	55 (°C)			60 (°C)			65 (°C)		
	pH 4.5	5.0	5.5	pH 4.5	5.0	5.5	pH 4.5	5.0	5.5
10%	23.08 ^{nop}	66.87 ^{de}	54.92 ^{lgh}	53.61 ^{ghi}	65.48 ^{def}	150.79 ^a	83.72 ^c	98.15 ^b	103.70 ^d
20%	13.43 ^{pqrs}	17.92 ^{opqr}	21.23 ^{nopq}	21.77 ^{nopq}	30.17 ^{lmn}	57.87 ^{efgh}	56.02 ^{efgh}	63.16 ^{efg}	75.00 ^{de}
30%	14.57 ^{opqrs}	9.13 ^{rs}	14.63 ^{opqrs}	9.52 ^{rs}	22.41 ^{nop}	35.80 ^{kim}	56.61 ^{efgh}	34.57 ^{kim}	50.93 ^{hij}
40%	10.73 ^{qrs}	4.81 ^s	10.80 ^{qrs}	7.14 ^s	15.97 ^{opqrs}	42.46 ^{ijk}	54.17 ^{lgh}	25.04 ^{mno}	39.81 ^{ijkl}

Means with the same superscript letter in the table are not significantly different ($P > 0.05$). Values in the Table are means of three replications; Milk clotting activity was expressed as MCU/ml.

Generally, camel milk clotting activity increased with increasing temperature. However, an inverse relation was observed between the concentration of ginger crude extract and milk clotting activity, where milk clotting activity tended to decrease with increasing concentration of crude extract (Figure 1a). On the other hand, camel milk clotting activity increased up to pH of 5.0 then decreased at pH of 5.5 for all crude extract levels (Figure 1b).

Curd firmness of camel milk

An apparent difference ($P < 0.05$) in gel strength (load at yield) was observed between camel milk samples subjected to different combinations of temperature, pH and crude extract concentration

levels (Table 3). The control treatment (milk treated with camel chymosin) had higher curd firmness compared with milk samples treated with ginger crude extracts (Table 3). Milk samples treated with ginger crude extract at pH of 5.0, temperature of 65°C and crude extract concentration of 0.1ml/ml resulted in strong (firm) curd formation (Table 3). On the other hand, camel milk samples subjected to ginger crude extract at pH of 4.5, temperature of 65°C and crude extract concentration of 10% by volume showed the weakest gel strength. Thus, treatment of camel milk with 10% (by volume) inclusion of ginger extract at pH value of 5.0 and temperature of 65°C can be used to coagulate camel milk and thus enable production of cheese from camel milk.

Curd firmness of cow milk

The gel strength of cow milk treated with crude extracts at different temperatures and pH of milk showed a significant difference ($P < 0.05$) (Table 4). Cow milk subjected to camel chymosin resulted in firm curd. This might be attributed to the specific property of kappa casein and milk coagulation potential of camel chymosin. Crude extract concentration of 10% by volume used at pH of 5.5 and temperature of 60°C resulted in higher curd firmness in cow milk compared with curds formed by the other pH and temperature combinations (Table 4). Weak gel strength was recorded at pH 4.5, temperature of 65°C and crude extract concentration of 10% by volume

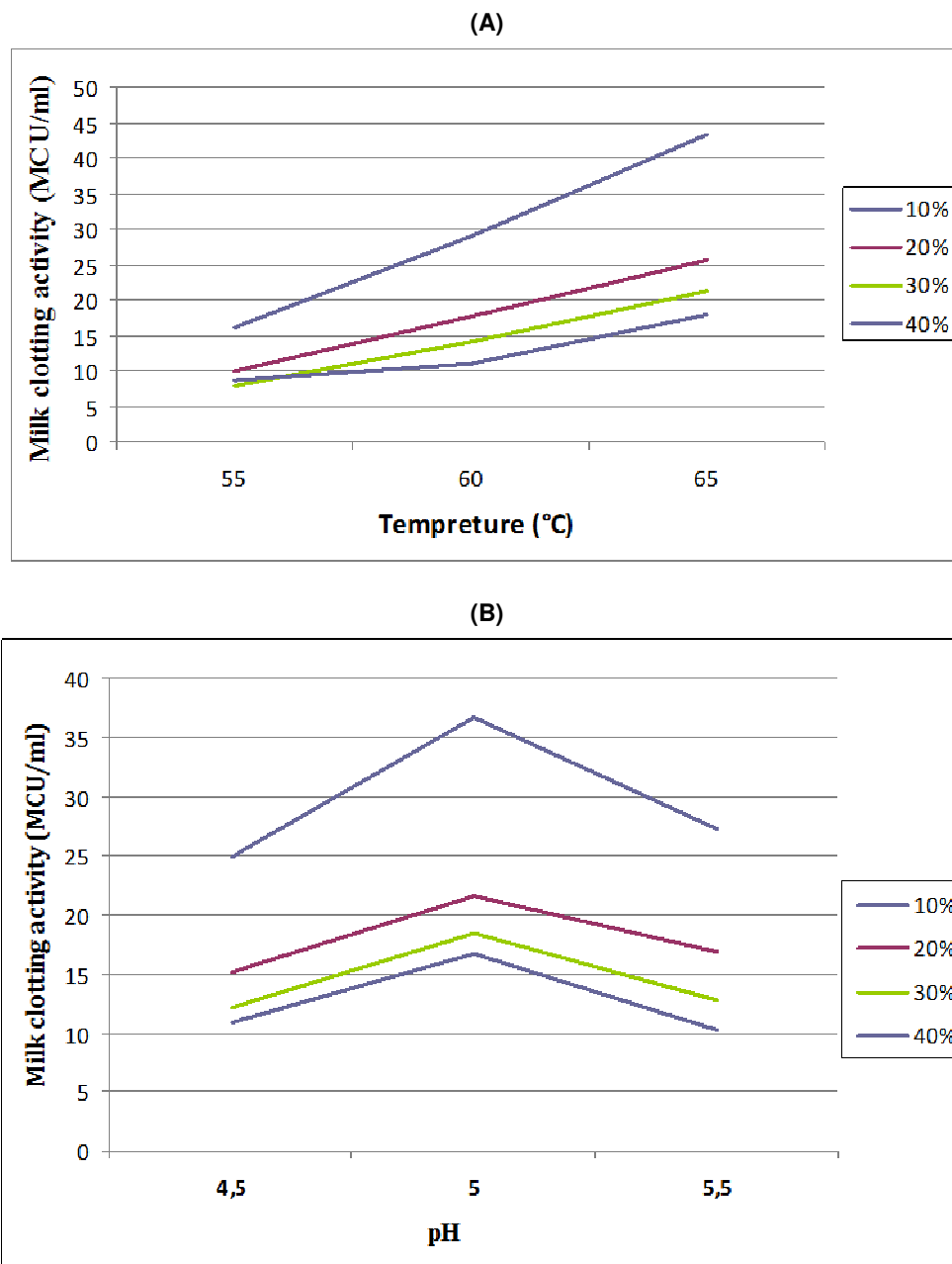


Figure 1. Clotting activity of camel milk (A = Effect of temperature and crude extract concentration on clotting activity (MCU/ml); B = Effect of pH and crude extract concentration on milk clotting activity (MCU/ml)).

DISCUSSION

The high clotting activity of camel milk observed in the current study might be attributed to the combined effect of the three parameters (temperature, pH and GCE concentrations); the active catalytic property of the crude extract, and the inherent characteristics of the substrate (milk). Earlier studies showed highest clotting activity of bovine milk by cysteine protease from ginger at high temperature (60°C) and high pH (5.5) (Hashim et al., 2011a; Huang et al., 2011). In addition, Huang et al. (2011) indicated that ginger protease showed optimal

proteolytic activity at temperature range of 40 to 60°C with maximum activity observed at 70°C. They also reported that 70% of milk clotting activity was retained when the temperature was increased to 65°C and ginger protease exhibited higher specificity for κ - casein with increase in temperature. Lowering the pH of dromedary milk delayed the solubilisation effect on casein micelles in which a transition state for solubilisation was observed at pH 5.0, and soluble casein amount in dromedary milk decreased at a pH below 5.0 (Kheroutou et al., 2003). Dromedary milk casein maintains its integrity till pH value of 5.5; however, below pH 5.0, caseins undergo

Table 3. Firmness of curd obtained by coagulation of camel milk with ginger crude extract and camel chymosin

Treatments	Load at yield (Newton)
T1	0.055416 ± 0.003633 ^b
T2	0.037709 ± 0.001759 ^{cd}
T3	0.044587 ± 0.003169 ^c
T4	0.033901 ± 0.002371 ^d
T5	0.087534 ± 0.007502 ^a

T1 = (pH 5.5, temp 60°C and 10% by volume inclusion of ginger crude extract), T2 = (pH 5.0, temp 65°C and 10% by volume inclusion of ginger crude extract), T3 = (pH 5.5, temp 65°C and 10% by volume inclusion of ginger crude extract), T4 = (pH 4.5, temp 65°C and 10% by volume inclusion of ginger crude extract), T5 = Control (pH 6.3, temp 36°C and 15% by volume inclusion of camel chymosin/litre of milk); Means with the same superscript letters within a column are not significantly different ($p > 0.05$); values in the Table are mean ± SD of three replications.

Table 4. Firmness of curd obtained by coagulation of cow milk with ginger crude extract and camel chymosin.

Treatment	Load at yield (Newton)
T1	0.045035 ± 0.001701 ^b
T2	0.036774 ± 0.002478 ^c
T3	0.040498 ± 0.005020 ^{bc}
T4	0.074687 ± 0.001252 ^a

T1 = (pH 5, temp 65°C and 10% by volume inclusion of ginger crude extract), T2 = (pH 4.5, temp 65°C and 10% by volume inclusion of ginger crude extract), T3 = (pH 5, temp 60°C and 10% by volume inclusion of ginger crude extract), T4 = Control (pH 6.3, temp 36°C and 15% by volume of camel chymosin/litre of milk). Means with the same superscript letter within a column are not significantly different ($p > 0.05$); Values in the table are mean ±SD of three replications.

extensive structural and biochemical modifications, and maximum solubilisation takes place at pH value of 4.9 (Attia et al., 2000).

Coagulation activity strongly depends on the pH and temperature of milk (Soledad et al., 2007; Mohammed et al., 2010). Optimum proteolytic activity of *Cynaracran dunculuson* bovine casein occurred at pH range of 5.1 to 6.0 (Garcia et al., 2011). Llorente et al. (1997) also indicated that crude extract of the upper (violet) part of mature flowers of *Cynara scolymus L.* exhibited optimum clotting activity at acid pH ranging from 3.5 to 5.0 for bovine milk and showed low thermal stability at temperatures above 45°C. Protease from *Cynara scolymus* exhibited maximum activity at 70°C for clotting cow milk (Sidrachet et al., 2005). Maximum MCA of crude and pure extracts of *Jacaratia corumbensis* for cow milk was also reported to occur at a temperature of 55°C for both extracts, while the optimum pH for crude and partially purified extracts was 6.5 and 7.0, respectively (Rodrigues et al., 2009).

As indicated by Attia et al. (2000) and Kheroutou et al. (2003), gel strength of camel milk may have an effect on the solubilization point of camel milk casein. A special redistribution of dromedary milk casein was reported by Kheroutou et al. (2003) where permanent bonds were created between casein fractions at pH 5.0 and a loose network was formed at low pH (4.4) that led to a pseudo curd formation. Attia et al. (2000) and Kheroutou et al. (2003), on the other hand, indicated that separated micelles start to gather together in globular or linear

shape as the pH approaches to 5.5 and at pH 5.0 and complete fusion and three dimensional networks are observed during acidification. Camel chymosin had three-fold higher catalytic efficiency on camel κ -casein as compared to cow κ -casein (Kappeler et al., 2006).

Dilution of ginger crude extract had effect on milk clotting activity where MCA increased with decreasing crude extract concentration. Earlier reports indicated that bovine milk clotting time is affected by the type and protein content of the coagulant. Mehaia (1997), for instance, indicated that clotting time of bovine milk can be longer when concentration ratio of the protein content of the coagulant is increased as a result of increasing effective collision due to decreasing aqueous phase. Mariela et al. (2010) also reported that higher dilution of hieroymain fruit extract prolonged bovine milk clotting time. In the contrary, a significant decrease in bovine milk clotting time was observed with increasing the amount of *Solanum macrocarpon* extract (Guama et al., 2010b).

CONCLUSION

Coagulation of camel milk could be achieved using ginger rhizome crude extract and improved firm curd could be obtained at a pH value of 5.0, a temperature of 65°C and crude extract concentration of 10% by volume. Therefore, the coagulation of camel milk using ginger crude extract suggests the possibility of making cheese from camel milk using locally available coagulants. Further studies

are needed to confirm the result by separating the pure milk clotting protease enzyme from ginger rhizome.

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