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Impact of Using Milk Clotting Enzyme From Seeds of *Moringa Oleifera* on Coagulation Properties of Curd Made From Camel's Milk

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THIS STUDY was conducted to assess the clotting properties of camel's milk using moringa seeds enzyme (MSE) as rennet substitute. Camel's milk total solids (CMTS) content was increased using buffalo's milk. The results indicated that milk clotting time (MCT) and curd firmness (CF) improved greatly with the increase of (CMTS) and when (MSE) were used as coagulant. The coagulum properties improved successfully by replacement of camel's milk with buffalos' milk and with the addition of (MSE). Therefore, it is possible to make good cheese from camel's milk by replacing up to 40% buffalo's milk with camel's milk and using a milk-clotting enzyme from the seeds of *Moringa oleifera* at 1.5% level.

Keywords: Camel's milk , Moringa seeds enzyme , Clotting time , Properties of the coagulum.

Introduction

Camel dairy farming is an alternative to cow dairy farming in dry regions of the world where bovine farming consumes large amount of water and electricity to power air-conditioned halls and cooling sprinkler systems.

Camel milk has enough nutrients to sustain a person through a day; it provides a nutritious and balanced diet to nomadic desert people under harsh conditioned. Camel milk differs in chemical composition from other ruminants. It contains a high percentage of immunoglobulin, vitamins (B2, A, C and E), minerals (potassium, sodium, magnesium, iron, zinc and copper) and insulin. On the other hand it is low in sugar, cholesterol and protein (Kamal et al., 2007 and Al-Hashem, 2009). Camel milk is three times higher in vitamin C than cow milk and 10 times higher in iron.

In spite of these huge benefits of camel milk, camel milk is more technically difficult to process than milk of other domestic dairy animals and some researchers have been claimed that camel milk cheese could be impossible to produce. This is unlike milk of cow and small ruminants, camel milk doesn't readily coagulate by rennet due to its low total solids content, unique composition and casein properties and also due to its greater amount of lysozyme, lactoferrin, lactoperoxidase and immunoglobulin than cow or buffalo's milk. These properties disturbed the enzymatic activity and gelation process of camel milk (El-Agmy et al., 1992).

Farah et al. (1990) found that the consistency of fermented camel milk was thin and a precipitate in the form of flocks. However, efforts have been made for cheese preparation from camel milk (Farah et al., 1990, Ramet, 1987, Mehaia et al., 1988 and Mohammed, 1990). However, success was achieved when pH of milk was lowered and calcium chloride was added prior to rennet addition. Haiderkhan and Aslam, (2004) successfully made cheese from camel milk by direct acidification and by adding starter culture of lactic acid bacteria. Recently, camel chymosin was developed using recombinant DNA technology by Danish scientists (Kappeler et al., 2006).

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. Several researchers tested the coagulation potential of bovine milk using different plant extracts (Abdalla et al., 2011

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and Garcia et al., 2011). Crude extracts of ginger was used to coagulate cow milk (Llorente et al., 1997) The clotting ability of ginger extract was higher than that of calf rennet and papain for cow milk; however, it is lower than mucor rennet. Recently, Tajalsir et al. (2014) used partially purified milk clotting enzyme from the seeds of *Moriga oleifera.* The high specificity of this enzyme in terms of its high ratio of milk-clotting to proteolytic activity could pave the way for its use in cheese industry as alternative to calf rennet.

In this study, the effect of increasing total solids of camel milk with buffalo's milk and partially purified milk clotting enzyme from the seeds of *Moringa oleifera* (MSE) for the enhancement of the coagulation properties of camel milk was studied.

Materials and Methods

Materials

Moringa oleifera seeds were obtained from Faculty of Agriculture & Natural Resources Farm, Aswan University. All fresh samples were dried for two days at room temperature. Thereafter, samples were ground using house grinder and stored in closed containers in the freezer at - 20°C until use.

Fresh camels' milk was obtained from the herd at Maryout Research Station, Desert Research Center (DRC) during winter season from December – March. Milk samples were immediately stored under refrigerated conditions until the transferring it to the laboratory. Buffalo's milk was collected from the local market. Calcium chloride ($CaCl_2$) was obtained from El-Nasr Pharmaceutical Chemicals Co., Cairo, Egypt. Animal rennet was obtained from Rhone-poulenc Maraschall products (MADISONWI).

Treatments

Buffalo's milk was used to increase the total solids of camel's milk. Camel's milk was replaced with buffalo's milk at different levels:

T1= 60% camel's milk +40% buffalo's milk
T2= 50% camel's milk+50% buffalo's milk
T3= 40% camel's milk+60% buffalo's milk
T4= 30% camel's milk+70% buffalo's milk
C1= 100% buffalo's milk
C2= 100% camel's milk

The effect of the MSE on the milk clotting times, curd firmness and syneresis were studied in the presence of 50% and 60% buffalo's milk in camel's milk. The enzyme was used at different concentrations at each level of the mixture , *i.e.* 0.5, 1.0 and 1.5%.

Preparation of curd

All milk samples from different treatments were heated in a water bath at 75±1°C for 15 sec. After cooling to 45°C , 0.02% (v/v) of rennet ± moringa seeds enzyme +0.02% $CaCl_2$ were added at 42±1°C until complete coagulation.

Enzyme extraction from moringa seeds

Enzyme extraction was done according to the optimum conditions with the procedure described by Tajalsir et al. (2014) and Mohamed Ahmed et al. (2010), using 5% NaCl in sodium acetate buffer (pH 5.0). *Moringa oleifera* was prepared and 5.0 g finely ground by grinder and extracted with 50 ml of the extractant for 4h with stirring. The extract was filtrated through cheesecloth and centrifuged at 12,000 × *g* for 20 min. The supernatant was dialyzed overnight at 4^0C against 0.1 mmol/L sodium acetate buffer, pH 5.0. The solution was centrifuged to remove any solid particles and then the activity was measured. Ammonium sulfate fractionation was carried out following the method described by (Mohamed Ahmed et al., 2009). Milk-clotting and proteolytic activities in the extract were determined.

Methods

Chemical analysis

The chemical composition of camel's, buffalo's milk and their mixtures were determined according to A.O.A.C (2012). Total solids content was determined by air-oven drying at 105˚C overnight. Total nitrogen content was determined using Kjeldahl method**.** The conversion factor of 6.38 was applied in this respect.

Determination of milk-clotting and proteolytic activities

Milk clotting time (MCT) was recorded as the time elapsed for the sign of clotting after the addition of the enzymes. Milk-clotting activity was determined according to the methods described by Arima et al. (1970), MCA (U/ml) = (2400⁄clotting time (sec)) × Dilution factor. Protease activity was determined as Unit/ ml described by Sarath et al. (1989).

Curd properties

Curd firmness was determined according to Shalabi (1987) while curd syneresis was measured using the draining method as described by Mehanna and Mehanna (1989).

Statistical analysis

All experiments were conducted in triplicates by means of analysis of variance (ANOVA) with statistical analysis system. The obtained data was carried out according to the method described by Clarke and Kempson (1997).

Results and Discussion

Chemical composition of camel's , buffalo's milk and their mixtures

Camel's milk doesn't readily coagulate by rennet due to its low total solids content, unique composition and casein properties (El-Agmy et al., 1992). Results in Table 1 confirmed this property, as camel's milk (C2) showed lower total solids content than buffalo's milk (C1), it was 11.73 % vs 17.44%, respectively. This decrease was mainly in protein, ash and fat which are the most variables responsible for curdling of milk. This result is in line with the results mentioned by Farag et al. (2015) and Kula & Tegegne (2016).

Results in Table 1 showed an increase in total solids, protein, ash and fat in the mixture as the added buffalo's milk increased. This may be due to the high total solids such as protein, fat and ash contents in buffalo's milk. These results agree with Farag et al. (2015).

TABLE 1. Chemical composition% and Acidity% of milk mixture from different treatments.

Treatments*	T. S	Ash	T.P	Fat	Acidity (%)
T1	13.40^{c}	0.74^{b}	4.00^{b}	5.20^{c}	0.17^{a}
T2	13.54^{c}	0.74^{b}	4.10^{b}	5.40^{bc}	0.17^{a}
T3	15.32^{b}	0.75^{b}	4.12^{b}	6.00^{b}	0.18^{a}
T4	16.35^{ab}	0.77^{a}	4.23^{ab}	6.00^{b}	0.18^{a}
C1	17.44^{a}	0.83^{a}	4.45^{a}	8.00^{a}	0.18^{a}
C2	11.73^{d}	0.70^{b}	3.50^{c}	3.30^{d}	0.13^{b}

*^{abcd} letters indicate significantly differences between treatments
T1= 60% camel's milk +40% buffalo's milk
T2= 50% camel's milk+50% buffalo's milk
T3= 40% camel's milk+60% buffalo's milk
T4= 30% camel's milk+70% buffalo's milk
C1= 100% buffalo's milk
C2= 100% camel's milk

Coagulation properties of camel's milk

The clotting and proteolytic activities of MSE recorded 13404.5 and 2.5 Unit/ml, respectively. This is in line with the data reported by Tajalsir et al. (2014), but the rennet recorded 249.6 and 0.05 Unit/ml, respectively. These results agree with Mohamed Ahmed et al. (2010) and Tajalsir et al. (2014). The coagulation properties of renneted camel milk supplemented with buffalo's milk are shown in Table 2. These included clotting time (MCT), curd firmness (CF) and curd syneresis (CS) of the mixtures of camel's and buffalo's milk compared to the control camel's and buffalo's milk were studied. Results exhibited poor rennetability of raw camel milk, as it is difficult to coagulate comparing to coagulation ability of buffalo's milk. The poor rennetability of camel's milk is due to its casein properties and its low κ-casein content (Farag et al., 2015). By increasing the total solids of camel's milk with buffalo's milk, the coagulation properties of the mix were improved. As the camel's milk replaced with 40% buffalo's milk, the MCT being 70 min., while it decreased profoundly with the increase in buffalo's milk content in the mixture, it decreased to 50 min. when buffalo's milk increased to 70%. These results concluded the mprovement of rennet clotting ability of camel milk in the presence of buffalo's milk. Similar findings were noticed in curd firmness (CF) which increased in the presence of buffalo's milk. It increased proportionally with the level of buffalo's milk added to camel milk. On the other hand, an opposite trend was observed for the curd syneresis of the mixture. The trend of the syneresis decreased with the increase of buffalo's milk content in the mixture. Similar results were given by Farag et al. (2015), who reported that cheese made by replacing up to 40% buffalo's milk with camel's milk were not significantly different from the control cheese which made from buffalo's milk.

Effect of enzyme from moringa seeds on coagulation properties of camel's milk mixed with buffalo's milk

Although the rennet clotting ability of camel's milk was enhanced in the presence of buffalo's milk but the curd appeared very weak compared

with the curd of the control buffalo's milk. So, the effect of enzyme extracted from MSE which has high clotting ability (Tajalsir et al., 2014) was used.

Table 3 shows the effect of enzyme from moringa seeds (MSE) on the milk-clotting activity, curd firmness and curd syneresis of camel's , buffalo's milk and their mixture. The results in Table 3 showed that although the MCT of milk mixtures in the presence of MSE improved greatly compared with the MCT of the mixtures without the enzyme, the camel's milk showed difficulty to coagulate (Tables 2 and 3). The results also indicated that the MCT of the mixtures decreased proportionally with the corresponding increase in the enzyme concentration. At 1.5% MSE, the MCT of the mixtures more or less similar to the MCT of control buffalo's milk.

Also, the results showed an increase in the curd firmness and curd syneresis by increasing the ratio of the MSE in all replacement ratios. These results agree with Tajalsir et al. (2014) who reported that MSE had high milk clotting ability, and it was more active compared to calf rennet. But, the results in Table 3 indicated a decrease in firmness and an increase in curd syneresis values by adding the enzyme vs the results in Table 2, this may be due to the high proteolytic activity of MSE comparing with rennet and the poor rennetability of camel's milk that due to also casein properties , low κ-casein content and limited ability for acidification by lactic acid bacteria which helps the enzyme coagulation activity. This contributes to the destabilization of the casein micelles (Ramet, 1994 and Oumelkheir et al., 2005). The high ratio of milk-clotting to proteolytic activity of the partially purified (MSE) indicates the potential of this enzyme as suitable rennet substitute in cheese making.

Conclusion

The present study confirmed the previous studies that camel's milk has poor coagulation propriety, which is the key to cheese making. Such property can be improved if it is mixed with other milk ruminants and with using moringa seeds enzyme (MSE) . Also, it could be concluded that, application of the MSE in dairy industry will be beneficial as rennet substitute cheese making.

TABLE 2. Clotting time, curd firmness and syneresis of the resultant curd.

Treatments*	Clotting time min.	Curd firmness gm.	Syneresis ml.
T1	70^{a}	16^{d}	400^{a}
T2	60^{b}	19^{c}	300^{b}
T3	55^{c}	19^{c}	285^{d}
T4	50^{c}	21.5^{b}	290^{c}
C1	30^{d}	45^{a}	280^{d}
C2	NC	NC	NC

*See foot note the Table 1 while rennet and $CaCl_2$ were added at levels (0.02% and0.02%) in order. NC= Not Coagulated.

TABLE 3. Effect of the enzyme extracted from moringa seeds on clotting time, curd firmness and syneresis of camel's milk mixed with buffalo's milk.

Treatments*	Clotting time min.	Curd firmness gm.	Syneresis ml 800/ml
T2 a	45^{a}	13^{c}	380^{d}
T2b	40^{b}	14^{c}	450^{ab}
T2c	35^{c}	17^{b}	500^{a}
T3a	40^{b}	13^{c}	430^{b}
T3b	35^{c}	13^{c}	450^{ab}
T3c	30^{d}	14^{c}	420^{c}
C1	30^{d}	24^{a}	260^{e}
C2	NC	NC	NC

*^{abcde} letters indicate significantly differences between treatments
T2a=50% buffalo's milk + 50% camel's milk + 0.02 % rennin + 0.5 % MSE +0.02% $CaCl_2$
T2b=50% buffalo's milk + 50% camel's milk + 0.02 % rennin + 1 % MSE +0.02% $CaCl_2$
T2c=50% buffalo's milk + 50% camel's milk+ 0.02 % rennin + 1.5 % MSE +0.02% $CaCl_2$
T3a=60% buffalo's milk + 40% camel's milk + 0.02 % rennin + 0.5 % MSE +0.02% $CaCl_2$
T3b=60% buffalo's milk + 40% camel's milk + 0.02 % rennin + 1 % MSE +0.02% $CaCl_2$
T3c=60% buffalo's milk + 40% camel's milk + 0.02 % rennin + 1.5 % MSE +0.02% $CaCl_2$
C1=100 buffalo's milk + 0.02 % rennin + 1 % MSE +0.02% $CaCl_2$
C2=100% camel's milk + 0.02 % rennin + 1 % MSE +0.02% $CaCl_2$

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تأثير إستخدام إنزيم مجبن للبن من بذور المورينجا على خصائص الخثرة المصنعة من لبن الإبل

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أجريت هذه الدراسة لتقييم تجبن لبن الإبل من خلال إستخدام إنزيم بذور المورينجا كبديل للمنفحة الحيوانية حيث تم رفع نسبة المواد الصلبة للبن الإبل بإستخدام اللبن الجاموسي و أظهرت النتائج أن زمن تجبن اللبن وقوة الخثرة قد تحسنت بشكل كبير مع زيادة نسبة المادة الصلبة في لبن الإبل عن طريق إضافة اللبن الجاموسي وعند إستخدام إنزيم بذور المورينجا كمجبن كما تحسنت خصائص التجبن بصفة عامة أيضاً كان لإستخدام إنزيم بذور المورينجا تأثيراً واضحاً ومعنوياً ، حيث زاد معامل التجبن وقل الزمن اللازم للتجبن مما يمهد الطريق لإستخدام هذا الإنزيم في صناعة الجبن كبديل للمنفحة الحيوانية . أوضحت النتائج أيضاً أنه من الممكن إنتاج خثرة جيدة عن طريق إستبدال اللبن الجاموسي بلبن الإبل بنسبة ٤٠٪ وإستخدام إنزيم بذور المورينجا بنسبة ١,٥٪ , حيث أدي إلى الحصول على صفات خثرة جيدة و تقليل وقت تجبن اللبن.