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RESEARCH ARTICLE

EFFECT OF CALCIUM CHLORIDE SALT ON THE CURDLING OF CROSS BREED COW MILK- A CASE REPORT

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ABSTRACT

A case of cross breed cow milk having problem of curdling of milk on incubation was reported in the polyclinics of college of Veterinary Science and Animal Husbandry, U. P. Pt. Deen Dayal Upadhyay Veterinary University and Go Anusandhan Sansthan,Mathura, U. P. India and milk samples were send to the Department of Livestock Products Technology of the same university. The chemical composition of milk was normal and the health status of cow was also normal. The case was suspected for lack of calcium contents. So the three treatments were given to the milk during traditional routine curdling process. The samples in which calcium chloride was added gave normal curd whereas; other treatments showed the problem of curdling and no curd was formed in such treatments. It indicates that calcium is having significant role in the curd formation.

Key words: Cross breed cow, lactose, calcium, curdling, milk

INTRODUCTION

India is having largest dairy herd population in the world and ranks first in production of milk and dairy products. However, demand of these products is still higher than the production due to massive increase in human population (Sachan and Singh, 2010). Most of the milk produce in India is mainly consumed as liquid milk (46%) and the rest is processed into various milk products such as butter, yogurt, milk powder, etc. Among these products Yogurt is consume (7.0%) of the total milk production in India (India's Milk Products Mix-2009). Indian curd, known as dahi, is well-known fermented milk product consumed by large sections of the population throughout country, either as a part of the daily diet or as a refreshing beverage (Caballero *et al*., 2010). Curd increases the food and nutritive value as comparable to the original milk. It is more palatable, easily digestible than milk and exerts a therapeutic value in stomach and intestinal disorder due to its antibiotics content. There are various constituents of milk like fat, proteins and other constituents have significant effect on curd formation and qualities (Everard *et al*., 2011). Numerous heat induced changes in milk play an important role in the heat-induced coagulation of milk, most notably heat-induced reduction in pH, heat-induced denaturation of whey proteins and their subsequent association with casein micelles, heat-induced precipitation of calcium phosphate onto the casein micelles and heat-induced dissociation of κ-casein from the micelle. Extensive reviews of heat induced changes in milk and their influence on heat stability of unconcentrated milk is described by O'Connell and Fox (2003) and Singh and Creamer (1992). The heat stability of concentrated milk is comprehensively reviewed by Singh (2004). After heat treatment, the milk base is cooled to the incubation temperature used for growth of the starter culture. An optimum temperature of the thermophilic lactic acid bacteria, i.e., *Streptococcus* subsp. *thermophilus* and *Lactobacillus delbrueckii* subsp. *bulgaricus*, is around 40- 45°C. Bacterial fermentation converts lactose into lactic acid, which reduces the pH of milk.

During acidification of milk, the pH decreases from 6.7 to ≤4.6. Gelation occurs at pH 5.2 to 5.4 for milk that was given a high heat treatment (Lee and Lucey, 2010). Physical properties and microstructure of yogurt are influenced by incubation temperature. The use of high incubation temperature resulted in a decrease in gelation time and G′ values at pH 4.6, and an increase in LTmax, *B*, and whey separation compared with yogurt gels incubated at low temperature (Lee and Lucey, 2003, 2004). This result indicates that gels formed at high temperature are weak and have a coarse gel network due to extensive rearrangement resulting in the formation of large pores and greater whey separation (Lucey, 2004). During the formation of yogurt gels at a low incubation temperature, slow protein aggregation occurs resulting in the formation of a large number of protein-protein bonds and less rearrangement of the particles/clusters. A highly cross-linked and branched protein network that had small pores was observed in micrographs of

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yogurt gels incubated at low temperature (Lee and Lucey, 2003, 2004). At lower incubation temperature, there is an increase in the voluminosity of casein particles, which results in an increase in the area of the junctions between aggregated casein particles. Increased contact area between casein particles could contribute to the increased stiffness of gels observed at low temperature (Walstra, 1998). Higher viscosity was observed in stirred yogurts that had been incubated at lower temperatures (e.g. <40°C) compared to gels incubated at high temperature (e.g. >40°C) (Beal *et al*., 1999; Martin *et al*., 1999; Sodini *et al*., 2004; Lee and Lucey, 2006). As incubation temperature increased, there was a decrease in the sensory attributes such as mouth coating and smoothness of stirred yogurts (Cho-Ah-Ying *et al*., 1990; Martin *et al*., 1999). Recently, a novel two-stage incubation temperature method was proposed. Peng *et al*., (2010) reported that if incubation temperature was changed after gelation, the textural properties of yogurt became similar to those of yogurts made at that new temperature for the entire fermentation process. It may be possible to use high incubation temperature for the initial stage of fermentation to facilitate rapid growth of the starter cultures and then slowly reduce the incubation temperature at some stage to achieve better textural properties.

Only 25 % of the calcium and 44% of the phosphorus are in a soluble form whereas the total amount of the other major mineral constituents is in insoluble form. The calcium and magnesium in the insoluble form is in chemical or physical combination with caseinate, phosphate and citrate (Mwaura and Akinsoyinu, 2010). With an increasing addition of CaCl2, the time of the start of coagulation is getting shorter. The time of the start of coagulation $t0$ was the shortest for the CaCl2 dose of 7% volume, i.e. by 72% in relation to time $t0$ for the dose of calcium chloride of 2% volume. Also decreasing in the time constant of congealation τ (Fig. 3) – by 73%. Landfeld *et al*., (2002) also reported that the process of coagulation starts and ends in a faster way with the increasing additions of calcium chloride, while the final limit value of the gel rigidity is not influenced practically. Between pH 5 and 8, there is no significant difference ($p<0.05$) between milk clotting time of goat and cow milk under the actions of kid and calve rennet.

In the presence of milk added CaCl2.2H2O (0 – 20 mM), the milk clotting time varies significantly ($p<0.05$) according to goat or cow milk. Between 20 – 30 mM CaCl2.2H2O added milk, Ca2+ ions delays milk clotting time. During milk pH modification, the main phenomena should be casein demineralisation followed by its hydrolysing (O'Connel and Fox, 2000). The increasing of calcium concentration in goat or cow milk results in milk clotting time decreasing when using either kid or calve rennet. During milk clotting, calcium ions makes bridges between micelles of para casein indicating that, the more there are calcium ions (0 – 20 mM) in milk, the more there will be linkage and the faster will be milk clotting. At concentrations higher than 20 mM added CaCl2.2H2O, Ca2+ inhibits milk clotting network. When using kid or calve rennet, milk clotting time decreases faster when using cow milk than goat milk. Goat milk micelles are bigger than cow milk micelles. Milk clotting time due to chymosine depends on micelles size (Ekstrand *et al*., 1980; Omar, 1985; O'Connel and Fox, 2000); it decreases while micelles size increases. For the same volume, there are more bridges between para casein micelle in cow milk than in goat milk. Thus to solve the problem of curdling of milk various combinations were tried and ultimately the combinations has been found to solve the problem.

Case History

Sri Virendra Singh, resident of Mathura, U. P., India, an owner of individual cow reported that his cross bred cow running in third lactation and having 5 years of age. The health status of animal was good and production of milk was normal. Animal was free from infections in the udder in particular free from mastitis and any other disease of the udder. Feeding of animal was with proper amount of feed and mineral regularly to the cross bred cow. The problem was the non-clotting of milk and non formation of curd/dahi from the milk of same animal by the conventional curd making method. The problem of curdling of milk from this animal was since first lactation.

METHODS

One litre freshly let down milk of cross breed cow was boiled for 15 minute and then cooled at 30^0c. The whole boiled milk was divided in to four different groups in equal amount (250ml each). The first group was considered as control and that was treated only with starter culture @ 2% of the milk sample and incubated at 37^0c for 16 hours. The second group was treated with the calcium chloride salt i.e. 500mg in 250 ml of milk along with starter culture @ 2 % of the milk sample and incubated at 37^0c for 16 hours. The third group was treated with the lactose i.e. 500mg in 250 ml of milk and starter culture @ 2 % of the milk sample and incubated at 37^0c for 16 hours. The fourth group was treated with the lactose plus calcium chloride i.e. 500mg each calcium chloride and lactose both in 250 ml of milk and starter culture @ 2 % of the milk sample and incubated at 37^0c for 16 hours.

RESULTS AND DISCUSSION

On the physico-chemical analysis of cross bred cow milk we found 3.5% fat, 4.95 lactose, 3.93% protein and 9.7% SNF. pH of milk was 5.96 with the specific gravity of 1.026 mg/ml. the colour and appearance of milk was normal yellowish white with the pleasant sweet taste. After incubations period of 16 hours for different groups in curd/ dahi making process we found that the only two groups second (calcium chloride added) and fourth (treated with both calcium chloride and lactose) showed curdling. Whereas, there was no curd formation as usual in the rest groups (control and lactose treated). So the results indicated that addition of calcium chloride facilitates the curdling. It also indicated that the milk sample was deficient in calcium content which plays an important role in milk coagulation as in the cheese formation. However, in sample number first and third only fermented smell (sour) was noticed during the curd formation. There were three trials conducted on each group of curd and the average values obtained is depicted in Table1. The fat and protein contents were almost similar in all treated group as compared to the control. The content of lactose was same in the control and calcium chloride treated group while the concentration of lactose was

Table 1: Physico-chemical composition of milk/curd of control, treated with calcium, lactose and calcium with lactose

Group	Fat %	Protein %	Lactose %	SNF %	Specific gravity (mg/ml)	pH
Control	3.1	4.0	4.6	10.2	1.0267	3.88
$Cacl_2$ treated	2.9	4.2	4.4	12.3	1.0386	3.55
Lactose treated	3.0	4.2	5.7	11.4	1.0327	3.41
$Cacl_2$ + lactose	3.3	3.8	5.5	16.5	1.0366	3.32

higher in a group treated with calcium plus lactose as compared to control and calcium treated group. It may be due to the external addition of lactose in these groups. The solid not fat (SNF) content was higher in the treated groups as compared to control that may be due to increase in solid content through addition of $Cacl_2$, lactose, in the groups. The specific gravity of treated group was higher in comparison to the control. The higher vale of specific might be due to addition of calcium chloride and lactose. The pH of control and calcium chloride treated group is more as comparison to the lactose and calcium chloride plus lactose treated group. It may be due lactose fermentation because these groups were treated with lactose and it forms lactic acid after fermentation.

Bencini, 2002 reported that addition of calcium chloride did not affect the clotting properties of sheep milk but in cows' milk it decreased renneting time and rate of firming and increased curd consistency. Similar report was also published by Mc Mahon *et al*., 1984 they concluded that coagulation time initially decreased upon calcium addition and reached a minimum at .05 *M* calcium. At high calcium (.4 *M*), coagulation time was severely retarded. Curd firming rate increased and reached a maximum at .01 *M* calcium. At higher calcium curd firmness was reduced. The role of calcium is also justified by the Balcones *et al*., 1996, Lucey and Fox, 1993, Montilla *et al*., 1995; they defined that Ca play an indirect role in primary hydrolysis of k-casein by reducing pH, which can enhance the activity of enzyme. Furthermore, addition of calcium can reduce the degree of k-casein hydrolysis required for the onset of coagulation and increase the rate of development of firmness of the coagulum through neutralisation of the negative change and the formation of calcium bridges; however the rate of gel firming is reduce when excessive concentrations of Ca ($\geq$ 0.3m) is used (Mc Mahon *et al*., 1984; Tamime *et al*., 2007).

The addition of $Cacl_2$ also decreased pH of milk, resulting in increased protein aggregation (Gastaldi *et al*., 1994). Fagan *et al*., 2007 suggested that temperature and calcium chloride addition level affect the light backscatter profile during coagulation whereas the light backscatter profile during syneresis was a function of temperature and cutting time. Shattered, mealy curd, sediment on the bottom of the vat, and slow acid production were associated with agglutinating strains of mixed cultures (Lee *et al*., 2011). Milk proteins are widely used as ingredients in prepared foods, in which they perform a wide range of key functions, including emulsification, thickening, gelling and foaming. An important functionality of milk proteins in food colloids is their ability to facilitate the formation and stabilisation of oil droplets in emulsions (Singh, 2011).

Conclusions

It is concluded that contents of calcium is necessary for the curdling of milk. In the study we revealed that without proper contents of calcium salt curd formation may be difficult. The other factors required for proper curd formation is also equally important.

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