INFLUENCE OF PASTEURIZATION TEMPERATURE, pH, CaCl₂ AND BLENDING OF BUFFALO MILK ON THE RENNET COAGULATION TIME (RCT), YIELD AND TEXTURE OF CAMEL MILK CHEESE

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Camel milk coagulation for the production of cheese with higher yield and good texture is a complex process as compared to cow and buffalo’s milk. Objective of current investigation was to study the combined impact of pH (5.3, 5.5 and 5.7), CaCl₂ (0.04, 0.06 and 0.08%), pasteurization temperature (60, 65 and 70°C) and addition of buffalo milk (0, 10 and 20%) on the rennet coagulation time (RCT), yield and texture of camel milk cheese. The effects of all these parameters were interpreted by response surface methodology (RSM system). The temperature and pH have pronounced effect on coagulation time as compared to CaCl₂, while the interaction of temperature with pH and CaCl₂ showed the significant effect on yield. The firm texture was obtained as the concentration of the buffalo milk increased at pH 5.5 and 65°C temperature.

Keywords: κ-casein, total solids contents, gel formation, processing parameters.

INTRODUCTION

Long-time requirement for rennet coagulation (RCT) is the major obstacle in manufacturing of cheese from camel milk which result in lower yield and textural defects of end cheese (Farah, 1996). Coagulation time of camel milk (Ramet, 2001) as well as bovine milk cheese yield (Daviau et al., 2000) can be altered by manipulating some parameters like temperature of pasteurization, pH and CaCl₂ concentration during cheese manufacturing. The impacts of these parameters have been widely studied for cow and buffalo milk, but a little work has been conducted for camel milk. Castillo et al. (2000); Daviau et al. (2000) have revealed the influence of individual factors on coagulation time and yield of camel milk; hence the combined effect of these parameters is not clearly elaborated. Milk coagulation is (Gunasekaran and Ay, 1996) highly influenced by pasteurization temperature which greatly affect the protein denaturation, aggregation and gel formation rates (Al haj et al., 2011). Most of the published material mainly focused on the coagulation of milk with heat (Laporte et al., 1998; Daviau et al., 2000; Lucey et al., 2000) but not on the effect of pasteurization temperature on RCT and finally recovered yield.

The pH is another influencing factor of coagulation time and yield. The reduction of pH from 6.5 to 5.2 decreases the RCT (Farah, 1996; Bai and Zhao, 2015). It is reported that κ-casein optimally hydrolyse (van Hooydonk et al., 1987; Bai and Zhao, 2015) and micellar calcium phosphate solubilize when there is lowering of pH of the milk, which ultimately (Kherouatou et al., 2003) reduces the casein molecule net charge and dissociation of casein from the micelles (Gastaldi et al., 1994; van Hooydonk et al., 1987). Lower concentration of κ-casein and larger size of casein micelle and other structural differences (Ramet, 2001; Kappeler et al., 2003; Farah and Fisher, 2004; Al haj and Al Kanhal, 2010) of camel milk casein as compared to other dairy animal’s milk could be responsible for the longer RCT with lower yield. By increasing the concentration of calcium and pH lowering, resulted improved firmness of curd of camel milk cheese (Ramet, 2001; Daviau et al., 2000). Yet, the more effective coagulation was observed in camel milk at pH 5.5 as compared to bovine milk (Farah and Fisher, 2004).

Addition of CaCl₂ to milk as an effective tool to decrease the RCT with the improvement of camel milk yield and texture during cheese manufacturing has been studied by various scientists (Farah and Bachmann, 1987; Lucey and Fox, 1993; Balcones et al., 1996; Ramet, 2001; Mahaia, 2006). The added CaCl₂ lowers the milk pH and promotes the aggregation of protein (Mahaia, 2006; van Hooydonk et al., 1987; Gastaldi et al., 1994). However, it also was reported that the higher doses of CaCl₂ produced firm curd from camel milk but bitter in taste (Farah and Bachmann, 1987; Balcones et al., 1996; El-Zubeir and Jabreel, 2008).

The lower level of total solids in Camel’s milk also effect coagulation time negatively as well as yield and texture. To overcome this problem blending of other animal’s milk which contains higher total solids may be the trick to solve this problem. The addition of buffalo milk possibly will result in the decrease of coagulation time and higher yield being owing to its higher solid contents. This improvement associated with the level of mixing of both milk (Inayat et al., 2003; Mahaia, 2006; Shahein et al., 2014; Brezoveckí et al., 2015).
Considering the issue of longer RCT and lower yield with poor texture, the study was conducted to evaluate the combined effect of CaCl₂, pH, pasteurization temperature and blending of camel milk with buffalo milk.

MATERIALS AND METHODS

Whole fresh buffalo and camel milk were procured from a private farm near Faisalabad city of Pakistan. Pre-sterilized airtight glass bottles were used to bring the milk samples within one hour after morning milking and kept in refrigerator before the production of cheese. The production of cheese and its characterization were carried out in the Laboratories of National Institute of Food Science and Technology (NIFSAT), University of Agriculture, Faisalabad-Pakistan. Camel chymosin (Powder form, FAR-M® Sticks, Material no. 147028) was supplied by the CHR Hansen, Denmark on request. Thermophilic (Lactobacillus therophilus and Lactobacillus bulgaricus) starter cultures were used in the cheese production obtained from SACCO (Lyofast Y 082 D). All the chemicals used in the study were from Sigma-Aldrich (St. Louis, MO, USA) and Fisher scientific (CHEMTREC®, USA). The Food grade anhydrous CaCl₂ with 96% purity (Muby Chemicals, India) was used. The camel and buffalo milk were analysed for fat (no. 2000.28) and milk solids not fat before using in cheese production following the methods of AOAC (2012). Camel milk cheese was manufactured (in triplicate) according to the method as described by Ong et al. (2015) with some modifications. Preparation of camel milk for the production of cheese was carried out stepwise (Fig. 1).

RESULTS AND DISCUSSION

The variables such as temperature of pasteurization (T), addition of CaCl₂ (C), reduction in pH (P) and blending of camel milk with buffalo milk (B) have significant (p<0.01) effect on the RCT, yield and texture of camel milk during manufacturing of cheese (Table 1). It is obvious from the results that all interactive effect among variables showed the significant (p<0.01) influence on RCT except the CxB. The interactions, CxB, BxT, PxCrX&B and PxBxT effected the both yield and texture non-significantly (p>0.05), but CxBxT effected (0>0.05) only the texture. The PxT, CxT, PxCrBxT interactions showed the significant (p<0.01) influence on yield and texture. The other interactions which showed the significant impact on the texture were the PxB and PxT. The Figure 2a-f represent the interactive effect of two variables for RCT. From the interaction (Fig. 2a) of pH and CaCl₂ it is evident that at 0.04% and 0.08% CaCl₂ concentrations; the response of coagulation time was almost similar compared to 0.06% CaCl₂. At each concentration of CaCl₂ there was relatively lower RCT for pH 5.5, followed by pH 5.3 and 5.7 respectively. The similar trend was obtained in the interaction of pH (Fig. 2b) with buffalo milk blend. The interaction of temperature with pH, CaCl₂ and buffalo milk blend as mentioned in Figures 2c, e and f illustrated the increase in RCT of camel milk (80-100 min) when pasteurized at 70°C temperature at all levels of pH, CaCl₂ and blending of buffalo milk.
Table 1. Mean squares for coagulation time, yield and texture for camel milk cheese.

<table>
<thead>
<tr>
<th>Source of variation</th>
<th>Coagulation time</th>
<th>Yield</th>
<th>Texture</th>
</tr>
</thead>
<tbody>
<tr>
<td>pH (P)</td>
<td>310.4**</td>
<td>1848.38**</td>
<td>5.21**</td>
</tr>
<tr>
<td>CaCl₂ (C)</td>
<td>232.6**</td>
<td>164.01**</td>
<td>1.90**</td>
</tr>
<tr>
<td>Blend (B)</td>
<td>285.3**</td>
<td>106.55**</td>
<td>0.92**</td>
</tr>
<tr>
<td>Temp (T)</td>
<td>71079.8**</td>
<td>227.87**</td>
<td>16.33**</td>
</tr>
<tr>
<td>P x C</td>
<td>65.7**</td>
<td>20.81**</td>
<td>0.06**</td>
</tr>
<tr>
<td>P x B</td>
<td>19.1**</td>
<td>0.91 NS</td>
<td>0.03**</td>
</tr>
<tr>
<td>P x T</td>
<td>140.6**</td>
<td>2.13*</td>
<td>0.06**</td>
</tr>
<tr>
<td>C x B</td>
<td>1.8 NS</td>
<td>1.37 NS</td>
<td>0.06 NS</td>
</tr>
<tr>
<td>C x T</td>
<td>43.3**</td>
<td>10.16**</td>
<td>0.08**</td>
</tr>
<tr>
<td>B x T</td>
<td>6.8**</td>
<td>1.22 NS</td>
<td>0.06 NS</td>
</tr>
<tr>
<td>P x C x B</td>
<td>7.3**</td>
<td>1.18 NS</td>
<td>0.008 NS</td>
</tr>
<tr>
<td>P x C x T</td>
<td>51.3**</td>
<td>5.89**</td>
<td>0.05**</td>
</tr>
<tr>
<td>P x B x T</td>
<td>3.9**</td>
<td>0.74 NS</td>
<td>0.06 NS</td>
</tr>
<tr>
<td>C x B x T</td>
<td>6.3**</td>
<td>1.50 NS</td>
<td>0.06 NS</td>
</tr>
<tr>
<td>P x C x B x T</td>
<td>9.5**</td>
<td>2.34**</td>
<td>0.06**</td>
</tr>
</tbody>
</table>

NS = Non-significant (P>0.05); * = Significant (P<0.05); ** = Highly significant (P<0.01)

Figure 2. Effect of two-two parameters on coagulation time.

The graph clearly shows that the coagulation time tremendously reduced at both 60 and 65°C temperature of pasteurization. The interaction of CaCl₂ with blending of buffalo milk (Fig. 2d) showed the small decrease in RCT with increase in the concentration of buffalo milk and CaCl₂. Cumulative effect of all parameters (Fig. 3) also illustrated the negative impact on rennet coagulation time at 70°C than 60 and 65°C; however, 65°C proved better than 60°C. It is observed from the results that coagulation time of camel milk adversely prolonged with increase in the temperature. These findings of the present work are supported by the studies of various scientists (Farah and Ruegg, 1989; Farah and Atkins, 1992; O’Connell and Fox, 2000; Al haj and Al Kanhal, 2010) who reported that the camel milk has low heat stability than cow and buffalo milk. The difference in casein micelles size of camel milk (200-500 nm) with cow’s milks (220-300 nm) could be the other reason to low heat stability (Van Hooydonk, 1986; Al-Saleh, 1996). The higher the size of casein micelle, the lower is the concentration of k-casein in the camel milk to protect the micelles (Al haj et al., 2011).

Figure 3. Cumulative effects of parameters (T, C, pH, B) on coagulation time.

The interactive effect of 3 levels of CaCl₂ represented in Figure 3 a-f, showed almost the same response at all levels with camel milk while addition of buffalo milk with the change in pH alter coagulation time. Addition of 0.08% CaCl₂ with 20% buffalo milk to camel milk obtained the least coagulation time. Soodam et al. (2015) noted the similar type of observations with pH and CaCl₂ interaction and recommended that the addition of calcium lowers the drainage pH which is effective to lower coagulation time during manufacturing of cow’s milk cheddar cheese and found most effective pH was 5.5. Attia et al. (2000) and Kherouatou et al. (2003) reported the similar findings. They recommended that at pH 5.5, the camel milk integrity is maintained while lower pH resulted in transitional biochemical modifications which effected coagulation time and coagulum texture. Farah and Atkins (1992) also noted the same observation, while Daviau et al. (2000) correlated the CaCl₂ effect with pH and their combined impact on gel firmness. Na’jera et al. (2003) studied the impact of ripening temperature on RCT instead of pasteurization temperature. They reported the shorter RCT at 44°C than at 28°C. Their finding contradicts the present finding, because they reported the lower RCT at high pH (6.8) with 18 mM of CaCl₂.

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The camel milk blend with buffalo milk improves the coagulation time (Fig. 2 and 3), regardless of the other factors. This finding is supported by the results of Eyassu et al. (2007); Shahein et al. (2014). They all found that an increase in total soluble solid contents of camel milk reduces the camel milk coagulation time by mixing the milk of other species. From the result, it is obvious that camel milk coagulation time was highly influenced by all the two factors interactions except the combined effect of C×B and (pH× C× T×B). The results of Castillo et al. (2000) are partially close to the present work. They concluded that gel firming rate in goat’s milk clotting is significantly influenced by the interaction of pH and coagulation temperature. It is an important parameter regarding the profitability of cheese industry. Higher the percentage of solids in milk, greater is the amount of cheese obtained and ultimately more gains in economic terms (Fox and McSweeny 1998). The influence of interaction between two variables for yield is depicted in Figure 4 a-f. The interaction of pH (5.3, 5.5 and 5.7) with different concentrations of CaCl₂ (0.04, 0.06 and 0.08%) present in Figure 4.3a indicates a very low yield at pH 5.3. The cheese yield was high at 5.5pH with three concentrations of CaCl₂ hence maximum was noted at 0.08% CaCl₂. While the yield of cheese was affected non-significantly by the interaction of pH and blending of buffalo milk. As the concentration of buffalo milk increased, the yield also increased at all pH values. The interaction of temperature with pH and CaCl₂ showed a significant effect on yield (Fig. 4c and e) while non-significant with blend (Fig. 4f). The interaction of blend with CaCl₂ present in Figure 4d also showed the increase in yield with the addition of buffalo milk relatively high at 0.08% CaCl₂. The lower yield was recorded at 70°C while relatively higher yield was estimated at 65°C.

The cumulative effects of all parameters for yield presented in Figure 5 illustrated that pH 5.3 had least effect regarding the yield as compared to pH 5.5 and 5.7 in all interactions of CaCl₂, temperature and buffalo milk blend. The highest yield was recorded at 65°C temperature, 0.08% CaCl₂ and pH 5.5. Regarding the addition of buffalo milk, it is visible in Figure 4, 5 and 7 that with the increase in concentration of buffalo milk there was increase in yield.

It is clear from the present findings that reduction in RCT and higher yield with improved texture was achieved at 5.5 pH. This aspect was also studied by Attia et al. (2000) and Kherouatou et al. (2003). They related this aspect with Dromedary camel milk’s micelle which seemed to maintain its integrity until about pH 5.5, below that they undergo through biochemical and structural modifications which resulted in the loose microstructure, micelle hydration and apparent viscosity. The pH 5.0 would be a transition pH between micelle structure and coagulum structure. Similar observation about camel milk was also noted by Farah and Atkins (1992). The finding of Najera et al. (2003) also depicted that 5.0 was the optimum pH for the enzymatic hydrolysis phase which affect the coagulation time. Camel milk is known for its stronger buffering capacity compared to bovine milk, therefore, buffering capacity of milk may influence many of its physico-chemical properties (Bai and Zhao, 2015).

Cheese texture is one of the basic signal (along with food appearance) encountered by consumers during food eating. Furthermore, it is a well-known food characteristic that
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Influence preference or acceptability of products (Hicsasmaz et al., 2000). The graphical manipulation of interaction between two variables for texture is provided in Figure 6a-f. The interactive effect of pH and CaCl$_2$ (Fig. 5a) showed that increase in the concentration of CaCl$_2$ improves the texture at every pH (5.3, 5.5, 5.7); however, more firm texture was observed at pH 5.5 and 0.08% CaCl$_2$. A similar trend was observed in the interaction of pH with blend (Fig. 6b). The firmness of texture was elevated as the concentration of the buffalo milk increased hence highest firmness was obtained at 20% blend and pH 5.5. The interaction of temperature with pH and CaCl$_2$ depicted (Fig. 6c and e, respectively) that firmer texture was obtained with increasing the temperature from 60 to 65°C regardless of pH and CaCl$_2$ concentration. The collective effect of all parameters for texture describe that high pasteurization temperature (70°C) is not in favour of firm texture for camel milk cheese while high concentration of CaCl$_2$ and buffalo milk improve the texture at 5.5 pH.

The results of Daviau et al. (2000) are similar to the present study, they concluded that gel firmness of fermented camel milk was affected by Calcium strength which was correlated by pH. Na’jera et al. (2003) concluded that high temperature (44°C) produced faster gel firming rate than low temperature (28°C) regardless of pH, however effect of CaCl$_2$ correlated with pH. Moreover, their multi-factorial study indicated that coagulation time was most affected by pH and CaCl$_2$, while curd firmness was influenced by temperature.

![Interaction Plot for Texture Data Means](image)

Figure 6. Effect of two-two parameters on texture (N).

Weak and fragile curd with low yield is an additional feature in camel milk cheese production which is likely be due to the low total solids content of the coagulum, especially low in casein contents (Ramet, 2001; El-Zubeir and Jabreel, 2008). The rate of rennet coagulation as well and yield and texture are also affected by concentration of calcium and pH. In present work the least RCT, high yield and firmer texture was observed at 0.08% CaCl$_2$ with addition of 20% buffalo milk to the camel milk. These findings are related with the work of Soodam et al. (2015) and Ong et al. (2015). They used the CaCl$_2$ and pH parameters to lower the RCT in cheddar cheese manufacturing from cow’s milk. These results were also in conformity with the findings of Castillo et al. (2000) who evaluated the relationship between pH and coagulation temperature for gel firmness rate in the clotting of goat milk.

![Figure 7. Cumulative effects of parameters (T, C, pH, B) on texture (N).](image)

Conclusion: For the manufacturing of cheese, camel milk should be first pasteurized at 65°C, then lower the pH to 5.5 and add the CaCl$_2$ 0.06% which would result in lowering the camel milk coagulation time from 5 hours to one hour. Moreover, addition of 20% buffalo milk can further reduce the coagulation time with camel milk to 50 min with the improved yield and good texture.

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