The effect of urea and formaldehyde on heat coagulation time (HCT) of camel milk

Metwalli, A. A. M[1,2].; Ismail*, E. A[1,3]; Alhaj, O.A[1]; Saleh, K. A[2] and Ibrahim, F.S[2]

[1]Department of Food Science & Nutrition, College of Food and Agricultural Sciences, King Saud University, P.O. Box 2460, Riyadh 11451, Saudi Arabia.; [2]Department of Dairy Science, College of Agriculture, Minia University, Egypt; [3]Department of Food Science, Faculty of Agriculture, Benha University, Egypt.
sayed1973@yahoo.com

Abstract: Heat coagulation time-pH curve (HCT/pH) of camel milk in the range of pH 6.4-7.2 was determined at 120 ^{0}C. The effect of dialysis of camel milk against Jennes and Koops buffer on HCT was determined. Moreover, the addition of camel milk whey and casein addition as well as urea, formaldehyde and mixture of both was studied. Results showed that camel milk has poor heat stability. However, this stability was markedly improved by adding formaldehyde at a concentration of 7.5 mM; while urea showed to have little effect on camel milk stability at a concentration of 30 mM. A mixture of urea and formaldehyde at a concentration of 5 mM/each was shown to have great a synergistic effect on HCT of camel milk. Dialysis of camel milk against J & K buffer has also shown to increase HCT of camel milk consequently improves heat stability. Heat coagulation time was found to decrease when camel whey proteins concentration was increased. Results showed that camel milk heat stability behavior differs from that of cow milk.
[Metwalli, A. A. M; Ismail, E. A; Alhaj, O. A; Saleh, K. A and Ibrahim, F.S. **The effect of urea and formaldehyde on heat coagulation time (HCT) of camel milk.** *Life Sci J* 2013; 10(4): 45-50] (ISSN: 1097-8135). http://www.lifesciencesite.com. 7

Key Words: camel milk, heat stability, urea, formaldehyde, J & K buffer.

1. Introduction:

Heat coagulation of milk proteins is affected by many factors such as heating temperature, pH, association of whey proteins with casein micelles, and some additives such as urea, formaldehyde and $CaCl_2$ (Singh, 2004). These factors either change the structure of milk proteins and/or the state of milk serum (Van Boekel *et al*, 1989 Metwalli *et al*. 1996a; Metwalli *et al.* 1996b). Heat coagulation of cow milk has been extensively studied by Fox and Moressey, (1977); and Fox, (1981). Heat stability was between 60 and 120 °C for different times after addition of Calcium chloride to skim milk powder (Sievanen *et al.*, 2008 Tsioulpas *et al.,* 2010, On-Nom *et al.*, 2012,). Sikand *et al.*, 2010, studied the effects of standardization material, protein content, and pH on the heat stability of reconstituted milk. Metwalli *et al.* (1996a) has shown that addition of urea to cow milk increased its heat stability at maximum pH (6.4 – 6.7) and alkaline region but not at minimum pH (6.6 – 6.9). Furthermore, Formaldhyde addition at the concentration of 1mM or above greatly increased the heat stability of cow milk at pH range of 6.4 – 7.2 (Metwalli and van Bockel, 1995). It has been reported that 140 °C is the standard protocol temperature used to study heat coagulation time of cow milk (Rose, 1961; 1963; Singh and Fox, 1987; Metwalli *et al.,* 1996a). However, camel milk heat stability has been given little attention in the literature. Farah and Atkins, (1992) reported that camel milk was shown to coagulate after a short time (heating up time ~ 2 minutes) at a temperature of 140 °C and no solid conclusion could be withdrawn at this standard protocol. The aim of this study is to determine the effect of adding whey proteins, casein, urea, formaldehyde and mixture of both on heat coagulation time (HCT) of camel milk at 120 ^{0}C.

2. Materials and Methods

Chemicals used

Urea, sodium azide and Formaldehyde were purchased from merk and Sigma (UK). Membrane film MW 6000-8000 was purchased from Fisher scientific.

Milk samples

Camel milk was collected from Alkharj in the central region of Saudi Arabia. Sodium azide was added to the milk as preservative at a concentration of 0.02 % and then freeze dried. However, camel milk was reconstituted in distilled water (12% w/v), then stirred for 24 hrs at room temperature. The pH of camel milk was adjusted to the desired pH (6.4-7.2) by adding either 1M NaOH or 1M HCL and then kept at room temperature for at least two hours, pH readings were recorded directly before heating.

Heat coagulation test

The heat stability of adjusted camel milk along with additives were carried out in thermostatically controlled oil bath, temperature was maintained at 120 ^{0}C according to the method described by Davies and White (1966). Milk samples (~ 3 mL) were heated in rubber stoppered glass tubes. Heat coagulation time (HCT) was calculated once the tubes immersed into oil bath; the end point of HCT was that of clear signs of coagulation were appeared at the wall of the tubes.

Addition of urea and formaldehyde

Solid urea was added to camel milk at different concentrations of 10-30 mM and allowed

to dissolve. Similarly, formaldehyde was added to camel milk at different concentrations of 3.0, 5.0 and 7.5 mM after which pH was adjusted.

Modification of salt composition

Reconstituted camel milk was dialyzed against 20 volumes of Jennes and Koops buffer (1962) for 24 h at 4 ^{0}C using number 1 spectra/ membrane MW 6000-8000.

Preparation of camel acid casein and whey proteins

Acid casein was prepared by acidifying raw camel skim milk to pH 4.3 with 1 M HCl. then precipitated using centrifugation and washed three times with water. Casein was re-dissolved in NaOH 1 M at pH 7.0 and re-precipitated at pH 4.3 and freeze dried. The lyophilisate was reconstituted in Jennes & Koops buffer (1962) to have a final casein concentration of 2.5g 100mL. To prepare camel whey proteins ammonium sulfate was added into obtained whey in concentration of 20% saturation followed by centrifugation, the obtained pellet was discarded and the saturation of ammonium sulfate increased into 80% and re-centrifuged again. The obtained pellet (whey proteins) was dialysed against distilled water for 48 hrs at 4°C to remove residual ammonium sulfate. The dialysed solution was freeze-dried and kept frozen till used.

Preparation of cow milk acid whey

Acid whey was obtained by acidifying raw cow skim milk to pH 4.6 with 1 M HCl, then caseins were precipitated using centrifugation and the resultant acid whey was used. Camel acid casein was suspended in cow acid whey in final concentration 2.5%

3. Results and Discussion

Heat coagulation time (HCT) of camel milk

Effect of adding NaOH/pH on HCT of camel milk

In the current study, a positive correlation was found between HCT of camel milk and increasing pH until reached pH 6.9; where HCT was decreased thereafter as shown in Fig 1. Singh (2004) and Singh and Fox (1985) reported that negative charges on κ-casein micelles increase at higher pH values, consequently increase milk heat stability. This was different in camel milk where samples having a pH value above 6.9 were found to have less HCT after heating temperature of 120^{0}C, hence exhibited less heat stability (Fig 1). This could be attributed to the low percentage of κ-casein (3.47% of the total casein) in camel milk (Kappeler *et al.*, 2003; Al haj and Al kanhal, 2010) compared with 13% in cow milk (Davies and Law, 1980).The results also showed that maximum HCT of camel milk was in the pH value of 6.9 as shown in Fig. 1. While the HCT was lower in samples having pH range of 6.4-6.8 as shown in Fig. 1. These results are in agreement with that reported by Alhaj *et al.* (2011). At low pH (6.4-6.8) the calcium ion activity (Ca^{+2}) is high enough to cause coagulation during heating. The calcium ion concentration of cow milk at physiological pH (pH 6.6) is ranged from 1.26 mM (Tanaka *et al.*, 2011) to 3.0 mM (Silanikove *et al.* 2003), or slightly lower of 2.0 mM (Holt *et al.*, 1981).

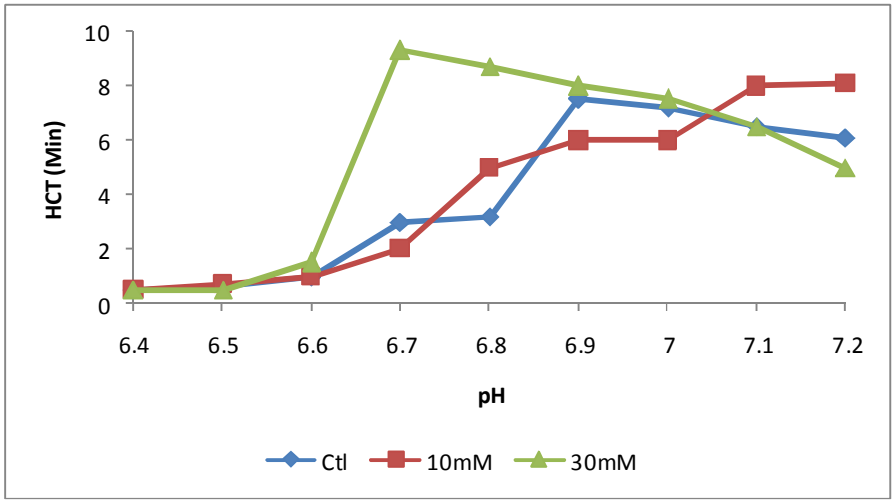

Figure 1: Effect of urea addition on heat coagulation time of camel milk at 120^{o}C.

Difference between these results might be derived from environmental temperature and physiological condition of the cows and also on the difference of temperature during the calcium ion concentration analysis. It seems that the ratio between calcium ion activity and κ-casein concentration plays the crucial factor in heat coagulation time of milk. The concentration of total calcium in camel milk is almost the same of cow milk; while, the κ-casein concentration is about one third of that of cow milk (Al haj and Al kanhal, 2010).

Effect of adding urea and formaldehyde on HCT of camel milk

Urea has been reported to naturally exist in cow milk as a part of non-protein nitrogen and its concentration is about 3-10 mM (Walstra and Jennes, 1984). The concentration of urea in camel milk is very low compared to cow milk. Faye *et al*. (2010) reported that the average concentration of urea in camel milk is 81.6 ± 60.4 mg/L (equivalent to 1.36 mM ± 1.007 mM) ranged from 0-290.5 mg/L (0-4.8 mM). The addition of urea at a concentration of ~3 mM was shown to increase the heat stability of cow milk (Metwalli *et al.*, 1996b), due to the formation of NH_3 which delay the reduction of pH (Fox and McSweeney, 1998). The effect of adding urea on heat coagulation time (HCT) of camel milk was not studied yet. Hence, two concentrations of urea; 10 mM and 30 mM were added to camel milk to determine its effect on HCT. However, the results in Fig. 1 show that the urea addition at a concentration of 30 mM has a slight increase on HCT on camel milk with shifting the maximum heat stability towards acid side (from pH 6.9 in control to 6.7 in 30 mM urea treated samples). This high concentration of urea (30 mM) greatly improved heat stability of cow milk at pH 6.2 – 7.2 (converted it from type A to type B milk) as reported by Muir and Sweetsur (1977). While, lower concentration of 10 mM has no noticeable effect on HCT at the minimum pH region (6.8-6.9) or at lower pH. than 6.4. Hereby, urea has no effect on heat coagulation time of camel milk, unless high concentration of urea is added. It seems that the heat coagulation promoting factors in camel milk is faster than the reaction of urea with lactose to formlactosyl urea which increases the buffering action of the system.

The addition of more than 1 mM of formaldehyde was reported to increase the heat stability of cow milk (Metwalli and Van Boekel, 1995).The effect of formaldehyde on camel milk heat stability was found to depend on the added concentration. Where, the addition of 3 mM of formaldehyde was noticed to have no effect on camel milk heat stability (Fig. 2). While, higher concentration of 5 mM was shown to slightly increase heat stability of camel milk at pH values 6.7-6.9. However, increasing the concentration of formaldehyde to 7.5 mM were markedly increased HCT at pH 6.7-7.2. These results are in agreement with those obtained by Singh and Fox (1985) in bovine milk. The HCT increased at pH 6.8 up to 40 min compared to 3.7 min in control as shown in Fig 2.

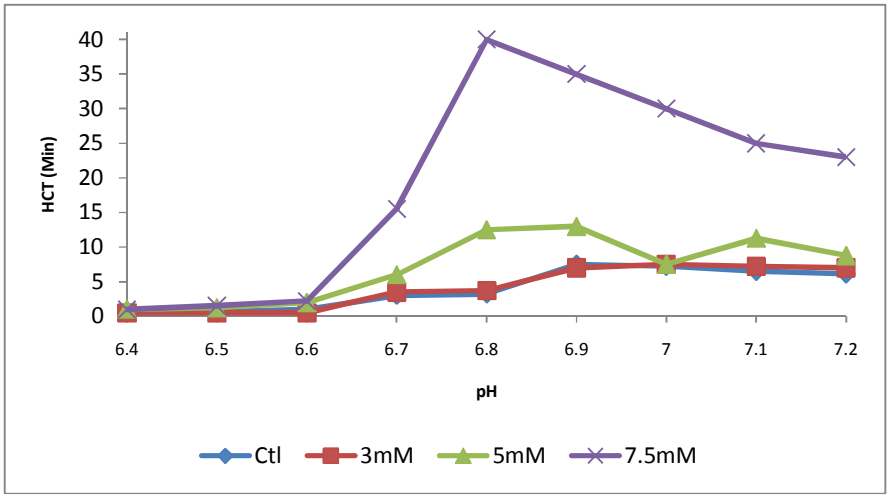

Figure 2: Effect of formaldehyde on heat coagulation time of camel milk at 120°C.

Further increasing of formaldehyde to 10 mM has shown to increase the HCT of camel milk to more than 2h (data not shown).

On the other hand, the addition of both, urea and formaldehyde, at a concentration of 5 mM for each and 5 mM have shown to increase HCT of camel milk to 20 min (Fig 3).

This might be due to the synergistic effect of urea together with formaldehyde. Moreover, increasing the concentrations of both urea and formaldehyde to 10 mM has shown to increase the HCT to more than 2hrs (data not shown). Such a synergistic effect on the heat stability of camel milk could be crucially depending on the concentration of urea and formaldehyde. Also formaldehyde reacts with free amino acids in milk proteins especially of lysine (Metwalli and VanBoekel, 1995) consequently, increases the net negative charges on the molecules leading to more stability. Moreover, Shalabi and Fox, (1982) reported a synergic action of urea and carbonyl compounds on heat stability of milk. The synergic action of urea and formaldehyde can be explained via the increase of net negative charges on protein molecules by formaldehyde postpone the heat coagulation time giving the chance for urea to react with lactose to

form lactosyl urea and increase the buffering action. Al-Saleh & Hammad, (1992) reported that the buffering action of camel milk is lower than that of cow milk. Addition of urea to camel milk may increase its buffering action and improves heat stability (Van Boekel *et al*., 1989; Metwalli *et al.*, 1996b; Singh, 2004). Reaction of urea with lactose yields lactosyl urea from which NH3 is released more slowly increasing the buffering action of milk (Fox and McSweeney, 1998).

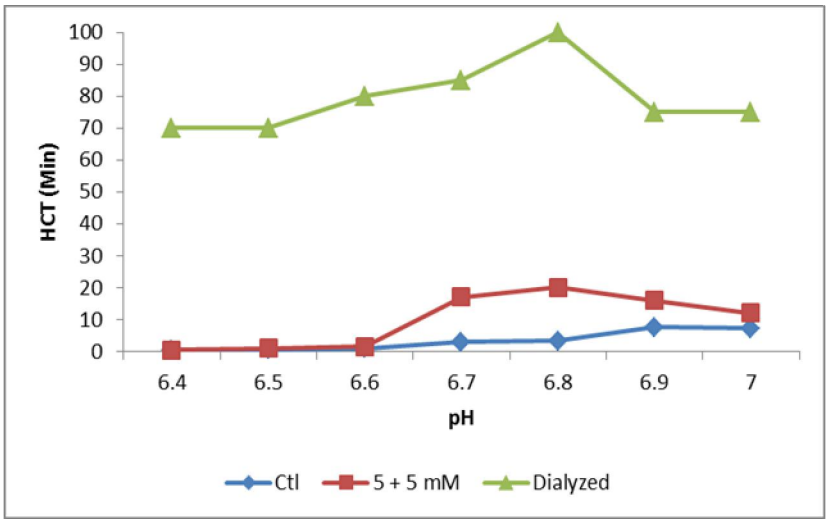

Figure 3: Effect of dialysis of camel milk against J & K buffer as well as formaldehyde and urea mixture on heat coagulation time of camel milk at 120°C.

Effect of serum salt on heat coagulation time (HCT) of camel milk

The main components of camel milk were reported to be relatively similar to that of cow milk (Al haj and Al kanhal, 2010). However, the content of κ- casein in camel milk is less than that of cow milk (Kappeler *et al.,* 2003), while the ash content is higher about 0.79 % (Al haj and Al kanhal, 2010) 0.83% Elamin and Wilcox (1992) and 1.3% Meiloud *et al*., 2011. The calcium concentration in camel milk is about the same as of cow milk 120 mg/100gm milk (Mehaia *et al.,* 1995). The composition of both κ- casein and ash content especially calcium could play a crucial role in heat coagulation of milk proteins (Singh and Fox, 1987; Van Boekel *et al.,* 1989). The effect of serum salt on heat stability of camel milk will be discussed in this part. Results in Fig. 3 show that compared to control, HCT of dialyzed camel milk against Jennes and Koops buffer (1962) was greatly increased with increasing pH until reached 100 min at pH 6.8 and then declined thereafter at 120°C. These findings are in disagreement with those reported by Al-Saleh (1996) who found that dialysis of camel milk against cow milk for three days has no effect on HCT of camel milk on the other hand, decreased HCT of cow milk. These variations could be attributed to the difference between cow milk and J & K buffer composition. To explain the effect of serum salt on heat coagulation time (HCT) of camel milk, it seems that the ratio between κ- casein as stabilizing agent and calcium ion as promoting heat coagulation agent is the crucial factor. κ- casein in camel milk represents 3.47% from total casein (Al haj and Al kanhal, 2010; Kappeler *et al.*, 2003) compared with 13% in cow milk (Davies and Law, 1980) while, the calcium concentration in both milks are the same (120 mg/100mL milk) as reported by Mehaia *et al*., (1995) and Al haj and Al kanhal, (2010). Moreover, during heating high percentage of κ- casein dissociate from casein micelles into milk serum (Van Boekel *et al.,* 1989; Metwalli *et al.* 1996a) and these κ- casein depleted micelles coagulate during short time where calcium ion still high to cause heat coagulation. On-Nom *et al.* (2012) reported that the reconstituted cow milk (9% total solids) do not coagulate on heating if the calcium ion concentration was <0.5 mM and pH was >6.3.

Effect of camel milk whey proteins addition on heat coagulation time (HCT) of camel milk casein suspension

In this experiment camel milk casein of a concentration 2.5% was suspended in J & K buffer (1962), camel milk whey proteins has been added in different concentrations of up to 1%. Heat coagulation time was found to decrease when camel whey proteins concentration was increased within pH studied (6.4-7.2) (Fig. 4).

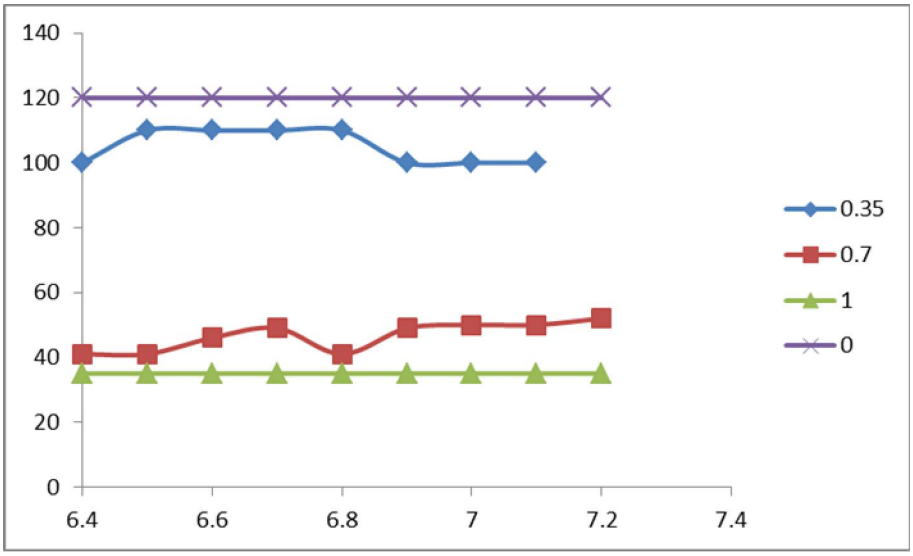

Fig. 4: Effect of camel milk whey proteins addition on heat coagulation time (HCT) of camel milk casein suspension.

Heat coagulation time of samples containing no camel whey proteins were extended to more than 120 min. While, the addition of 0.35% camel whey proteins to casein solution decreased HCT to about 100 min. On the other hand, the HCT greatly decreased to about 45 min when whey protein concentration was increased to up 0.7%. Moreover, HCT shown to decrease to about 34 min when 1% camel whey protein was added. Findings in this experiment have shown that pH values have no pronounced effect on HCT when whey proteins added at different concentrations. This could be attributed to the whey constitutes, where α – lactalbumin is the main whey proteins in camel milk whey; while β-lactoglobulin is the main part in bovine whey. However, the HCT of cow or camel casein suspended in Jennes and koops buffer (1962) behave as type B milk (Van Boekel *et al.,* 1989; Metwalli *et al.,* 1996b). On addition of cow whey proteins into cow casein suspension, the heat coagulation converts from type B to type A milk. In these experiments addition of camel whey proteins into camel casein caused heat instability.

Effect of cow milk acid whey addition on heat coagulation time (HCT) of camel milk casein

In this experiment casein from camel milk (2.5%) was suspended in cow milk acid whey and Jennes and Koops buffer. HCT of camel milk casein which suspended in Jennes and Koops buffer was shown to coagulate after about 120 min. However, HCT of camel milk casein which suspended in acid cow milk whey was coagulated during heating up time in less than 2 min (data not shown). This could be due to the high calcium activity present in acid whey which contains almost total calcium in milk. Obviously, all calcium content in milk was dissolved in cow milk acid whey at pH 5.2 (Metwalli *et al.,*1996a and Patocka *et al.,* 1993**)**. On the other hand, HCT was increased when casein from camel milk (2.5%) was suspended in cow milk rennet whey, compared to that suspended in cow milk acid whey, because rennet whey contains only the soluble calcium, while colloidal calcium retained with the curd.

Conclusion

In general, camel milk exhibited poor heat stability compared to other mammalian such as cow milk. The addition of neither urea at concentration of 10 mM nor formaldehyde at a concentration of up to 5 mM alone has no effect on HCT of camel milk heat stability. However, combining both urea and formaldehyde at a concentration of 5 mM have shown to increase HCT of camel milk due to the synergistic effect between them. Moreover, dialysis of camel milk against J & K buffer has also shown to increase HCT of camel milk consequently improves heat stability. Heat coagulation time of camel casein suspended in Jennes and Koops buffer 1962 is very stable at 120°C and decreased with addition of camel whey proteins.

Acknowledgment

The authors would like to extend their sincere appreciation to the Deanship of Scientific Research at King Saud University for its funding of this research through the Research Group Project no. RGP – VPP – 232.

References

1. Al haj, O. A., and Al Kanhal, H. A. (2010). Compositional, Technological and Nutritional aspects of Dromedary Camel Milk - a Review. *International Dairy Journal*, 20 811-821.
2. Alhaj O. A., Metwalli A. A. M., and Ismail E. A., (2011) Heat Stability of Camel Milk Proteins After Sterilization Process. *Journal of Camel Practice & Research,* 18 277-282.
3. Al-Saleh, A. A. (1996). Heat coagulation of camel milk. Journal of King Saud University, 8, 107-117.

4. Al-Saleh, A. A., & Hammad, Y. A. (1992). Buffering capacity of camel milk. Egyptian
5. Journal of Food Science, 20, 85e97.
6. Davies, D. T., & Law, A. J. R. (1980). Content and composition of protein in creamy
7. milk in South-West Scotland. Journal of Dairy Research, 47, 83-90.
8. Davies, D. T., and White, J. C. D. (1966).The stability of milk proteins to heat. 1. Subjective measurements of heat stability of milk. *Journal of Dairy Research*, 33 67-81.
9. Elamin, F.M and Wilcox C. J. (1992). Milk Composition of Majaheim Camels. Journal of Dairy Science, Vol. 75. 3155-3157
10. Farah, Z., and Atkins, D. (1992).Heat coagulation of camel milk. *Journal of Dairy Research,* 59 229-231.
11. Faye, B. ;Konuspayeva, G. and Loiseau, G. (2010). Variability of urea concentration in camel milk in Kazakhstan. Dairy Sci. Technol. 90, 707-713.
12. Fox, P. F. 1981. Heat-induced changes in milk preceding coagulation. J. Dairy Sci. 64:2127–2137.
13. Fox, P. F., and P. A. Morrissey (1977). Reviews of the progress of dairy science: The heat stability of milk. J. Dairy Res. 44:627–646.
14. Fox, P. F., and P. L. H. McSweeney (1998).Dairy Chemistry and Biochemistry. Blackie Academic & Professional, an imprint of Chapman & Hall, London.
15. Holt, C., D. G. Dalgleish and R. Jenness. (1981). Calculation of the ion equilibria in milk diffusate and comparison with experiment. Anal.Biocem. 113:154-163.
16. Jenness, R., & Koops, J. (1962). Preparation andproperties of a salt solution which simulates milkultrafdtrate. Netherlands Milk Dairy Journal, 16, 153.
17. Kappeler, S., Farah, Z., & Puhan, Z. (2003). 5-Flanking regions of camel milk genes are highly similar to homologue regions of other species and can be divided into two distinct groups. Journal of Dairy Science, 86, 498-508.
18. Mehaia, M. A; Hablas, M. A.; Abdel-Rahman, K. M.Samy and El-Mougy, A. (1995). Milk composition of Majaheim, Wadah and Hamra camels in Saudi Arabia.Vol.52, 115-122.
19. Meiloud, G. M.; Bouraya I.N. O; Samb, A. and HoumeidA. A. (2011) Composition of Mauritanian Camel Milk: Results of First Study. *Int. Journal of Agric. Biol., 13: 145–147.*
20. Metwalli A. A. M. and Van Boekel M. A. J. S. (1995). Effect of formaldehyde on heat stability of milk.Netherlands Milk and Dairy Journal. 49, 177-189.
21. Metwalli, A. A. M.; Metwalli N. H. and Van Boekel M. A. J. S. (1996a).Effect of urea on heat-induced changes in milk. *Netherlands Milk and Dairy Journal* 50,427-457.
22. Metwalli, A. M. and Van Boekel, M. A. J. S. (1996b).Effect of urea on heat coagulation of milk. Netherlands Milk and Dairy Journal 50, 459-476.
23. Muir, D. D. and A. W. M. Sweetsur (1977).Effect of urea on the heat coagulation of the caseinate complex in skim-milk. Journal of Dairy Research, 44: 249-257
24. On-Nom N., Grandison A. S., and Lewis M. J. (2012) Heat stability of milk supplemented with calcium chloride. Journal of Dairy Science 95 :1623–1631
25. Patocka,G., Jelen P. and KalabM. (1993) Thermostability of skimmilk with modified casein/whey protein content. International Dairy Journal 3, 35-48
26. Rose, D. (1961) Factors affecting the pH-sensitivity of the heat stability of milk from individual cows. Journal of Dairy Science 44 1405–1413.
27. Rose, D. (1963) Heat stability of bovine milk: a review. Dairy Science Abstracts 25 45–52.
28. Shalabi, S. I. and. Fox, P. F. (1982). Heat stability of milk: synergic action of urea and carbonyl compounds. Journal of Dairy Research. 49, 197-207.
29. Sievanen, K., T. Huppertz, A. L. Kelly, and P. F. Fox. 2008. Influence of added calcium chloride on the heat stability of unconcentrated and concentrated bovine milk. Int. J. Dairy Technol. 61:151–155.
30. Sikand, V., P. S. Tong, and J. Walker. 2010. Heat stability of reconstituted, protein-standardized skim milk powders. Journal of. Dairy Science. 93 :5561–5571.
31. Silanikove, N., F. Shapiro and A. Shamay. 2003. Use of an ionselective electrode to determine free Ca ion concentration in the milk of various mammals. J. Dairy Res. 70:241-243.
32. Singh, H. and Fox, P. F. (1987). Heat stability of milk: role of β-lactoglobulin in the pH-dependent dissociation of micellar κ-casein. Journal of Dairy Research 54, 509–521.
33. Singh, H. and Fox, P. F. (1985) Heat stability of milk: the mechanism of stabilisation by formaldehyde. Journal of Dairy Research 52, 65–76.
34. Singh, H. (2004) Heat stability of milk. International Journal of Dairy Technology, vol. 57, 111-119.
35. Tanaka, M. Suzuki, Kotb1, S and Kamiya, Y. (2011). Relation of Calcium Activity in Milk and Milk Production of Holstein Cows in Hot Season. Asian-Aust. J. Anim. Sci.Vol. 24, No. 10 : 1372 – 1376.
36. Tsioulpas, A., A. Koliandris, A. S. Grandison, and M. J. Lewis. 2010. Effects of stabiliser addition and in-container sterilisation on selected properties of milk related to casein micelle stability. Food Chem. 122:1027–1034.
37. Van Boekel, M.A.J.S.; Nieuwenhuijse, J.A.; Walstra, P. (1989). The heat coagulation of milk. 1. Mechanisms. Netherlands Milk and Dairy Journal, 43, 97 – 127.
38. Walstra. P. & Jenness, R., 1984. Dairy Chemistry and Physics; John Wiley & Sons: New York.