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INFLUNCE OF PASTEURIZATION TEMPERATURE, pH, $CaCl_2$ 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), $CaCl_2$ (0.04, 0.06 and 0.08%), pasteurization temperatures (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 $CaCl_2$, while the interaction of temperature with pH and $CaCl_2$ 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 $CaCl_2$ 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 $CaCl_2$ 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 $CaCl_2$ lowers the milk pH and promotes the aggregation of protein (Mehaia, 2006; van Hooydonk *et al.*, 1987; Gastaldi *et al.*, 1994). However, it also was reported that the higher doses of $CaCl_2$ 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; Mehaia, 2006; Shahein *et al.*, 2014; Brezovecki *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 $CaCl_2$, 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 thermophilus* and *Lactobaccilus 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 $CaCl_2$ 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).

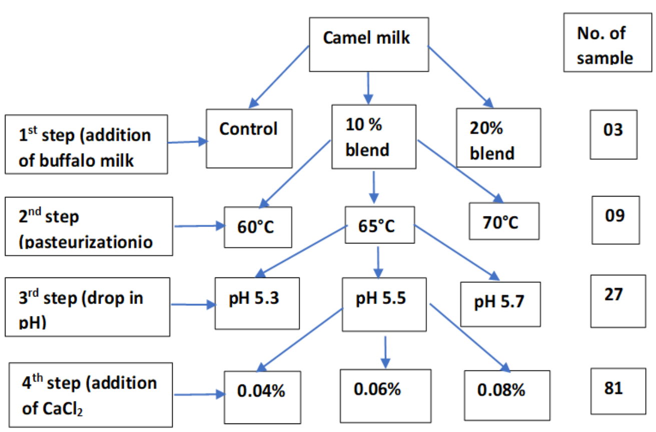

Figure 1. Treatments plan for the camel cheese manufacturing.

In 1st step, buffalo milk was added at 0 (control), 10 and 20 % to camel milk (blends). In 2nd step, control and blend milk were pasteurized at 60, 65 and 70°C for 30 min. In 3rd step, pH of each milk samples was dropped to 5.3, 5.5, and 5.7 after cooling to inoculation temperature (37°C) by the drop wise addition of citric acid (10%). In 4th step, $CaCl_2$ (0.04, 0.06 and 0.08%) was added in all pasteurized and acidified milk samples before the production of cheese. After the addition of starter cultures and rennet, the coagulation time was noticed after every ten minutes till the curd form and start to leave the wall of the beaker (visual examination). The curd was cut into 1.0 cm^3 with a wire cutter and scalding was carried out for 10 min at 42°C before the removal of whey. As mentioned in Figure 1, 81 different samples of cheese were produced on laboratory scale in 500 mL beakers to investigate the combined effect of buffalo milk addition, change in pH, pasteurization temperature and CaCl2 on the RCT, texture and yield. Cheese samples (1 cm in thickness and 5 cm dia) from each treatment were sealed in plastic bags and stored in bags at 8°C for twelve hours before the determination of texture hardness using Stable Micro System Texture analyser TA.XT (Guinee *et al.*, 2000).

Cheese yield was calculated after drainage following the equation as given below

$$\text{Yield (\%)} = \frac{\text{Cheese weight (kg)}}{\text{Milk weight (kg)}} \times 100$$

Data was statistically evaluated by using analysis of variance (ANOVA) at 95% level of significance and combined interactions were studied using response surface methodology (Minitab software).

RESULTS AND DISCUSSION

The variables such as temperature of pasteurization (T), addition of $CaCl_2$ (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 C×B. The interactions, C×B, B×T, P×C×B and P×B×T effected the both yield and texture non-significantly ($p>0.05$), but C×B×T effected (0>0.05) only the texture. The P×T, C×T, P×C×B×T 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 P×B and P×T.

The Figure 2a-f represent the interactive effect of two variables for RCT. From the interaction (Fig. 2a) of pH and $CaCl_2$, it is evident that at 0.04% and 0.08% $CaCl_2$ concentrations; the response of coagulation time was almost similar compared to 0.06% $CaCl_2$. At each concentration of $CaCl_2$ 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, $CaCl_2$ 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, $CaCl_2$ and blending of buffalo milk.

Table 1. Mean squares for coagulation time, yield and texture for camel milk cheese.

Source of variation	Coagulation time	Yield	Texture
pH (P)	310.4**	1848.38**	5.21**
$CaCl_2$ (C)	232.6**	164.01**	1.90**
Blend (B)	285.3**	106.55**	0.92**
Temp (T)	71079.8**	227.87**	16.33**
P x C	65.7**	20.81**	0.06**
P x B	19.1**	0.91^{NS}	0.03**
P x T	140.6**	2.13*	0.06**
C x B	1.8^{NS}	1.37^{NS}	0.06^{NS}
C x T	43.3**	10.16**	0.08**
B x T	6.8**	1.22^{NS}	0.06^{NS}
P x C x B	7.3**	1.18^{NS}	0.008^{NS}
P x C x T	51.3**	5.89**	0.05**
P x B x T	3.9**	0.74^{NS}	0.06^{NS}
C x B x T	6.3**	1.50*	0.06^{NS}
P x C x B x T	9.5**	2.34**	0.06**

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

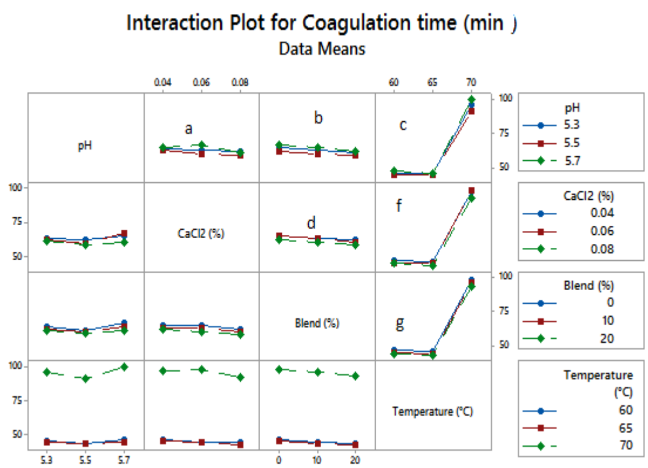

Figure 2. Effect of two-two parameters on coagulation time.

The graph clearly shows that the coagulation time tremendously reduced at both 60 and 65°C temperature of pasteurization. The interaction of $CaCl_2$ with bending of buffalo milk (Fig. 2d) showed the small decrease in RCT with increase in the concentration of buffalo milk and $CaCl_2$. 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'Connel 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).

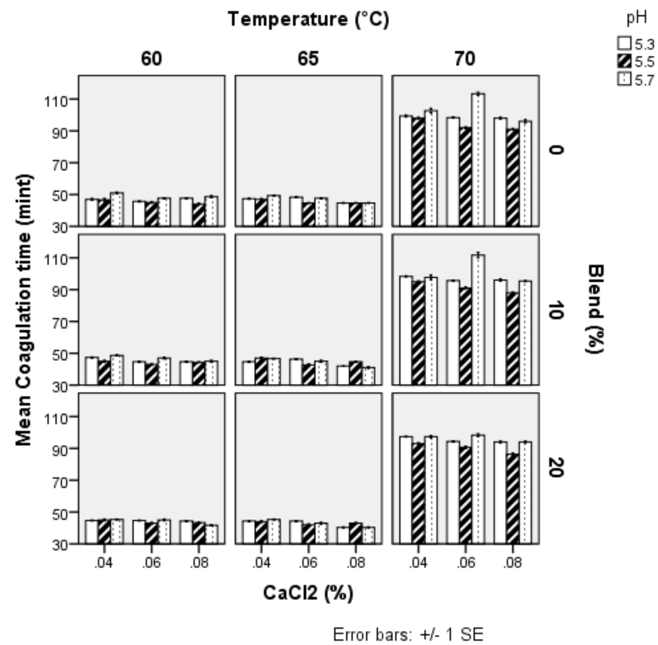

Figure 3. Cumulative effects of parameters (T, C, pH, B) on coagulation time.

The interactive effect of 3 levels of $CaCl_2$ 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% $CaCl_2$ 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 $CaCl_2$ 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 $CaCl_2$ 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 $CaCl_2$.

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 $CaCl_2$ (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 $CaCl_2$ hence maximum was noted at 0.08% $CaCl_2$. 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 $CaCl_2$ showed a significant effect on yield (Fig. 4c and e) while non-significant with blend (Fig. 4f). The interaction of blend with $CaCl_2$ present in Figure 4d also showed the increase in yield with the addition of buffalo milk relatively high at 0.08% $CaCl_{2.}$ The lower yield was recorded at 70°C while relatively higher yield was estimated at 65°C.

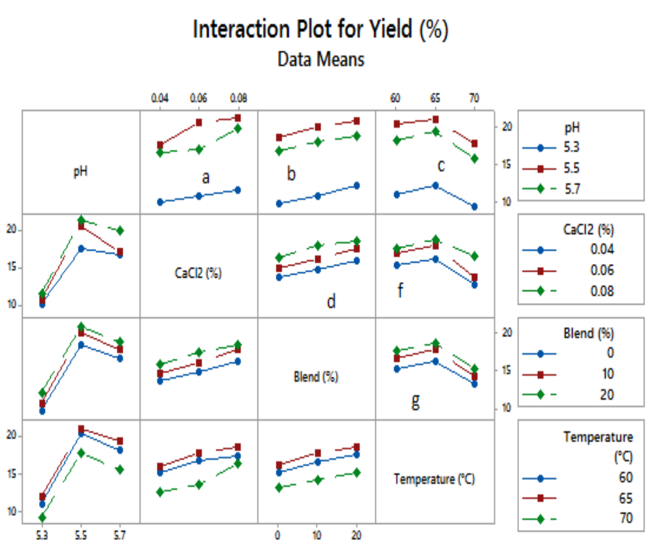

Figure 4. Effect of two-two parameters on yield (%).

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 $CaCl_2$, temperature and buffalo milk blend. The highest yield was recorded at 65°C temperature, 0.08% $CaCl_2$ 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.

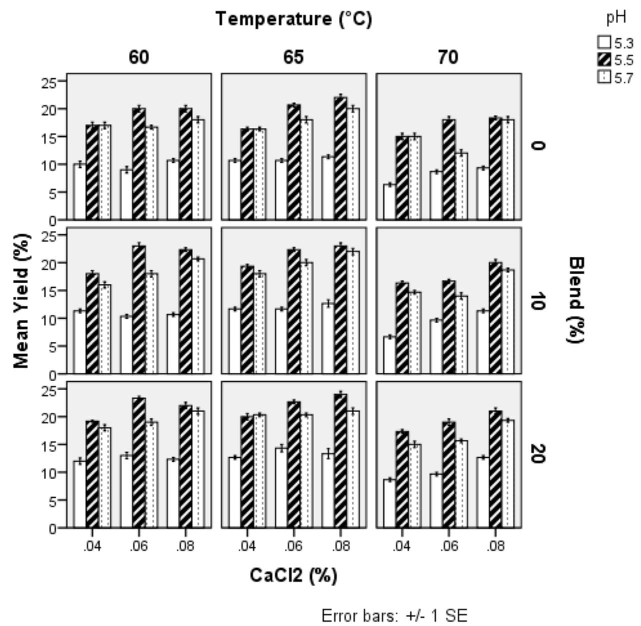

Figure 5. Cumulative effects of parameters (T, C, pH, B) on yield (%).

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 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.

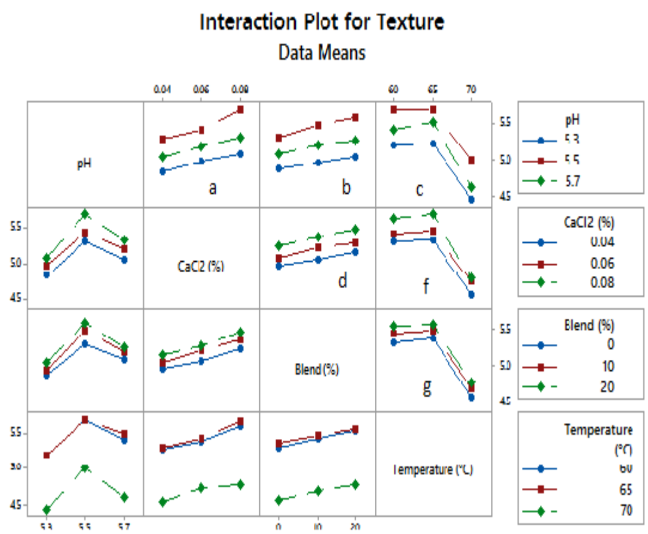

Figure 6. Effect of two-two parameters on texture (N).

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 as 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.

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.

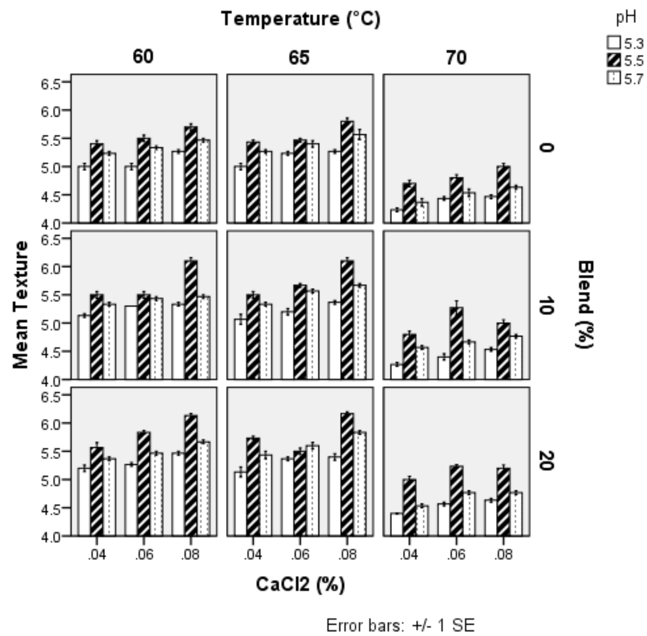

Figure 7. Cumulative effects of parameters (T, C, pH, B) on texture (N).

***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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