

## 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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**T**HIS 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 , 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 *Moringa 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 ( $\text{CaCl}_2$ ) 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  $75\pm 1^\circ\text{C}$  for 15 sec. After cooling to  $45^\circ\text{C}$  , 0.02% (v/v) of rennet  $\pm$  moringa seeds enzyme +0.02%  $\text{CaCl}_2$  were added at  $42\pm 1^\circ\text{C}$  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  $12,000 \times g$  for 20 min. The supernatant was dialyzed overnight at  $4^\circ\text{C}$  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  $105^\circ\text{C}$  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),  $\text{MCA (U/ml)} = (2400/\text{clotting time (sec)}) \times \text{Dilution factor}$ . 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 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 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 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%
<b>T1</b>	13.40 <sup>c</sup>	0.74 <sup>b</sup>	4.00 <sup>b</sup>	5.20 <sup>c</sup>	0.17 <sup>a</sup>
<b>T2</b>	13.54 <sup>c</sup>	0.74 <sup>b</sup>	4.10 <sup>b</sup>	5.40 <sup>bc</sup>	0.17 <sup>a</sup>
<b>T3</b>	15.32 <sup>b</sup>	0.75 <sup>b</sup>	4.12 <sup>b</sup>	6.00 <sup>b</sup>	0.18 <sup>a</sup>
<b>T4</b>	16.35 <sup>ab</sup>	0.77 <sup>a</sup>	4.23 <sup>ab</sup>	6.00 <sup>b</sup>	0.18 <sup>a</sup>
<b>C1</b>	17.44 <sup>a</sup>	0.83 <sup>a</sup>	4.45 <sup>a</sup>	8.00 <sup>a</sup>	0.18 <sup>a</sup>
<b>C2</b>	11.73 <sup>d</sup>	0.70 <sup>b</sup>	3.50 <sup>c</sup>	3.30 <sup>d</sup>	0.13 <sup>b</sup>

\*abcd letters indicate significant 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 highest, 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  $\kappa$ -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 improvement 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 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 milk mixed with buffalo's milk

Although the rennet clotting ability of camel milk enhanced in the presence of buffalo's milk

but the curd appeared very weak compared to the curd of control buffalo's milk. So, the effect

of enzyme extracted from MSE which has high clotting ability (Tajalsir et al., 2014) was used.

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

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

\*See foot note the Table 1 while rennet and CaCl<sub>2</sub> were added at levels (0.02% and 0.02%) in order. NC= Not Coagulated.

Table 3 show 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 show 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 decreasing in firmness and increasing 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  $\kappa$ -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.

**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 <sup>a</sup>	13 <sup>c</sup>	380 <sup>d</sup>
T2b	40 <sup>b</sup>	14 <sup>c</sup>	450 <sup>ab</sup>
T2c	35 <sup>c</sup>	17 <sup>b</sup>	500 <sup>a</sup>
T3a	40 <sup>b</sup>	13 <sup>c</sup>	430 <sup>b</sup>
T3b	35 <sup>c</sup>	13 <sup>c</sup>	450 <sup>ab</sup>
T3c	30 <sup>d</sup>	14 <sup>c</sup>	420 <sup>c</sup>
C1	30 <sup>d</sup>	24 <sup>a</sup>	260 <sup>c</sup>
C2		NC	

\*abcde letters indicate significant differences between treatments

T2a=50% buffalo's milk + 50% camel's milk + 0.02 % rennin + 0.5 % MSE +0.02% CaCl<sub>2</sub>

T2b=50% buffalo's milk + 50% camel's milk + 0.02 % rennin + 1 % MSE +0.02% CaCl<sub>2</sub>

T2c=50% buffalo's milk + 50% camel's milk+ 0.02 % rennin + 1.5 % MSE +0.02% CaCl<sub>2</sub>

T3a=60% buffalo's milk + 40% camel's milk + 0.02 % rennin + 0.5 % MSE +0.02% CaCl<sub>2</sub>

T3b=60% buffalo's milk + 40% camel's milk + 0.02 % rennin + 1 % MSE +0.02% CaCl<sub>2</sub>

T3c=60% buffalo's milk + 40% camel's milk + 0.02 % rennin + 1.5 % MSE +0.02% CaCl<sub>2</sub>

C1=100 buffalo's milk + 0.02 % rennin + 1 % MSE +0.02% CaCl<sub>2</sub>

C2=100% camel's milk + 0.02 % rennin + 1 % MSE +0.02% CaCl<sub>2</sub>

NC= Not Coagulated

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## 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.

## References

- Abdalla, M.O.M., Kheir, S.E.O and El-Owni, O.A.O. (2011) Effect of extraction method, ammonium sulphate concentration, temperature and pH on milk clotting activity of *Solanumdubium* fruit extract. *Advanced J. Food Sci. Technol.* **3**, 40-44.
- Al-Hashem, F. (2009) Camel milk protects against aluminium chloride-induced toxicity in the liver and kidney of white albino rats. *Am. J. Biochem. Biotechnol.* **5**, 98-108.
- A.O.A.C. (2012) *Official Methods of Analysis of The Association of Official Analytical.* Washington, DC, USA.
- Arima, K., YA, J. and Iwasaki, S. (1970) Milk-clotting enzyme from *Mucor pusillus* var. Lindt. In: *Methods in Enzymology*, (Ed.: Pearlman, E. G., and Lorand, L.). Academic Press, New York, pp. 446-459. ISBN: 978-0-12-181881-4.
- Clarke, G. M. and Kempson, R.E. (1997) *Introduction to the Design and Analysis of Experiments.* Arnold, a Member of the Holder Heading Group. 1<sup>st</sup> EDS, London, UK.
- El-Agamy, E. I., Ruppanner, R., Ismail, A., Champagne, C. P. and Assaf, R. (1992) Antibacterial and antiviral activity of camel milk protective proteins. *J. Dairy Res.* **59**, 169-175.
- Farag, S.I., Kebary, K.M.K., Zedan, A.N., El-Sonbaty, A.H. and Helal, S.H.S. (2015) Impact of replacing buffalo's milk with camel's milk on the quality of Domiati like cheese. *12 th Egypt. Conf. Dairy Sci. & Technol., Cairo*, 9-11 Nov. pp. 95-104.
- Farah, Z. Streiff and Bachmann, M.R. (1990) Preparation and consumer acceptability tests of fermented camel milk in Kenya. *J. Dairy Res.* **57**, 281-283.
- Garcia, H.S, Hernandez, A.L. and Hill, C.G. (2011) Enzyme technology-Dairy industry applications. *Comprehensive Biotechnol.* **4**, 567-574.
- Haiderkhan, I.H.A. and Aslam, M. (2004) Evaluation of cheese prepared by processing camel milk. *Pakistan J. Zool.*, **36**, 323-326.
- Kamal, A. M., Salama, O. A. and El-Saied, K. M. (2007) Changes in amino acid profile of camel milk protein during the early lactation. *Int. J. Dairy. Hepatol.* **32**, 39-47.
- Kappeler, S.R., Van den Brink, H.J.M., Rahbek, N.H., Farah, Z., Puhan, Z., Hansen, E.B. and Johansen, E. (2006) Characterization of recombinant camel chymosin reveals superior properties for the coagulation of bovine and camel milk. *J. Biochem. Biophys. Res. Commun.* **342**, 647-654.
- Kula, J. and Tegegne, D. (2016) Chemical composition and medicinal values of camel milk. *Inter. J. of Res. Studies in Biosci. (IJRSB)* Vol. 4, Issue 4, 13-25.
- Llorente, B.E., Brulti C.B., Natalucci, C. L. and Caffini, Y. O. (1997) Partial characterization of a milk clotting proteinase isolated from Artichoke (*Cynara scolymus* L., Asteraceae). *Acta Farmaceutica Bonaerense*, **16**, 37-42.
- Mehaia, M. A., Abou EL-Kheir, A. M., and Hablas, M. A. (1988) Enzymatic coagulation of camel milk. A study using soluble and immobilized chymosin. *Milchwissenschaft*, **43**, 438e441.
- Mehanna, N.M. and Mehanna, A.S. (1989) A study on the use of stabilizer for improving some properties of cow's milk yoghurt. *Egyptian J. Dairy Science.* **17**, 289.
- Mohammed Ahmed, I. A., Morishima, I., Babiker, E. E. and Mori, N. (2009) Characterization of partially purified milk-clotting enzyme from *Solanum dubium* Fresen seeds. *Food Chem.* **116** (2), 395-400.
- Mohamed Ahmed, I. A., Babiker, E. E. and Mori, N. (2010) pH stability and influence of salts on activity of a milk-clotting enzyme from *Solanum dubium* seeds and its enzymatic action on bovine caseins. *LWT of Food Sci. & Technol.* **43**, 759-764.
- Mohammed, M. A. (1990) Camel milk: Chemical composition of casein and Preliminary trial of cheese-making properties. P.31.
- Oumelkheir, S., Abderahmane, M., and Brahim, H. (2005) Improvement of the coagulation properties of camel (*Camelus dromedarius*) milk : use of coagulating enzyme extracts from the stomach of dromedaries . *Rivista Italiana Eppos*, **39**, 15.
- Ramet, J.P. (1987) Use of bovine calf rennet to coagulation raw camel milk. *World Animal Rev.*, **61**, 11-17.
- Ramet, J. P. (1994) The scientific aspects and specific technology for the production of camel milk cheese. *Proceedings of the Workshop on Dromedary and Camel milk*, October, **26**, 1994, Nouakchott, Mauritania.



- Sarath, G., Motte, R.S., and Wanger, F.M. (1989) Protease assay methods. In R. J. Beynon, & J. S. Bond (Ed.), *Proteolytic enzyme - A practical approach* (pp. 25-55). Oxford: IRL Press.
- Shalabi, S.I. (1987) Milk clotting activity of commercial rennet substitute. A comparative study. *Minia J. Agric. Res. Dev.* **9**, 441-460.
- Tajalsir, A.E., Ebraheem, A.S.A., Abdallah, A.M., Khider F.J., Elsamani. M.O. and Ahmed, I.A.M. (2014) Partial purification of milk-clotting enzyme from the seeds of moringa oleifera. *J. of microb., Biotech. Food Sci.* **4**, 58-62.

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### تأثير استخدام إنزيم مجبن للبن من بذور المورينجا على خصائص خثرة الجبن المصنعة من لبن الإبل

أجريت هذه الدراسة لتقييم تجبن لبن الإبل من خلال استخدام إنزيم بذور المورينجا كبديل للمنفعة الحيوانية حيث تم رفع نسبة المواد الصلبة للبن الإبل باستخدام 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 استخدام 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 يمهّد الطريق لإستخدام هذا الإنزيم في صناعة الجبن كبديل للمنفعة الحيوانية . أوضحت النتائج أيضاً أنه من الممكن إنتاج خثرة جيدة عن طريق إستبدال اللبن الجاموسي باللبن الإبل بنسبة ٤٠ ٪ وإستخدام إنزيم المورينجا بنسبة ١,٥ ٪ , حيث أدي إلى الحصول على صفات خثرة جيدة و تقليل وقت تجبن اللبن.