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Effect of heating and cooling rates on recovery of milk components during heat-acid coagulation of milk for preparation of Chhana - an Indian soft cottage cheese

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Abstract: Effect of heat treatments i.e., combined heating and cooling rates of milk on recovery of milk components viz., fat, protein, lactose, and minerals were studied during heat-acid coagulation of milk for the preparation of chhana, an Indian soft cottage cheese. In the study, the heating rate was varied between 6.7 to 40^{o}C.min-1 whereas the cooling rate was varied between 8 to 30^{o}C.min-1. Area under the time-temperature curve was used as an index for measuring the time-temperature treatment given to milk prior to acidification. The study revealed that decreasing the combined effect of heating and cooling rates increased the recovery of total milk solids from 0.523 to 0.594 kg per kg milk solid, fat from 0.824 to 0.93 kg per kg milk fat, protein from 0.811 to 0.925 kg per kg milk protein, lactose from 0.084 to 0.102 kg per kg milk lactose and mineral from 0.38 to 0.593 kg per kg milk mineral. Also, the study revealed that the faster the rate of cooling, the recovery of total milk solid, fat, protein, and mineral in chhana were higher as compared to the heating. More ever, in case of lactose recovery, the faster heating rate was found to be better.

Keywords: Milk components, heat-acid coagulation, chhana, recovery of milk components

Introduction

Chhana, Indian counter part of soft cottage cheese, is a milk product obtained by acid coagulation of hot milk followed by drainage of whey. It is a rich source of milk fat, protein, carbohydrate and vitamins *A* and *D*. It is used extensively as base and filler material for the preparation of a variety of Indian milk products like paneer, rasogolla, gulamjanum etc. With high protein and low sugar content, *chhana* is highly recommended for diabetic patients (De, 1980).

According to the definition of Bureau of Indian Standard (1969), *chhana* should not contain more than 70% moisture and milk fat should not be less than 50% of the dry matter. According to the Prevention of Food Adulteration Rules (1976), *chhana* is defined as the milk product obtained by precipitating a part of milk solid by boiling whole milk of cow and or buffalo or a combination thereof by addition of lactic acid, citric acid or any other suitable coagulating agent and subsequent drainage of whey.

In the preparation of *chhana*, the recovery of total milk solid and yield of *chhana* is influenced by the heat treatment given to milk prior to acidification, acidity of milk-acid mixture at the time of coagulation and residence time of the coagulum before separation of milk solids, besides the type of milk and its initial composition (Jonkman and Das, 1998). The heat treatment of milk prior to acidification involves temperature to which the milk is heated, the rate of heating, temperature to which the milk is cooled and the rate of cooling. 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).

Heating causes denaturation of whey protein and

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they get associated with casein micelles. The degree of denatured whey proteins depends on the time-temperature combination during the heating and is mainly determined by the maximum temperature to which milk is heated. Although Burton (1988) suggested that a temperature of 90 °C with a holding time of 10 min is sufficient for complete denaturation of whey protein, Soni *et al*. (1980) and Jonkman and Das (1993) heated milk to a temperature of 95°C for complete denaturation of α-lactalbumin, the smallest and most thermostable protein in whey (Larson and Rolleri, 1955). Burton (1988) and Jonkman and Das (1993) described whey protein denaturation as the parameter for describing the rate of heating and cooling. Ray and De (1953) reported that in general, *chhana* retains about 90% of fat and protein, 50% of mineral and 10% of lactose of the fresh milk. No study has so far been reported on the effect of heating and cooling rate on the recovery of milk components viz., fat, protein, lactose, and mineral. Since milk solids in *chhana* constitute of mainly fat, protein, lactose and minerals, it is desired that the maximum recovery of individual components would maximize the yield and recovery of total milk solids in *chhana*.

The objective of the present paper is to study the effect of heating and cooling rates in term of area under the time-temperature curve during heat-acid coagulation of milk on recovery of milk components viz., fat, protein, lactose and minerals in *chhana*.

Materials and Methods

Raw materials

Cow milk was used in the present study. The milk obtained from a particular cow from the nearby cow yard at IIT Kharagpur, India was dried into powder using a buchi spray dryer (Model–456/R, USA). The milk was dried in order to maintain the compositions of its constituents same, which was further reconstituted by adding distilled water, immediate after drying. Fat content in the reconstituted milk was balanced at 4% making a mass balance over fat and solid-not-fat content (Sahu, 2007). The reconstituted milk samples were stirred at the ambient temperature to ensure complete equilibration for at least 10 hr before further treatment.

Citric acid was used as the coagulating agent for milk coagulation. The strength of the citric acid solution necessary for the milk coagulation was calculated from the following equation (Jonkman and Das 1993).

$$X_{ma} = \frac{C_m w_m + b\left(\frac{M_{la}}{M_{ca}}\right) w_{ca}}{w_m + w_{ca}} \quad (1)$$

where, X_{ma} (% lactic acid) is the acidity of milk-acid mixtre, C_m (% lactic acid) is the acidity of milk, w_m (kg) is the weight of milk, b (%) strength of the citric acid, M_{la} (gm per mole) is the molecular weight of lactic acid, M_{ca} (gm per mole) is the molecular weight of citric acid and w_{ca} (kg) is the weight of citric acid solution.

In the present study, ratio of weight of milk to citric acid solution was maintained at 5:1 (Choudhury *et al*., 1998). Acidity of milk-acid mixture at the time of coagulation was maintained at 0.52% lactic acid (Choudhury *et al*., 1998). Substituting $X_c = 0.4$, $C_m = 0.158$, $w_m = 1*1.028$, $M_{la} = 90$, $w_{ca} = 1*1.028 *0.2$ and $M_{ca} = 64$ into Eqn. (1), the strength of the citric acid solution *b* was calculated as 1.49%.

Preparation of chhana

200 ml of milk was taken in a 250 ml glass or stainless steel beaker and heated by using an electrical heater operated at 220 volts. By altering the vessel i.e., glass or stainless steel and by setting the heating control of the heater, the heating rate of milk could be varied in the range of 6.7 to 40 °C.min^{-1}. Time taken for the milk for raising every 5°C temperature was recorded. As the temperature of the milk reached 95°C, the beaker was kept inside a double walled stainless steel cooling vessel. The annular space between the beaker and the inner wall of the vessel was filled with water. Cooling of the heated milk could be carried out in the range of 8 to 30°C.min^{-1} by circulating cold water around the annular space of the vessel. Time taken for cooling the milk from 95 to 70°C was noted for every 5°C fall of temperature. In all the experiments, the temperature of milk at the time of coagulation was maintained at 70 ± 1°C (Sen and Rajorhia 1998). As the temperature of the milk reached 70 °C, 40 ml of citric acid solution previously heated to 70 ± 1°C was added to it. The mixture was stirred mildly until clear whey appeared and held for 1 min. The coagulated milk-acid mixture was then strained using a muslin cloth, tied up in a bundle and hung up in air for 20 min to allow gravity drainage of the whey. Utmost care was taken in transferring quantitatively the small pieces of *chhana* from the muslin cloth. All the experiments were carried out in triplets and representative samples of *chhana* was collected for further analysis.

Chemical analysis of milk and chhana

The moisture content was determined as per IS: 5162 (1980). Fat content was determined by Gerber method (ISI: 1224, 1977). Mineral content was determined according to ISI: 5162 (1980). Protein content was determined by Kjeldahl method using 6.38 as nitrogen to protein conversion factor (BIS 1981). Lane-Eynon method (BIS 1981) was used to determine the lactose content.

The recovery of total milk solid and components i.e. fat, protein, lactose, and minerals in *chhana* were expressed as kg of the component recovered in *chhana* per kg of the same component present in milk. In the calculation of the recovery of total milk solid, solid coming into the *chhana* from the acid solution was neglected, since in actual practice the amount of solid coming into the *chhana* from the acid solution is very negligible.

Heat treatment of milk prior to acidification

Extent of heat treatment of milk prior to acidification was expressed as the area X_t (°C.hr) under the curve of temperature T (°C) and time t (s). Since the heat treatment of milk involves heating of milk followed by cooling, the total heat treatment X_t was calculated as the sum of areas under heating X_h and cooling X_c curve.

The effect of milk temperature on structure and solubility of whey proteins are reversible upto 60°C and governed mainly by hydrophobic bonding (de Wit and Klarenbeek, 1983). This hydrophobic bonding is enhanced when temperature increases up to 60°C and is weakened as the temperature drops. As denaturation of whey proteins becomes noticeable above 60°C, the area under the heating curve above 60°C only was taken into calculation. Thus,

$$X_t = X_h + X_c = \frac{1}{3600}\left[\int_{60}^{95} T dt + \int_{95}^{70} T dt\right] \quad (2)$$

where, 95 °C is the maximum heating temperature of milk and 70 °C is the temperature of milk at the time of acidification.

In order to find the influence of heating, cooling, and total heat treatment of milk on the recovery of milk components viz., total solid, fat, protein, lactose and mineral, coding of independent variables X_h, X_c and X_t were carried out between +1 and –1 and designated by x_h, x_c and x_t respectively. If X_{max} and X_{min} are respectively, the maximum and minimum values of all the experimental data obtained for X, the expression for x_h, x_c and x_t are as follows;

$$x_h = \frac{2X_h - (X_{hmax} + X_{hmin})}{X_{hmax} - X_{hmin}} \quad (3)$$

$$x_c = \frac{2X_c - (X_{cmax} + X_{cmin})}{X_{cmax} - X_{cmin}} \quad (4)$$

$$x_t = \frac{2X_t - (X_{tmax} + X_{tmin})}{X_{tmax} - X_{tmin}} \quad (5)$$

For conversion of coded variables x (x_h, x_c or x_t) into their real values X (X_h, X_c or X_t), the following equation is used.

$$X = \frac{X_{max}(1 + x)}{2} + \frac{X_{min}(1 - x)}{2} \quad (6)$$

Linear and non-linear second order regression equations (Eqn. 7 and 8) were developed for the responses Y_R (R_s, R_f, R_p, R_l, and R_m) as the function of the coded values of the independent variables x (x_h, x_c and x_t).

$$Y_R = a_o + a_1 * x_h + a_2 * x_c + a_{11} * x_h^2 + a_{22} * x_c^2 + a_{12} * x_h * x_c \quad (7)$$

$$Y_R = b_o + b_1 * x_t + b_2 * x_t^2 \quad (8)$$

The adequacy of developed relationship between the predicted and actual values of response was estimated from relative deviation percent R_d (Lomauro *et al*., 1985).

$$R_d = \frac{100}{N}\sum_{i=1}^{N}\left|\frac{y_{ai} - y_{pi}}{y_{ai}}\right| \quad (9)$$

In general, it is considered that the value of R_d below 10% gives a very good fit (Lomauro *et al*., 1985).

Results and Discussions

Quality of milk

The average moisture, fat, protein, lactose and mineral content of the standardized milk were found to be 86.8 ± 1.21%, 4 ± 0.16%, 3.95±0.03%, 4.75 ± 0.05, and 0.735 ± 0.05%, respectively. Its acidity value was 0.168 ± 0.005% lactic acid. The pH and density of the milk was 6.7 and 1028 ±5 $kg.m^{-3}$, respectively at 21°C.

Heat treatment of milk prior to acidification

From the time-temperature data recorded during the heat treatment of milk, it was observed that the value of heat treatment during heating X_h ranged between 1.142 and 6.665 °C.hr, and that of during cooling X_c ranged between 1.124 and 4.308 °C.hr. The value of the total heat treatment X_t during heating and cooling varied between 2.267 and 10.974 °C.hr. It must be noted that with the increase in the value of heat treatment, the value of area under the time-temperature curve decreases.

Recovery of total milk solids

The value of total milk solid recovery in *chhana* varied from 0.523 to 0.611kg per kg milk solid. The recovery obtained in the present study is higher than the values reported by Jonkman and Das (1993) while preparing *chhana* from low fat cow milk. The authors reported the value of total milk solid recovery between 0.433 and 0.482 kg per kg milk solid. Choudhary *et al*. (1998) observed that the recovery of total milk solid for cow milk ranged from 0.512 to 0.649 kg per kg milk solid. Figure 1 shows the response surface for the recovery of total milk solids as affected by the heat treatments during heating x_h and cooling x_c. The figure shows that the recovery of total milk solid decreased with the increase in the values of x_h and x_c.

Following regression equations were developed using the coded (x_h, x_c, x_t) values of independent variables using step-down regression method, where the coefficients with *F*-value less than 1 were deleted as described by Snedecor and Cochran (1967).

$$R_s = 0.564-0.032x_t+0.005x_t^2 (R_d=1.66\%, R^2=0.965) \quad (10)$$

$$R_s = 0.613 - 0.011x_h - 0.0198x_c - 0.054x_h^2 + 0.0058x_c^2$$
$$(R_d = 8.36\%, R^2 = 0.986) \quad (11)$$

Low value of R_d i.e. 1.66% and high value of R^2 i.e. 0.965 indicate that the Eqn. (10) fitted adequately to the experimental data. Negative sign of the coefficient of total heat treatment x_t in the linear term of Eqn. (10) reveals that the value of R_s increased with decreasing the value of x_t. From Eqn. (11), it is observed that both the heat treatments during heating and cooling are negatively correlated with the recovery of total milk solid. This implies that by decreasing the value of heat treatment i.e., lower area under the temperature-time curve during heating and cooling increased the recovery of total milk solids. In Eqn. (11), the higher value of coefficient of the heat treatment due to cooling x_c than the corresponding value of heating x_h indicates that heat treatment during cooling had greater effect than the heating on the recovery of total milk solid. The analysis of variance (Table 1) of Eqn. 11 indicates that all the terms of the equation significantly affect the recovery of total milk solid at 1% level of significant. Similar observation is reported by Choudhary *et al*. (1998) while preparing *chhana* from cow and buffalo milk.

Table 1. Analysis of variance for recovery of total milk solids

Effect	Sum of squares	DF	Mean sum of squares	F-value
x_h	0.1245	1	0.1245	32.25**
x_c	0.2245	1	0.2245	46.25**
x_h^2	0.2481	1	0.2481	52.66**
x_c^2	0.4592	1	0.4592	119.67**
Error	0.9125	22	0.1245	

F-value at 1% level 1, 22 = 7.94; **Significant at 1% level

Recovery of milk fat

Fat content of *chhana* was found to vary from 0.185 to 0.2 kg per kg *chhana* and the fat recovery in *chhana* between 0.815 and 0.929 kg per kg milk fat. The response surface of the recovery of fat during heating x_h and cooling x_c is shown in Figure 2. The recovery of fat increased with decrease in heat treatment due to heating and cooling. Following regression equations were developed between fat recovery in *chhana* in terms of coded values of independent variables using step-down regression method.

$$R_f = 0.915-0.022x_t-0.03x_t^2 (R_d=1.67\%, R^2=0.932) \quad (12)$$

$$R_f = 0.936-0.007x_h-0.013x_c-0.061x_h^2 - 0.002\, x_h x_c$$
$$(R_d = 8.86\%, R^2 = 0.961) \quad (13)$$

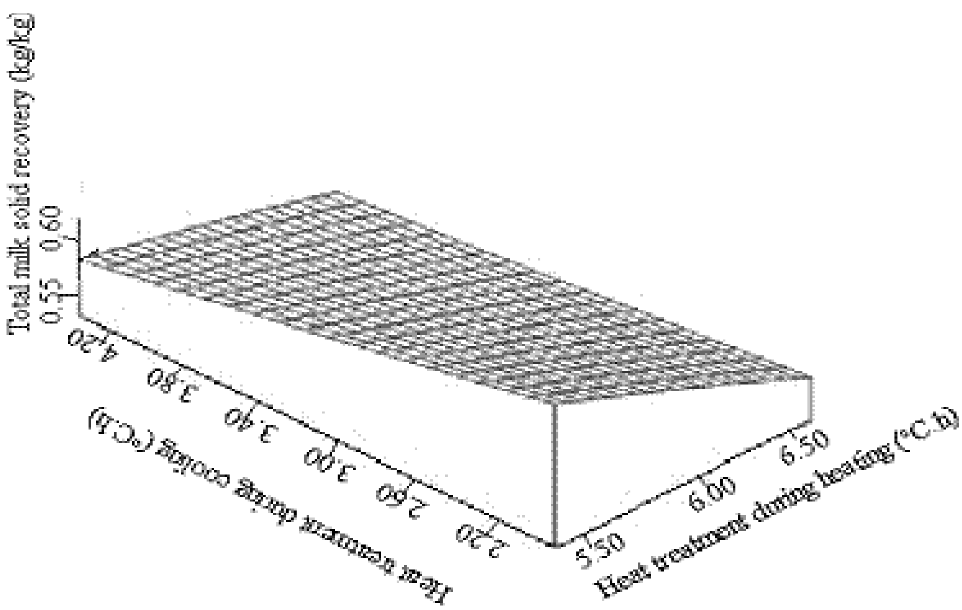

Figure 1. Effect of heat treatments during heating and cooling on recovery of total milk solid from milk to *chhana*

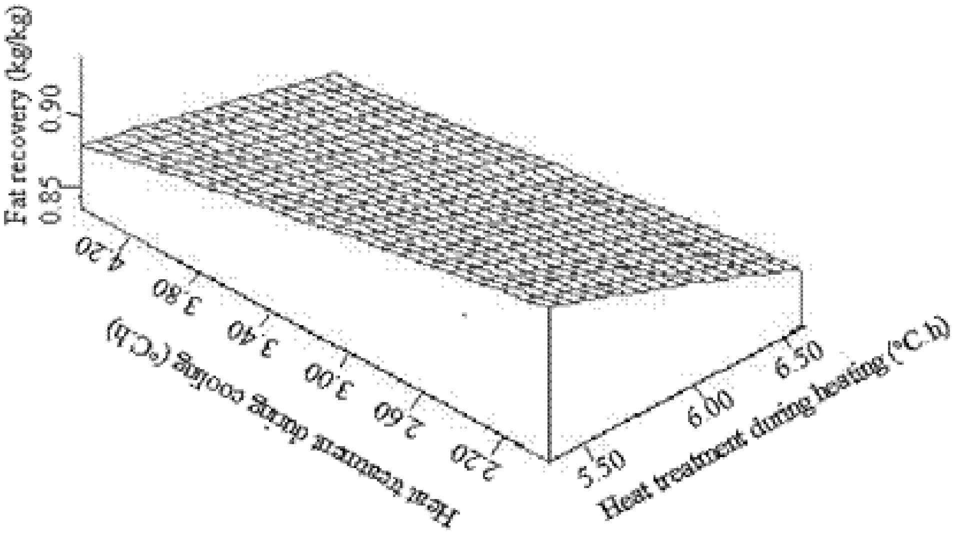

Figure 2. Effect of heat treatments during heating and cooling on recovery of fat from milk to *chhana*

The negative sign of the coefficient of x_t in Eqn. (12) shows that the recovery of fat R_f increased with decrease in the value of x_t. From Eqn. (13), the negative sign of the coefficients of x_h and x_c reveals that the value of R_f will increase with decrease in the heat treatment during heating and cooling. Higher absolute value of coefficient of cooling in Eqn. (13) than the corresponding value of heating indicates that the heat treatment due to cooling x_c will have the more influence than that of heating x_h on the recovery of fat. Low value of R_d = 8.86% and high value of R^2 = 0.961 obtained for the analysis indicates the adequacy of the equation for representing the recovery of fat in *chhana*. The analysis of variance given in the Table 2 shows that all the terms except quadratic term of x_h have significant (1% level) effect on the recovery of fat in *chhana*.

Table 2. Analysis of variance for fat recovery

Effect	Sum of squares	DF	Mean sum of squares	F-value
x_h	0.2422	1	0.2422	64.39**
x_c	0.1587	1	0.1587	45.09**
x_h^2	0.0581	1	0.0581	2.59^{ns}
x_hx_c	0.2153	1	0.2153	63.18**
Error	0.6689	22	0.6689	

F-value at 1% level 1, 22 = 7.94; **Significant at 1% level; ns: non-significant

Recovery of milk protein

Protein content of *chhana* varied between 0.174 and 0.195 kg per kg *chhana*. Its recovery varied from 0.841 to 0.92 kg per kg milk protein. Response surface for the recovery of protein due to the combined effect of heat treatment during heating x_h and cooling x_c is shown in Figure 3. The response surface indicates that milk subjected to lower value of x_h and lower value of x_c gave higher recovery of protein in *chhana*. Eliminating non-significant terms (*F*-value < 1), the following equations were fitted with coded values of independent variables.

$$R_p = 0.915-0.038x_t-0.003x_t^2 \quad (R_d = 1.13\%, R^2 = 0.968) \quad (14)$$

$$R_p = 0.931 - 0.016x_h - 0.0196x_c - 0.0615x_h^2 + 0.018x_c^2$$

$$(R_d = 4.82\%, R^2 = 0.929) \quad (15)$$

Eqn. (14) shows that the recovery of protein followed the similar trend as that of the recovery of total milk solids and fat. The higher absolute value of the coefficient of heat treatment during cooling than heating in Eqn. (15) indicates that the heat treatment due to cooling had the higher effect on the recovery of protein compared to heating. The analysis of variance (Table 3) showed that all the terms are significant for the recovery of protein at 1% level.

Table 3. Analysis of variance for protein recovery

Effect	Sum of squares	DF	Mean sum of squares	F-value
x_h	0.2896	1	0.2896	96.22**
x_c	0.1598	1	0.1598	79.15**
x_h^2	0.9875	1	0.9875	129.56**
x_c^2	0.1597	1	0.1597	81.07**
Error	0.9056	22	0.9056	

F-value at 1% level 1, 22 = 7.94; **Significant at 1% level

Recovery of milk lactose

Lactose content of *chhana* was found to vary between 0.024 and 0.026 kg per kg *chhana* and its recovery between 0.084 and 0.1 kg per kg milk lactose. Following regression equations were developed with coded values of the independent variables using step-down regression method by rejecting the terms having *F*-values less than one. Good fit was obtained with low value of R_d and high value of R^2, which shows that the equations developed are adequate to fit the experimental data.

$$R_l = 0.089 - 0.002x_t + 0.0002x_t^2$$

$$(R_d = 2.43\%, R^2 = 0.981) \quad (16)$$

$$R_l = 0.088 - 0.002x_h - 0.0005x_c + 0.002x_h^2 - 0.001x_c^2$$

$$(R_d = 1.65\%, R^2 = 0.935) \quad (17)$$

It is evident from Eqn. (16) that decreasing the value of x_t increased the value of lactose recovery. The higher absolute value of the coefficient of heat

Table 4. Analysis of variance for lactose recovery

Effect	*Sum of squares*	*DF*	*Mean sum of squares*	*F-value*
x_h	0.2684	1	0.2684	98.18**
x_c	0.0925	1	0.0925	11.69**
x_h^2	0.1586	1	0.1586	62.25**
x_c^2	0.14325	1	0.14325	32.01**
Error	0.95824	22	0.95824	

F-value at 1% level 1, 22 = 7.94; **Significant at 1% level

Table 5. Analysis of variance for recovery of mineral

Effect	Sum of squares	DF	Mean sum of squares	F-value
x_h	0.006581	1	0.006581	64.78**
x_c	0.001489	1	0.001489	16.52**
x_h^2	0.009264	1	0.009264	125.36**
x_c^2	0.001987	1	0.001987	8.95**
x_hx_c	0.002625	1	0.002625	36.25**
Error	0.009685	23	0.009685	

F-value at 1% level 1, 23 = 7.88; **Significant at 1% level

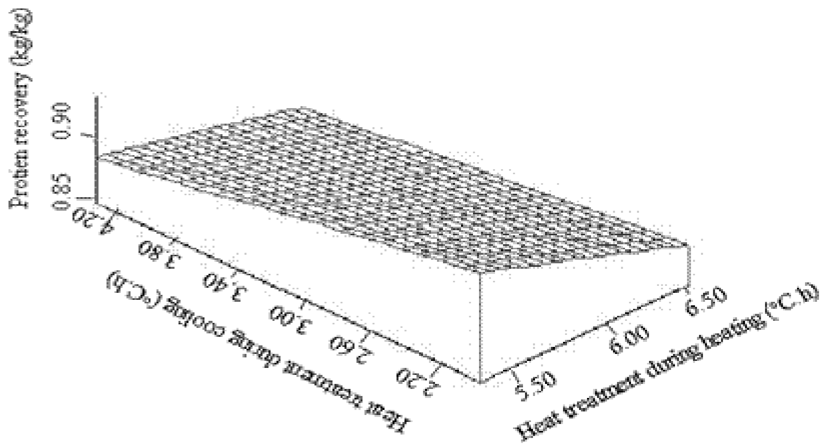

Figure 3. Effect of heat treatments during heating and cooling on recovery of protein from milk to *chhana*

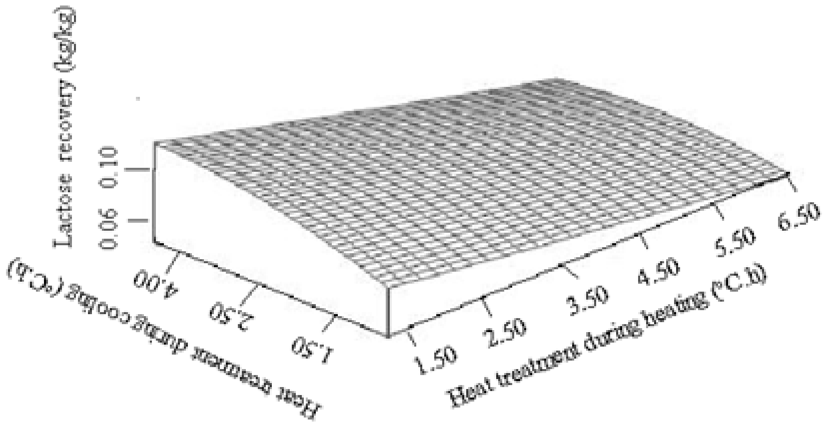

Figure 4. Effect of heat treatments during heating and cooling on recovery of lactose from milk to *chhana*

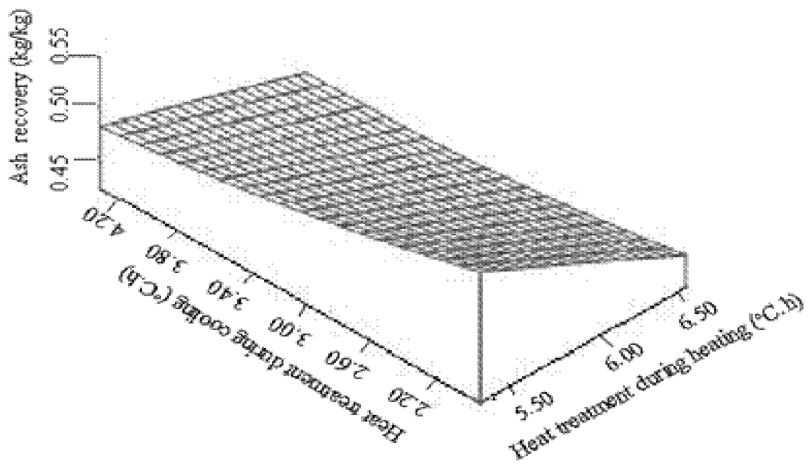

Figure 5. Effect of heat treatments during heating and cooling on recovery of mineral from milk to *chhana*

treatment due to heating x_h than cooling x_c in the Eqn. (17) reveals that the heat treatment due to heating had greater influence than the cooling on lactose recovery. The analysis of variance in Table 5 shows that all the terms have significant effect on the recovery of lactose at 1% level.

Figure 4 represents the response surface for the recovery of lactose due to the heat treatment during heating and cooling. The figure shows that the recovery of lactose follows the similar trend as that of the recovery of total milk solids, fat and protein. But, the higher slope of the response along x_h axis indicates that the heating will have higher effect on the recovery of lactose than cooling.

Recovery of milk minerals

Mineral content of *chhana* ranged between 0.012 and 0.018 kg per kg *chhana* and its recovery between 0.42 and 0.582 kg per kg milk mineral. Figure 5 represents the response surface for the recovery of mineral due to heat treatment of milk during heating x_c and cooling x_h. The figure shows that the recovery of mineral increases with the decrease in the value of the total heat treatment due to heating and cooling. This observation is similar to that obtained for the recovery of total milk solids, fat, protein and lactose. Relationship between the coded (x_h, x_c, x_t) values of independent variables on the recovery of mineral was developed by deleting the terms having the corresponding F-values less than 1.

$$R_a = 0.523-0.054x_t-0.501x_t^2$$

$$(R_d=4.74\%, R^2 = 0.962) \quad (18)$$

$$R_a=0.556-0.005x_h-0.043x_c-0.12x_h^2+0.012x_c^2+0.019\, x_h x_c$$

$$(R_d=2.26\%, R^2=0.899) \quad (19)$$

The absolute value of the coefficient of heat treatment during cooling in the Eqn (18) is greater than the heating. This had resulted in higher slope of the response surface along the x_c axis in Figure 5. Adequacy of the developed relationship agrees with the low value of R_d (= 2.26%) and high value of R^2 (= 0.899). The analysis of variance in Table 5 shows that all the terms have significant effect on the recovery of ash at 1% level.

Eqn. (10) to (19) were developed by using X_{tmax} = 10.974°C.hr, X_{tmin} = 2.267°C.hr, X_{hmax} = 6.665°C.hr, X_{hmin} = 1.142°C.hr, X_{cmax} = 4.310°C.hr, X_{cmin} = 1.124°C.hr and by following the conversions mentioned in Eqn. (3) through (6).

Theoretical recovery of maximum total milk solids

From the maximum recovery of fat, protein, lactose and ash content in *chhana* were 0.929, 0.921, 0.1 and 0.535 kg per kg *chhana*, respectively. Therefore, the maximum recovery of total milk solid in *chhana* was 0.04*0.929+0.0395*0.921+0.0475*0.11 +0.00735*0.535 = 0.08227 kg milk solid per kg milk. Based on this value, the maximum theoretical value of the recovery of total milk solid was 0.08227*(1-0.868)$^{-1}$ i.e., 0.623 kg per kg milk solid. This value of recovery is close to the maximum value of total milk solids i.e., 0.611 kg per kg milk solid obtained during the heat-acid coagulation process.

Conclusions

The general trends on effect of heat treatment on recovery of total milk solid in *chhana* during the heat-acid coagulation of milk are:

- Reducing the area under the time-temperature curve (i.e. low value of X_t) will increase the recovery of total milk solids in *chhana* as observed from Eqn. (10).
- Effect of low area under the time-temperature curve during cooling x_c is more than heating x_h on the recovery of the total milk solids in *chhana* as observed from Eqn. (11).
- Lower the area under the time-temperature curve during cooling (i.e. low value of X_c) increases the recovery of milk fat, protein, lactose, and mineral in *chhana* as observed from Eqns. (13), (15), (17) and (19).
- Lower the area under the time-temperature curve during heating (i.e. low value of X_h) increases the recovery of milk fat, protein, lactose, and mineral in *chhana* as observed form Eqns. (13), (15), (17) and (19).
- Effect of X_c is more than that of X_h in recovery of milk fat, protein and mineral in *chhana*. For the case of lactose, the effect of X_c is, however, less than X_h.

Literatures on the heating, cooling and acidification of milk show the following generalized trends.

- Heat-acid coagulation of milk is caused by chemical and physical changes in casein due to action of acid at high temperature (Singh, 1995). In the process, large structural aggregates of casein curd are formed from the normal colloidal dispersions of discrete casein micelles, where fat and coagulated serum proteins are entrapped with whey. During the process of boiling and subsequent coagulation, casein micelles loose a part of calcium, phosphate and other salts.

- The heat treatment of milk before acidification is characterized by the temperature to which milk is heated, rate of heating, the temperature to which the milk is cooled and the rate of cooling. Heating causes denaturation of whey proteins and they get associated with casein micelles (Kessler, 1985).
- The degree of denatured whey proteins depends on the time-temperature combination given to milk and is mainly determined by the temperature to which milk is heated (Waltra and Jennes, 1987; Fox, 1981a; Singh, 1995; O'Connell and Fox, 2003; Singh, 2004).
- Whey protein denaturation above 70°C is considered as a two-step process; an unfolding step that may be either reversible or irreversible and an aggregation step that mostly follows by irreversible unfolding. Irreversible changes of protein structure are influenced further by environment conditions such as pH, ionic strength and protein concentration of the milk-acid mixture (Singh, 2003; Fox and Creamer, 1997).
- de Wit and Klarenbeek (1983) observed through Differential Scanning Calorimetry that unfolding of globular proteins is accompanied by an endothermal heat effect. According to the same author α-lactalbulin is the only reversible heat denatured whey protein (reversibility > 90%), whereas all the other whey proteins, no longer show endothermal heat effect of unfolding.
- During the process of heat-acid coagulation, calcium and phosphate are transferred from solution to the colloidal state. Though these changes are irreversible, for α-lactalbulin, the relaxation time is long (Walstra and Jennes, 1983).
- Mc Grace (1999) reported that milk fat has little effect on the stability of milk. However, the constituents of fat globule membrane particularly phospholipids may influence the recovery of fat. Low recovery of milk solids and fat might be due to less number of fat globules in the milk, which could not trap sufficient amount of milk protein through their association with the fat globule membrane.
- Choudhary *et al*., (1998) reported that the rate of cooling had the most prominent effect on the recovery of total milk solids in *chhana* than the rate of heating.
- Singh and Fox (1987a) and Jang and Swaisgood (1990) reported that κ-casein on the surface of casein micelles is involved in the formation of a specific disulphide-linked complex with β-lactoglobulin. As the β-lactoglobulin aggregates or the monomers are considered to form the disulphide bonds with κ-casein, the cystine residues that are located in the para- κ-casein part of the protein must be relatively accessible to the protein of the coagulated milk-acid mixture. But, there is no plausible explanation about how whey protein aggregates form disulphide bonds of κ-casein.

Since milk solids in *chhana* constitute of mainly fat, protein, lactose and minerals, it is desired that the maximum recovery of individual components would maximize the yield and recovery of total milk solid in *chhana*. From the limited information, it is observed that no work in the past has been done on the rates of heating and cooling as the variables affecting changes in milk constituents and their migration to milk coagulum during the acidification of milk. No plausible explanation could, therefore, be given for the trends of milk components recovery that was observed on the present study. Moreover, from the generalized trends, it can be inferred that, since the recovery of milk lactose is very small compared to the recovery of milk fat, protein, and minerals, it is necessary that a very fast cooling rate must be employed for acidifying the milk in the preparation of *chhana*. Therefore, while developing a continuous heat-acid coagulation unit for continuous production of *chhana*, acid solution at normal or low temperature should be injected directly to the heated milk in order to allow instant cooling of the heated milk.

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