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Effect of heat assisted high pressure treatment on rate of change in pH and gel strength of acidified milk gel in the preparation of soft cheese

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Abstract

The effect of high pressure (200 – 600 MPa), pressurization time (0 – 60 min) and heat treatment temperature (15 - 65°C) on change in pH and storage modulus (G') of the acidified milk gel in the preparation of soft cheese was studied. The value of the pH of the heat assisted high pressure (HAHP) treated samples was observed to vary from 5.48 to 5.31 after 140 s of acidification, whereas it dropped from 6.38 to 5.16 after 240 s of acidification for the control. It was observed that higher pressure and temperature increased the pH values of the milk gel, whereas the values decreased with higher pressurization time. Similarly, the values of G' of the HAHP treated samples ranged between 384 Pa and 322 Pa after 200 s, compared to 361 Pa after 260 s for the control. High pressure increased the G' value of the HAHP samples, whereas the value decreased with high pressurization time and temperature.

Introduction

Soft cheese refers to the milk solids obtained by acid coagulation of hot milk and subsequent drainage of whey. According to the Prevention of Food Adulteration Act (PFA), 1954, soft cheese is defined as the product obtained from cow or buffalo milk or a combination thereof by precipitation with sour milk, lactic acid or citric acid solution. It should not contain more than 70 per cent moisture, and milk fat should not be less than 50 per cent of the dry matter.

In manufacturing of soft cheese, whole milk of cow and/or buffalo is heated to 95°C. The heated milk is allowed to cool suddenly to 70°C and an acid solution, previously heated to 70°C is added to coagulate milk (Sahu and Das, 2007). Coagulation of milk basically involves destabilization of casein micelles which leads to formation of large, firm and cohesive structural aggregates of casein micelles in which milk fat, other colloidal and soluble solids are entrained along with deproteinated whey (Lucey, 2003). The whey is drained out and the coagulated milk solids are pressed to obtain soft cheese. In the process, the heat treatment of milk prior to acidification is characterized by the temperature to which milk is heated, the rate of heating, the temperature to which the milk is cooled and the rate of cooling (Choudhury *et al.*, 1998). Heating causes denaturation of whey proteins and the degree of whey protein denaturation depends mainly on the time-temperature combination given to milk (O'Connell and Fox, 2003; Huppertz *et al.*, 2004a; 2005).

High pressure (HP) processing is now of great concern, because of its potential efficacy to achieve desired functional, structural, chemical and sensory attributes in the end product. Various applications of HP processing in dairy industry are systematically reviewed and reported by Lopez-Fandino (2006) and Rastogi *et al.* (2007). HP processing, unlike thermal treatment, has shown to accelerate the rate of gel formation and firmness of cheese (Pandey *et al.*, 2000; Ferragut *et al.*, 2004). HP- treatment also improves the coagulation properties, yield and micro-structure of cheese (Pandey *et al.*, 2003). However, there is limited literature characterizing influence of HP-induced milk processing on coagulation properties of acidified milk gel in preparation of soft cheese.

Heat treatment before acidification affects the coagulation properties of milk. Combined use of HP and moderate temperature can lead to efficient microbial inactivation comparable to that of thermal pasteurization. It has also been shown that changes in temperature of pressurization influence certain characteristics of milk such as hydrophobicity, coagulation characteristics, cheese micro-structure and average particle diameter (Gaucheron *et al.*, 1997). However, there is no pertinent study on effect of thermal assisted HP-induced change in acidity and

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strength of the acidified milk gel in preparation of soft Indian cheese. The specific objective of the present paper is to study the effect of HAHP-treatment on change in pH and gel strength of the acidified milk gel in preparation of soft Indian cheese. It is expected that findings of the study will help to develop a HP-assisted process technology for preparation of soft Indian cheese, which is yet to be introduced in Indian food industry.

Materials and Methods

Milk sample preparation

Cow milk was used in the present study. The milk obtained from a particular cow from the nearby dairy farm at the University of Reading, UK was dried into powder using a buchi spray dryer (Model – 456/R. USA). The powder was immediately reconstituted by adding distilled water. Fat content in the reconstituted milk was maintained at 4% making a mass balance over fat and solid-not-fat content of the initial and final milks. The reconstituted milk samples were stirred at room temperature of 18°C for 4 h before experimentation to ensure complete equilibration.

Moisture content of the milk sample was determined as per BIS (1981). Fat content was determined by Gerber method (IS: 1224, 1977). Lactose content (% lactic acid) and titrable acidity (% lactic acid) were determined by using Lane-Eynon method (BIS, 1981). Ash content was determined according to BIS (1981). Protein content was determined by Kjeldahl method using 6.38 as nitrogen to protein conversion factor (BIS, 1981). The proximate compositions of the milk sample as obtained from the measurements were: moisture 0.851±0.015, protein 0.045±0.008, fat 0.04±0.007, lactose 0.0358±0.002 and ash 0.011±0.001 kg per kg milk. Average titrable acidity of the milk sample was 0.158±0.005% equivalent lactic acid. Density and pH of the milk sample were 1013 kg.m^{-3} and 6.3, respectively at room temperature of 18°C.

Experimental design

A five level-three variable central composite rotatable design (CCRD) was employed to carry out the experiments at various combinations of high pressure (X_1), pressurization time (X_2) and heat treatment temperature (X_3). The maximum and minimum values of X_1, X_2 and X_3 selected were 200 to 600 MPa, 0 to 60 min, and 15 to 65°C, respectively. Changes in pH and storage modulus of the acidified milk gels were measured with coagulation time. A second order regression equation (Eqn. 1) was used to correlate the actual values of the independent variables X_i (X_1, X_2 and X_3) with the dependent variables Y_i (constant values of pH and storage modulus after acidification).

$$Y_i = A_o + \sum_{i=j}^{k} A_i X_i + \sum_{i=j}^{k} A_{ii} X_i^2 + \sum_{i=j}^{k}\sum_{i=j}^{k} A_{ij} X_i X_j \qquad (1)$$

(i = 1 - 3, j = 1 – 3)

where, A_o, A_i, A_{ii}, and A_{ij} are constants. The statistical software "Design Expert- 8.0" was used to fit the experimental data into the regression equation. The analysis of variance (ANOVA) was carried out to test the fitness of the developed equations.

Pressure treatment

An ABB Isostatic Press (Model-CIP 42260, ABB Autoclave System, OH) was used for subjecting milk to various treatment conditions. The test pouches (Koch vacuum pouches, size: 20 cm x 30 cm), filled with milk and sealed using vacuum sealing, were submerged in water in the pressure chamber and subjected to various experimental combinations. Adiabatic heating results in temperature rise in test samples and pressure medium (approx. 3°C/100 MPa). In order to maintain a constant temperature, the circulating water temperature was kept slightly below the treatment temperature.

Acid gel formation

Citric acid solution was used for coagulation of milk. Ratio of milk and acid solution during coagulation process was maintained at 5:1 (Choudhury *et al.*, 1998). The strength of the acid solution was maintained at 1.75 g per 100 g of citric acid solution (Sahu and Das, 2007). The HAHP treated samples were acidified immediately using the citric acid solution at 70±1°C and was stirred mildly for 30 s. In order to compare the effect of HAHP treated milk gels with that of the heat treated gel on change in pH and gel strength, a control sample was prepared by using conventional method. For the purpose, milk was heated to 95°C, allowed to cool to 70°C and added with the citric acid solution, previously heated to 70±1°C and stirred mildly for 30s (Sahu and Das, 2007). pH of the acidified milk gel with time was measured using a digital pH meter (Systronic, UK). Gel strength with time was measured in term of storage modulus using a Rheometer (TA, AR1000, UK).

Results and Discussion

Figure 1 shows the variation in pH and storage modulus of the HAHP treated acidified milk gel with time. In the figure, the milk was subjected to a pressure of 400 MPa for 30 min at 40°C. The initial

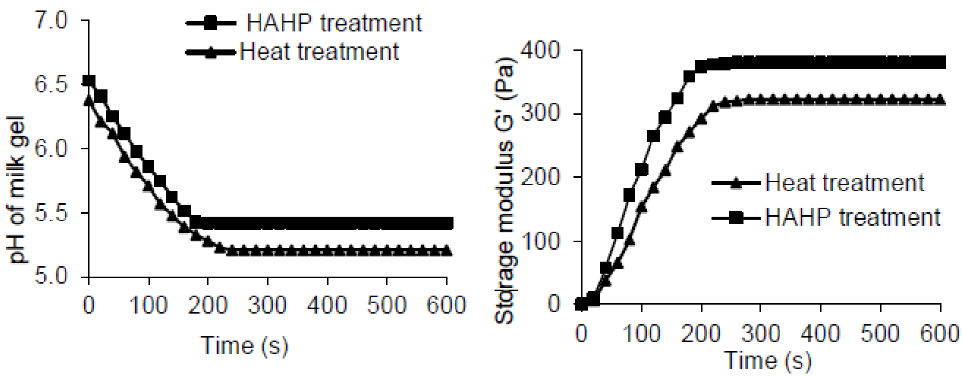

Figure 1 Variation in pH and storage modulus of the acidified milk gel with time at 400 MPa for 30 min at 40^{o}C

Table 1. Measured values of pH and storage modulus (G')

Expt. No	Actual values of independent variables X_1 (MPa)	X_2 (min)	X_3 (°C)	Responses pH	G' (Pa)
1	523.00	48.43	55.36	5.48	384
2	523.00	48.43	24.64	5.28	366
3	523.00	11.57	55.36	5.44	370
4	523.00	11.57	24.64	5.44	361
5	277.00	48.43	55.36	5.43	356
6	277.00	48.43	24.64	5.46	342
7	277.00	11.57	55.36	5.28	356
8	277.00	11.57	24.64	5.41	382
9	600	30	40	5.48	379
10	200	30	40	5.36	341
11	400	60	40	5.32	366
12	400	0	40	5.31	322
13	400	30	65	5.36	379
14	400	30	15	5.32	350
15	400	30	40	5.44	376
16	400	30	40	5.44	376
17	400	30	40	5.44	376
18	400	30	40	5.44	376
19	400	30	40	5.44	376
20	400	30	40	5.44	376
21	400	30	40	5.44	376

where, X_1 = high pressure, X_2 = pressurization time, and X_3 = heat treatment temperature

value of pH of the HAHP treated and control samples was 6.53 and 6.38, respectively and dropped to a value of 5.43 and 5.16 after 180 s and 240 s of acidification, respectively. As shown in the figure, initially, there was a faster rate of decrease in pH after acidification and thereafter, the pH remained constant. All the samples treated at different combinations of the independent variables (Table 1) showed the similar trends after acidification except the sample treated at 0 min. This sample showed the trend similar to the control sample, however, although the pH of the sample changed to 6.54 to 5.31 after 240 s of acidification, the storage modulus was observed to be very poor compared to other samples. This might be due to low temperature (40^{o}C) of heating without application of pressure treatment at which no whey protein denaturation occurs in milk. Also, the figure shows that although the HAHP treated and control samples follow the similar trend, however, the trend for the HAHP treated samples was higher than the control indicating that the high pressure treatment affected the change in pH and thus, the coagulation rate, and decreased the coagulation time. Similar observations were reported by Floury *et al.*, (2002) who suggested that physical phenomenon like cavitation, turbulence, impact and shear forces that occur during HP processing, cause reduction in droplet size in emulsion improving coagulation properties of milk and reducing the coagulation time.

The correlation between actual values of the high pressure, pressurization time and heat treatment temperature, and pH was developed by the following regression equation.

$$pH = 5.409 + 9.086^{*}10^{-5}X_1 - 8.195^{*}10^{-3}X_2 + 1.451^{*}10^{-3}X_3 + 5.514^{*}10^{-7}X_1X_2 + 3.308^{*}10^{-6}X_1X_3 + 2.782^{*}10^{-4}X_2X_3 + 1.296^{*}10^{-7}X_1^2 - 8.081^{*}10^{-5}X_2^2 - 1.141^{*}10^{-4}X_3^2 \quad R^2 = 0.97 \qquad (2)$$

where, X_1, X_2 and X_2 are the actual values of high pressure (MPa), pressurization time (min) and heat treatment temperature (°C) of milk, respectively. The analysis of variance (ANOVA) of Eqn. 2 indicated that the equation is statistically signifiact at $p < 0.05$ with an $r^2 = 0.97$. However, the associated F-value was non-significant at $p < 0.05$ for lack of fit of the equation indicating that the equation developed could be a good equation for predicting the value of pH.

The response surface plots (Figure 2) of high pressure, pressurization time, and heat treatment temperature on measured value of pH of the acidified milk gels showed that higher pressure and temperature increased the pH of the milk gel, whereas the value decreased with increasing the value of pressurization time. The result has a good agreement with the findings reported by Famelart *et al.* (1998). The authors showed that the HP-induced dissociation of κ-casein from micelles and disruption of micelles result in an increased micellar surface area with a reduced level of κ-casein available to provide steric stabilization which may lead to reduced coagulation time and increase the rate of gel formation (Landfeld *et al*., 2002).

The plot between the storage modulus (G') of the acidified milk gel with time shows the similar trends for both the HAHP and control samples. However, the HAHP treated samples had higher G' value than the control. In the early stage i.e. lag phase of the coagulation process, lower values of G' were observed and it increased rapidly up to 381 Pa and 318 Pa after 180 s and 220 s of acidification for the HAHP treated and control samples, respectively, indicating high pressure induced a rapid progress in gel formation. This period was followed by a constant rate period, where the G1 values remained constant. Similar trends were observed for all other HAHP treated samples.

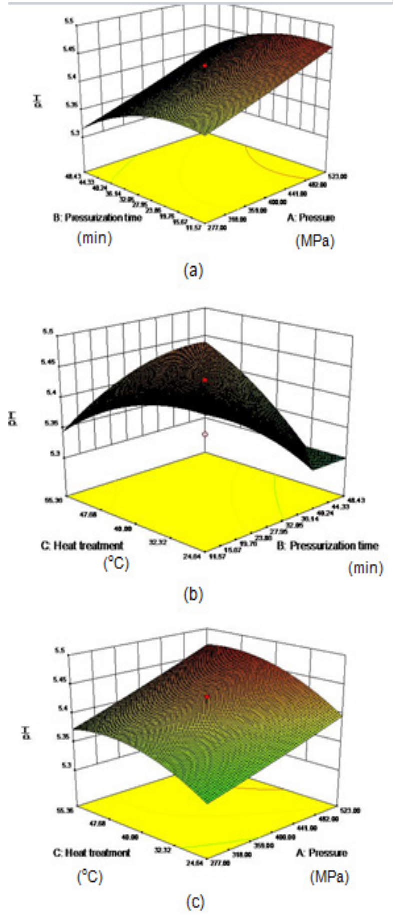

Figure 2. Response surface plots showing effect on pH of the acidified milk gels: (a) pressure and pressurization time, (b) pressurization time and heat treatment, and (c) pressure and heat treatment

The following regression equation was fitted to the measured values of the storage modulus with actual values of high pressure, pressurization time and heat treatment time.

$$G' = 385.225+0.139X_1-2.776X_2-0.836X_3+3.253^{*}10^{-3}X_1X_2+2.580*10-3X1X3+0.0216^{*} X_2 X_3 - 3.596^{*}10^{-4} X_1^2 +7.792^{*}10_{-3}X_2^{2-} 6.768^{*}10^{-3}X_3^2 \quad R^2 = 0.98 \qquad (3)$$

where, G' is the storage modulus (Pa), and X_1, X_2, and X_2 are the actual values of high pressure (MPa), pressurization time (min) and heat treatment temperature (°C) of milk, respectively. The analysis of variance (ANOVA) indicated that the developed equation was significant at $p < 0.05$ with an r2 value equal to 0.98. The lack of fit for the equation was also significant at $p < 0.05$.

The response surface plots of high pressure, pressurization time and heat treatment on the storage modulus of the acidified milk gels shows that higher pressure increased the values of G', whereas the value decreased with higher pressurization time and treatment temperature. Hayes and Kelly (2003a) reported that HP-treatment shortened the gelation time and increased gel firmness due to modifications in pH and particle size. Buffa *et al.* (2003) showed that the microstructure of HP treated rennet milk gel improved the casein–casein or fat–casein interactions. This induced a more compact casein matrix in the milk gel and thus, improving the gel strength. Furthermore, cheese prepared from heated milk has high moisture content due to association of whey proteins at casein micelle surface, which impedes fusion of para-casein network. This high moisture content in cheese might reduce the gel strength of the control sample.

Conclusion

The study concludes that the HAHP-induced changes reduced the pH in the acidified milk gel, thus, reduced the coagulation time. The gel strength of the HAHP-treated samples was higher than the control suggesting that the HAHP-induced disruption of casein micelles has a larger effect on the gel formation. HAHP-induced disruption of casein micelles and HAHP-induced dissociation of micellar k-casein appeared to greatly reduce coagulation time, increase the rate of gel formation and strength of the gel formed. It was also observed that the pressure and treatment temperature had the highest effect on reducing the pH of gel and thus, reducing the coagulation time. At the same time, they had the highest effect on gel strength or firmness. Therefore, while optimizing the process parameters, one should consider either lower coagulation time, so that the production rate will be higher or gel strength or firmness depending upon the consumers' preference.

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