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THE EFFECT OF PROCESSING TREATMENTS ON MILK COAGULATION WITH RENNET AS MEASURED BY VISCOSITY-TIME CURVE

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ABSTRACT

The change in milk viscosity upon addition of rennet was used to study the effect of various milk treatments on renneting reaction. The change in milk viscosity after the addition of rennet when recorded versus time gave a curve that passed through 4 turning points (T_1 to T_4). An induction period with no change in viscosity (T_1) ended with a rise in the viscosity to a first maximum (T_2) then viscosity decreased to a minimum with milk still in the liquid form (T_3) followed by a rapid increase to reach a final maximum forming a plateau (T_4) when coagulation occurred.

The effect of milk cooling, freezing, heat treatments, calcium addition, rennet concentration, addition of NaCl and homogenization on viscosity-time curve was studied.

Milk cold storage, freezing, heat treatments and the addition of NaCl elongated the periods of 4 phases periods than of raw milk periods. The delaying effect in descending order was the addition of NaCl > freezing > cooling > heat treatments. Most the delay was in the lag phase (T_1). Heat treatments delay of T_1 was proportional to the temperature. There was a good correlation with $r = 0.98$ ($p<0.01$) between T_1 and the temperature. Increasing the concentration of rennet was more effective in correcting the effect of heating than the addition of calcium. Heat treatments caused a large increase in viscosity of the first maximum than the raw milk value. Homogenization was very effective in correcting the effect of milk heat treatments and shortened T_1 than the value of raw milk. The end of the lag phase (T_1) leading to the first rise in viscosity occurred at a certain amount of casein hydrolysis depending on to milk treatment.

Keywords: Milk coagulation, Rennet, Viscosity, Milk heat treatments.

INTRODUCTION

The change in milk viscosity upon addition of rennet has been used to monitor the renneting reaction. Number of workers has reported that upon the addition of rennet, milk viscosity showed an initial decrease reaching a minimum value, and then it increased rapidly until gel is formed. Lomholt and Ovist (1997) found that the initial decrease in viscosity was a function of the degree of k-casein hydrolysis (α) and after the viscosity minimum, it was no longer a function of α. The minimum occurred when α was between 0.6 and 0.7.Rennet concentration affected rate of curd gel firmness.Dekruif *et al* (1992) forward an explanation for the steps of milk coagulation namely, the initial viscosity decrease, the increase in viscosity and then the aggregation. The initial decrease in viscosity was reasoned to be due to the decrease in the effective volume fraction of casein micelles on splitting the glycomacropeptide by rennet. The decrease was proportional to the relative hair length of the k-casein. They reported a decrease of 7%, claiming to be similar to the percent of hair removed. The removal of the hairs caused the loss of the main steric stabilization layer and this in turn caused the micelles to show concomitant mutual attraction with the Van der waals attraction

raising the viscosity. This was followed by aggregation process, which depends on, α and the collision frequency. Lomholt and Ovist (1997) showed that micelles aggregation occurs when α reached 60%. The above explanation was supported by Gastaldi et al (2003) who found that the net negative charge of casein micelles was reduced with increasing degree of k-casein hydrolysis and this was accompanied with a small but significant decrease in hydrodynamic diameter and micellar hydration.

The value of the initial decrease in viscosity varied between workers and affected by number of factors such as type of milk, type of rennet and heat treatment. While Dekruif *et al.* (1992) reported a 7% decrease in bovine milk, Lopez (1999) reported 22.3% decrease in raw goats' milk. While Guthy and Novak (1977), could not detect this initial decrease. Lopez (1999) found that heat treatment lessened the value of the initial viscosity decrease. The average decrease in raw goats' milk was 22.3% compared to 17 and 19% in pasteurized milk at 65 and 72°C respectively. This was reasoned that the heat treatment decreased the rate of k-casein hydrolysis due to the interaction with denatured β-lactoglobulin. Actually at a β-lactoglobulin denaturation levels greater than 60%, as occurs in UHT, milk was not coagulated. However, Schreiber (2001) using whey protein free milk concluded that heat induced changes in the calcium distribution between the micellar and serum phases were the main factor impairing rennet coagulation.

β-lactoglobulin and its heat denaturation play number of important roles other than the delaying of coagulation. Photchanachai and Kitabatake (2001) found that heating β-lactoglobulin showed two endothermic peaks. The first peak was below 100°C and about 80°C where the thermal denaturation of the protein, by aggregation and polymerization, caused viscosity to increase. β-lactoglobulin also plays role in casein heat stability it was reported by O'connell and Fox (2001) that at maximum heat stability, β-lactoglobulin chelated calcium ions (Ca^{2+}) and preventing k-casein from dissociation from the miceels at minimum stability, β-lactoglobulin sensitized casein micelles to heat by inducing precipitation of calcium phosphate by increasing micellar hydrophobicity.

Cooling and different additives such as Ca^{2+} or NaCl affect the whole coagulation process. Raynal and Remeuf (2000) reported that cooling caused the dissociation of casein fractions, the increase the in solubility of calcium phosphate and the decrease in micelle size. These changes increased milk clotting time by rennet, reduce gel-strengthening rate and curd firmness. Addition of sodium chloride to milk was found by Aoki *et al* (1999) to increase the soluble calcium and inorganic phosphate indicating of an increase in the solubility of micellar calcium phosphate and loosened the structure of casein micelles. This delayed milk coagulation and gave softer curd. Homogenization was reported to speed up the coagulation process. Ghosh and Kessler (2001) found that homogenization before and after heat treatment (80°C/3 min.) gave differences in milk rennetibility. A slightly higher rennet coagulation time, higher increase in viscosity, higher gel strength and syneresis were noticed in the milk homogenized before heating compared to

milk homogenized after heating. The difference was postulated to be due to the difference in the amount of denaturated whey protein adsorbed on globules newly formed membranes when milk homogenized before or after the heat treatment.

Transglutaminase enzyme (TGase) modifies proteins by cross-linking through covalent bonds between glutamine and ε-amino group of lysine amino acids. When heated milk was treated with TGase led to a decrease in rennetibility due to "surface sealing" of casein micelles cross-linked with β-lactoglobulin. The dissociation of casein micelles with urea and removal of colloidal calcium phosphate was reduced as reported by O'Sullivan *et al* (2002).

This research was carried out to use the change in milk viscosity diagram upon the addition of rennet to study the effect of number of milk treatments on the renneting reaction. The effect of milk cooling, milk freezing, various heat treatments, homogenization, salt addition, different rennet concentration, milk protein cross-linking and Ca^{2+} addition on renneting reaction were studied.

MATERIALS AND METHODS

Materials:

Buffaloes' milk was obtained from the faculty of Agriculture dairy herd. Calcium chloride (Merck, Darmstadt. Germany), rennet (0.2N) (from local source), TCA (Merck) and sodium chloride (Merck) were used. A microbial (*Streptoverticillium spp*) Transglutaminase was a gift from Ajinomoto Europe Sales (Stubbenhuk 3, D-20459, Hamburg, Germany); the declared activity of the preparation was approximately 1000 units/g.

Milk treatments:

Milk whether cooled or not was heat-treated either pasteurized at 63°C/30 min., 72°C/15s and 85°C/15s or boiled at 100°C/15s. Cooling milk was carried out at 5°C for 48 hours. Milk freezing was carried out at -15°C for 48 hours and thawed at room temperature. Sodium chloride was added to raw milk before the addition of rennet at a rate of 5 and 10%. Milk was homogenized at 13.8 and 3.4 Mpa in 1^{st} and 2^{nd} stages at 55°C. Calcium chloride 4% solution was add at a rate of 1 ml / 180 ml milk (equals to 0.02% $CaCl_2$ which is usually added to cheese milk when heated at high temperature) and this amount of $CaCl_2$ was doubled in the experiment of double Ca^{2+}. Milk was treated with Transglutaminase enzyme at a rate of 0.5g enzyme powder/Kg milk and was incubated at 50°C for 2 hours. All renneting reactions were carried out by adding 0.4 ml of 0.2 N rennet (per 180 ml milk) after 4 times-dilution with distilled water at 40°C.

Non protein nitrogen was determined in a 4% TCA milk filtrate measured at a wavelength of 210 nm (Lopez *et al.* 1999).

Viscosity measurements:

Milk viscosity measurements on renneting were carried out in triplicates over temperature of 40°C using a concentric cylinder Brookfield Programmable viscometer (Model DV -II+; Brookfield Engineering Laboratories, USA) with UL adaptor and ULA spindle over a shear rate of

12.2 s^{-1}. The milk samples were allowed to temper at 40°C for 10 min. prior to measurements. WinGather version 1.1 (Brookfield Engineering Laboratories, Inc., Copyright© 1995) software was used to collect, store and plot the data on a personal computer connected to the viscometer.

Statistical Analysis:

Numerical results were plotted as the arithmetic mean. Analysis of variance (one way ANOVA) was used for multiple comparisons over the different treatments. The statistical significance of the data was determined using Fisher's L.S.D. post hoc test. *P* value was equal to or less than 0.05 was considered sufficient to reject the null hypothesis. Statistical analysis was performed by running the SPSS 12.0 (SPSS Inc., Copyright© 2003, Chicago, IL, USA) package on a personal computer.

RESULTS AND DISCUSSION

Milk viscosity changes immediately after the addition of rennet was followed up and was drawn against time. Figure (1) shows the obtained viscosity change diagram of untreated raw milk renneting reaction. There were 4 turning points (T_1 to T_4) in the diagram. After the addition of rennet there was an induction period with no change in viscosity (T_1) after which viscosity rose sharply to achieve an initial maximum (T_2) followed by a viscosity minimum (T_3) with milk still in the liquid form. Finally, viscosity increased rapidly until coagulum was formed. At this stage a plateau in viscosity (T_4) was reached.

Workers have reported a viscosity diagram starting with a controversial initial viscosity decrease followed by an increase in viscosity till aggregation occurs (Dekruif *et al.* 1992 and Lopez *et al.* 1999). It seems that this 4 turning point diagram in this work shows the reaction behavior beyond the reported initial viscosity decrease or it is part of the lag phase. Actually some workers could not observe such initial decrease (Guthy and Novak, 1977).

During the lag phase, the main reaction would be the hydrolysis of *k*-casein with rennet causing a reduction in the charge and in the hydrodynamic diameter of casein micelles. The newly formed *para-k*-casein could be mutually attracted to each other with the decrease in charge and with some Ca^{2+} bridging. Also there are contributions of weak linkages such as hydrogen and hydrophobic bonds (Surel *et al.* 2003 and Marchesseu *et al.* 1998) these attractions compensated the reported decrease in viscosity which might occur due to the decrease in the hydrodynamic diameter of the hydrolyzed micelles.

At certain percent of *k*-casein hydrolysis (differs by different milk treatment), Ca-bridging and other attracting forces increases to reach a level causing the first increase in viscosity (T_1) thus ending the lag phase. Of course, rate of hydrolysis determines the rate of Ca^{2+} bridging. Therefore, when milk was treated with transglutaminase enzyme, which made *k*-casein bonds inaccessible for rennet hydrolysis, coagulation was not formed indicating that the rate and degree of *k*-casein hydrolysis determine the shape of the diagram.

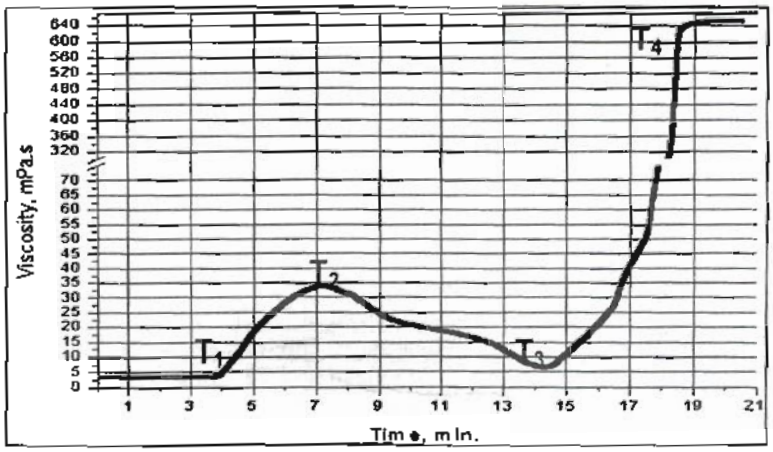

Fig. (1): Viscosity changes diagram of raw milk renneting reaction

At the beginning, the above attractions might be in the form of loose clusters that could be dispersed by stirring causing viscosity reduction to minimum (T_3). By appearance milk showed no visible change and was in the liquid form. As *k*-casein hydrolysis continues, Ca-bridging of *para-k*-casein and other attracting forces predominantes forming large number of aggregates causing the final rise in viscosity (T_4) ending with a viscosity plateau when milk coagulates. Non protein nitrogen (NPN) was determined as indicator of *k*-casein hydrolysis and the release of glycomacropeptides. Non protein nitrogen was determined at the four turning points and was calculated as a percent of total NPN released at complete coagulation, (T_4) and was reported in Table (1). The three turning points T_1, T_2 and T_3 occurred at 18, 36 and 49% NPN, respectively. At the end of the lag phase, the first increase in viscosity (T_1), few numbers of Ca-bridging occurred since only 18% of hydrolysis occurred. Viscosity reached its first maximum at 36% of hydrolysis. Probably the weakly attracted micelles were dispersed causing the dip of viscosity (T_3). At 49% of *k*-casein hydrolysis, large and stable aggregates were formed causing the large increase in viscosity to complete milk coagulation.

The above renneting reaction diagram was used to study the effect of milk treatments on the renneting reaction.

The effect of milk heat treatment was tested. Figure (2) shows the effect of milk heat treatments on the viscosity changes diagram of milk renneting reaction. Milk heat treatments elongated the lag phase (T_1), increased the level of the first maximum viscosity (T_2), and elongated milk coagulation time T_4. The elongation percent of both lag phase and final coagulation time were increased by temperature. The increase was significant at $p \leq 0.05$. The delay in the lag phase was 35%, 137%, 213% and 302% of raw milk lag phase for heating at 63, 72, 85 and 100°C, respectively. Figure (3) shows a strong correlation ($R^2 = 0.9708$) between heat treatment temperature and T_1, which means that T_1 could be used to indicate milk heat treatment. On heating milk at temperatures above 70°C two factors were

reported to cause the coagulation delay namely β-lactoglobulin-*k*-casein interaction and the decrease of Ca^{2+}.

Table (1): Non-protein nitrogen contents of milk at different coagulation phases and treatments.

Coagulation phase	Milk Treatment	NPN[1], %
End of lag phase (T_1)	Raw	18.3
First viscosity maximum (T_2)	Raw	36.4
Minimum of viscosity dip (T_3)	Raw	49.7
Plateau of final viscosity (T_4)	Raw	100
T_1	Pasteurized at 72°C/15s	45.3
T_1	Pasteurized and homogenized	28
T_1	Pasteurized and Ca^{2+} was added	45.3
T_1	Cooled and pasteurized	64.3
T_1	Pasteurized and 5% NaCl	52.3

1- NPN, % was calculated as percent of total NPN released at complete coagulation.

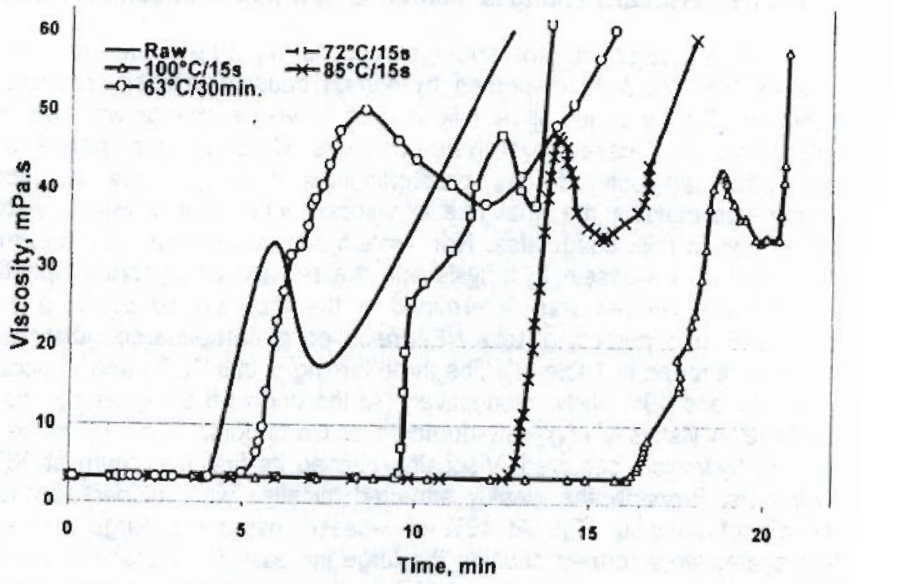

Fig. (2): The effect of milk heat treatments on viscosity changes diagram of milk renneting reaction.

Therefore, at 63°C, the decrease in Ca^{2+}, though slight, was the main delaying factor. Figure (4) illustrates the effect of various treatments run on milk that was pasteurized at 63°C for 30 minutes. The effect of the decrease in Ca^{2+} was tested by the addition of Ca^{2+} to the heated milk. This caused the lag phase to return back to the value of raw milk. However, the addition of Ca^{2+} to milk heated to temperatures higher than 63°C did not restore raw milk lag phase. This proved that the decrease in the concentration of calcium ions was the main factor in delaying T_1 at 63°C. The amount of Ca^{2+} added was 0.02%, the amount regularly used as additive for cheese milk that heated for high temperature (~80°C).

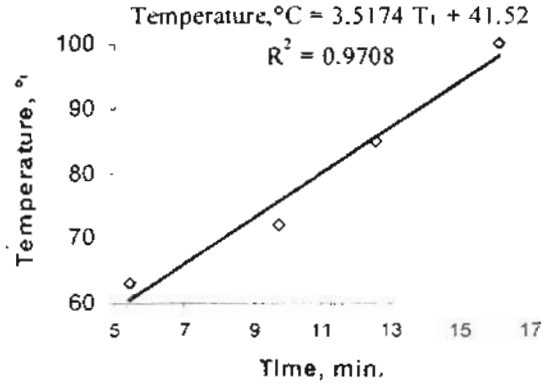

Fig (3): The relationship between temperature of heat treated milk (as independent variable) and time of lag phase.

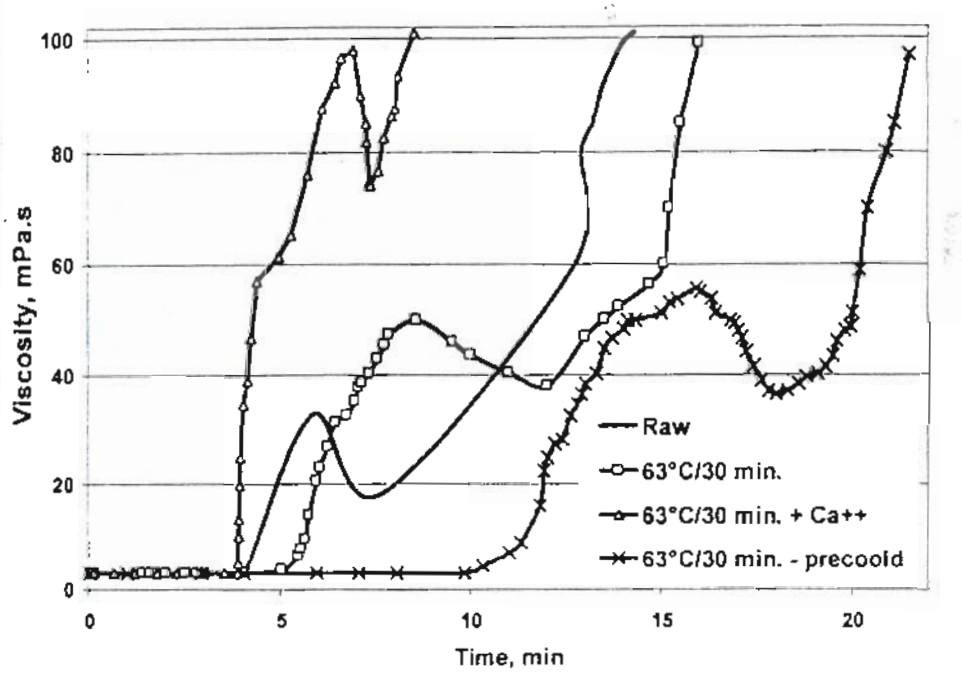

Fig. (4): Viscosity changes during renneting reaction of milk pasteurized at 63°C/30 min. as affected by cooling prior pasteurization and addition of Ca^{2+}.

Figure (5) shows the effect of various treatments ran on milk that was pasteurized at 72°C/15s. The treatment elongated T_1 by 237% of T_1 of raw milk and after the addition of Ca^{2+} became 178%. Only after the addition of double amount of Ca^{2+}, the 72°C-milk retained the value of T_1 of raw milk. At this temperature, protein interactions started to occur as well as the amount of insoluble Ca^{2+} increased than at 63°C. Not only that but also, β-lactoglubulin might chelate some of the Ca^{2+} (O'connell and Fox, 2001). The end of the lag period, T_1 occurred at NPN contents of 45% indicating the need for more casein to be hydrolyzed. Probably, the complex formed by proteins interaction, though allowed enough bonds to be hydrolyzed, sterically prevented Ca^{2+} to reach sites of bridging on *para-k*-casein. The increase in the amount of hydrolysis thus gave more particles increasing the chances for these sites to be exposed for bridging. This was supported by the fact that doubling amount of Ca^{2+} just restores the value of raw milk but the addition of rennet to double the amount used for the coagulation, greatly decreased T_1 than of the raw milk by 45% without the addition of Ca^{2+}. This pointed out that heating milk at 72°C and above, protein hydrolysis was the rate-limiting step in coagulation as compared to the shortage of calcium ions activity. Increasing the concentration of rennet and doubling Ca^{2+} reduced T_1 to one third of T_1 of raw milk.

Therefore, the correction of the effect of heat could be done by increasing rennet concentration, which corrects the effect of protein interactions. Actually, the decrease in coagulation time with the increase in Ca^{2+} activity was reported to be eveled off at high Ca^{2+} activity (Udabase *et al.* 2001).

Figure (6) reports the effect of heating milk at 85°C/15s on the renneting reaction diagram. The treatment delayed T_1 by 312% of T1 of raw milk, and the addition of Ca^{2+} reduced this delay into 230%.

Figure (7) shows the effect of heating milk at 100°C for 15s on the renneting reaction diagram. Heating elongated T_1 by 405% and the addition Ca^{2+} reduced the delay to 257% of raw milk. Heating at 100°C increased Ca^{2+} insolubility, the denaturation of β-lactoglobulin and started to affect the micelles integrity. Therefore the increase in Ca^{2+} activity alone was not enough to correct the effect of such high heat. There is minimum concentration requirements for colloidal calcium phosphate and casein in the micelles should be met before Ca^{2+} decreases coagulation time (Udabase *et al.* 2001).

The treatment that also corrects the delaying effect of heating milk is homogenization. Homogenization of milk pasteurized at 72°C/15s alleviated the effect of heating and shortened milk lag phase and complete coagulation by 5% and 46.3% of the values of raw milk (Figure 5). Homogenization mainly breaks fat globules as well as some of casein micelles into smaller sizes. Fat newly formed surfaces absorb casein particles and some whey proteins on their newly formed membrane to a degree that globules acquire some of the casein properties such as heat stability and coagulation. The dispersion of casein micelles and probably separate the β-lactoglobulin from the complex and their rearrangement by homogenization caused the

exposure of bonds of hydrolysis, thus not only overcome the complex formation of β-lactoglobulin-*k*-casein but also made the rest of the bonds more accessible for hydrolysis and the formed *para-k*-casein for Ca^{2+} bonding. However, homogenization could not do such effect when caseins cross-linked covalently with TGase. The TGase treated milk did not coagulate with rennet even after homogenization.

Milk heat treatment caused the first increase in viscosity to be significantly ($p \leq 0.05$) greater than raw milk. This might be due to casein micelles-β-lactoglobulin complex and the increase in β-lactoglobulin solution viscosity by heating (Photchanachai and Kitabatake, 2001 and Jeurnink and Dekruif, 1993) This increase was 171 and 143% of raw milk viscosity for 63°C and 72°C treatments, respectively (Figure 2). The highest increase was done by homogenization. The increase was 243% of raw milk viscosity for 72°C-pasteurized and homogenized milk. The addition of double Ca^{2+} and double rennet to pasteurized milk at 72°C caused 202% and 293% increase of raw milk viscosity, respectively. Once again the addition of rennet helped the coagulation and the increase in viscosity than addition of Ca^{2+}.

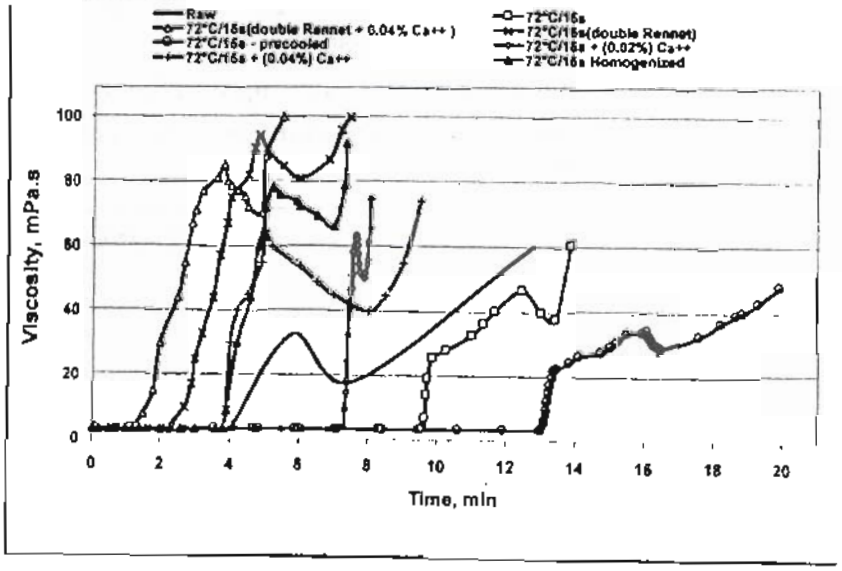

Fig (5): Viscosity changes during renneting reaction of milk pasteurized at 72°C/15s as affected by cooling milk prior pasteurization, the addition of different concentration of Ca^{2+} and rennet and homogenization.

The level of viscosity decrease (T_3) after its first maximum also was changed significantly ($p \leq 0.05$) by treatments. Raw milk showed the most decrease of about 44% and homogenized milk and milk with double rennet showed the lowest decrease of 6.4% and 7.4%, respectively. This means that homogenization made some arrangements for the micelles and their small aggregates so that viscometer stirring did not effectively disperse them. Also, the increase in hydrolysis by high rennet concentration helped in making large aggregates difficult to disperse.

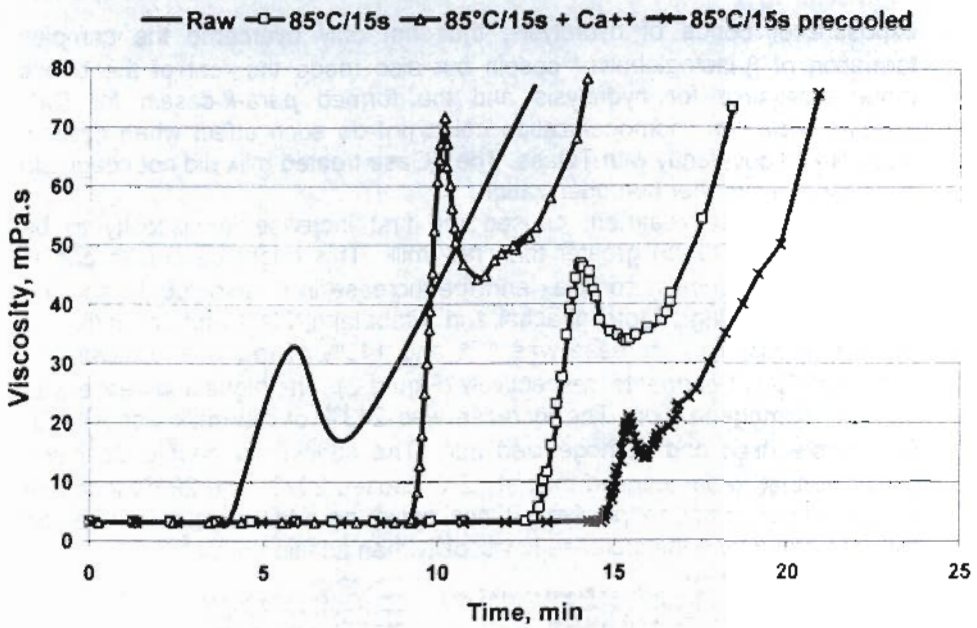

Fig. (6): Viscosity changes during renneting reaction of milk heated at 85°C/15s as affected by cooling prior pasteurization and addition of Ca^{2+}.

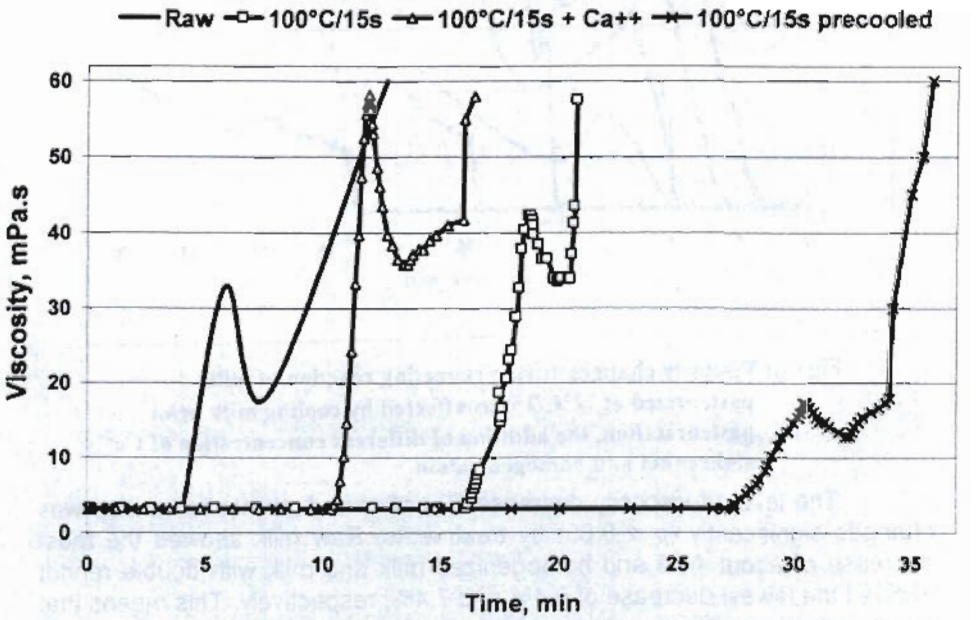

Fig. (7): Viscosity changes during renneting reaction of milk heated at 100°C/15s as affected by cooling prior pasteurization and addition of Ca^{2+}.

Milk cooling before heating, exerted more drastic delaying effect of the lag phase and milk coagulation time than heat treatments. The lag phase significantly ($p \leq 0.05$) increased by 41%, 15%, 157%, 267% and 585% of raw milk values for pre-cooled milk heated at 63, 72, 85 and 100°C (Figure 4 through Figure 7), respectively. The first and the second rise in viscosity were gradual, which means the reaction proceeded slowly. The end of lag phase and the first increase in viscosity was at 64.6% NPN. This means that a lot of hydrolysis was required for pre-cooled milk to coagulate as a result of the changes that were reported to be induced by cooling. Cooling was reported (Raynal and Remeuf, 2000) to cause the dissociation of casein fractions from the micelles especially β-casein, increase the solubility of calcium phosphate and the decreasing of micelles size. These changes increased in milk coagulation time, reduced gel strengthening rate and curd firmness. These detrimental effects occurred since the integrity of casein micelles and the presence of minimum requirements for colloidal calcium phosphate and caseins in micelles are required for proper coagulation. These workers reported that milk original properties could be partly restored by milk heating, slight acidification, addition of protein or calcium. In this work heating milk did not correct the changes caused by cooling. Actually, the elongation of T_1 and coagulation time (T_4) of pre-cooled raw milk was less than the elongation of pre-cooled heated milk.

Figure (8) shows the viscosity diagram of cooled and frozen raw milk. The T_1 delay percent for pre-cooled raw milk, frozen raw milk and cooled pasteurized milk at 72°C of cold milk were 41.7, 65 and 225%, respectively. Freezing, suppose to be more effective in the delay because freezing destabilizes fat emulsion and casein colloidal system.

One of other treatments that are practiced and affects milk coagulation is the addition of salt to milk before renneting such as in the Egyptian white cheese. Five and 10% of salt were dissolved in milk before renneting and viscosity diagrams were determined (Figure 9). Salt greatly and significantly ($p \leq 0.05$), delayed the lag phase as well as the coagulation time than all other milk treatments. The delayed effect significantly ($p \leq 0.05$) increased by salt percent. The lag phase delay percent of raw milk for 5 and 10% salt were 1694 and 2400%, respectively. The first increase in viscosity was very small and was at 52% NPN, which means a lot of hydrolysis, was required for salted milk to end the lag phase and start the increase in viscosity. This should be expected since NaCl replaces the calcium phosphate of colloidal part of the micelles causing the increase in the solubility of micellar calcium phosphate and loosens the structure of casein micelles thus giving the same detrimental effect on coagulation as described earlier. In another experiment salted milk was homogenized. Homogenization corrected a good part of the detrimental effect of salt addition. The lag phase was shortened to be 1117% of raw milk values compared to the 1694% before homogenization. Also, there was a good increase in the value of the first increase in viscosity. Once again, homogenization helped the coagulation through the physical rearrangement of casein micelles.

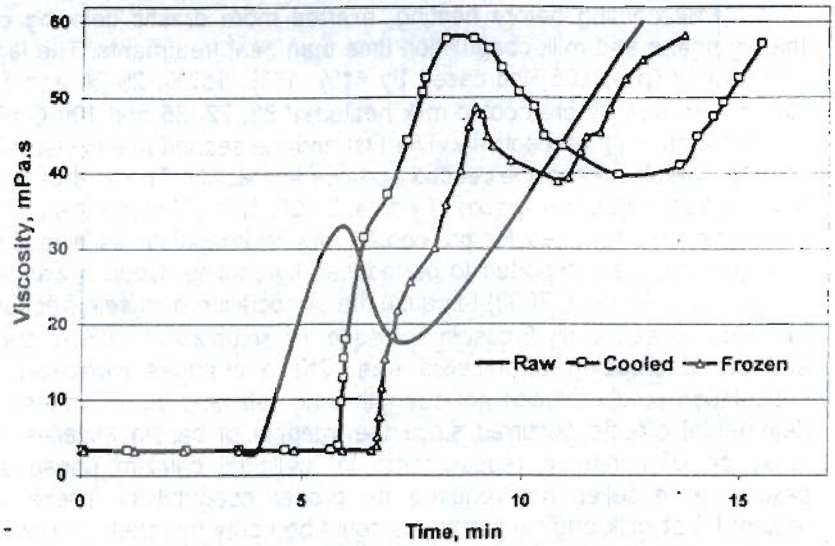

Fig. (8): The effect of raw milk cooling and freezing on viscosity diagram of milk renneting reaction.

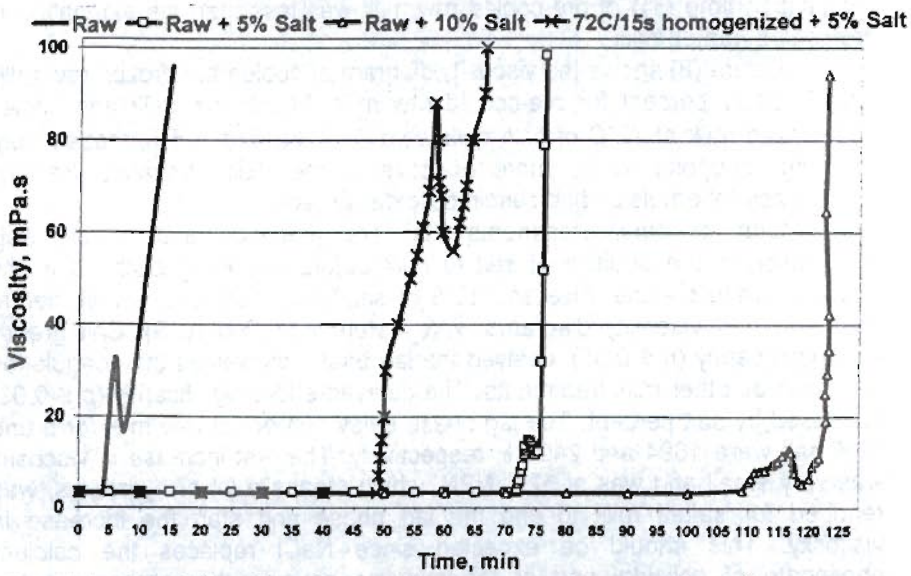

Fig. (9): Viscosity changes during renneting reaction of raw and homogenized milk as affected by salting with 5 and 10%.

The final viscosity measuments were not accurate since the coagulum was destroyed. This is why the plateau not recorded in the diagrams. However, the viscosity plateau of some samples would be indicative of the coagulum hardeness. Heat treatments at 63, 72, 85 and 100°C gave final viscosity readings of 580, 636, 310 and 250 mPa.s compared to 650 mPa.s for raw milk, respectively. Milk heated at the above

temperatures after cooling showed 400, 359, 241 and 166 mPa.s, repectively. Addition of NaCl to milk also gave low viscosity.

Though this method of measuring milk viscosity for monitoring renneting reaction was destructive for the sample it demonstrated the reaction phases successfully. This 4 phase diagram of renneting reaction was not reported before. The diagram lent itself for studying the effect of milk various treatments on renneting reaction by studying the effect on each phase. Milk heat treatment could be determined by measuring T_1 and vice versa. Timing of the incipient coagulation could be determined by timing the intersection of two straight lines, one is the extension of lag phase and the other is the linearity final increase in viscosity as suggested by Ay and Gunasekaran (1994).However,at this point of intersection milk appeared fluid.

REFERENCES

Aoki, T.; T. Umeda and T. Nakano (1999). Effect of sodium chloride on the properties of casein micelles. Milchwissenschaft 54: 91-93.

Ay, C. and S. Gunasekaran (1994). Ultrasonic attenuation measurements for estimating milk coagulation time.Transactions of the ASAE 37:857-862.

Dekruif, C. G.; T. J. M. Jeurinink and P. Zoon (1992). The viscosity of milk during the initial stages of renneting. Neth. Milk Dairy J. 46: 123-137.

Gastaldi, E.; N. Trial; Giullaume; E. Bourret; N. Gontard and J. L. Cug (2003) Effect of controlled *k*-casein hydrolysis on rheological properties of acid milk gels. J. Dairy Sci. 86: 704-711.

Ghosh, B. C. and H. G. Kessler (2001). Rennetability of whole milk homogenized before or after high heat treatment. Indian J. Dairy Sci. 54: 138-143.

Guthy, K. and G. Novak (1977). Observation on the primary phase of milk coagulation by rennet under standardized condition. J. Dairy Res. 44: 363-366.

Jeurnink, T. J. M. and K. G. Dekruif (1993). Changes in milk on heating: viscosity measurements. J. Dairy Res. 60: 139-145.

Lomholt, S. B. and K. B. Ovist (1997). Relationship between rheological properties and degree of *k*-casein proteolysis during renneting of milk. J. Dairy Res. 64: 541-549.

Lopez, M. B.; M. J. Jordan; M. V.Granados;J.C. Fernandez; M. Castillo and J. Laencina(1999).Viscosity changes during rennet coagulation of Murciano-Grandina goat milk. International J. Dairy Technology 53: 102-108.

Marchesseu, S ; A. Lagaudl; J. L. Cug; B. Tarodo de la Fuente; E. Lefebver-Cases; E. Gasotaldi and V. Vidal (1998). Identification of intractions among casein gel using dissonantly chemical agents. J. Dairy Sci. 81: 932-938.

O'connell, J. E. and P. F. Fox (2001_. Effect of β-lactoglobulin and precipitation of calcium phosphate thermal coagulation of milk. J. Dairy Res. 68:81-94.

O'sullivan, M. M.; A. L. Kelly and P. F. Fox (2002). Influence of transglutaminase treatment on some physico-chemical properties of milk. J. Dairy Res. 69: 433-442.

Photchanachai S. and N. Kitabatake (2001). Heting of β-lactoglobulin A solution in a closed system. Food Sci. 66: 647-652.

Raynal, K and F. Remeuf (2000). Effect of storage at 4°C on the physical and renneting properties of milk: a comparison of caprine, ovine and bovine milks. J. Dairy Res. 67:199-207.

Schreiber, R. (2001). Heat-induced modification in casein dispersions affecting their rennetability. International Dairy J. 11: 553-558.

Surel, D. and M. Famelart (2003). Heat induced gelation of acid milk: balance between weak and covelant bonds. J. Dairy Res. 70: 253-256.

Udabase, P; R. Mckinnon and M. A. Augustin (2001). Effects of mineral salts and calcium chelating agents on the gelation of renneted skim milk. J. Dairy Sci. 84: 1569-1575.

إستخدام التغير في منحنى العلاقة بين اللزوجة-الزمن في دراسة تأثير معاملات اللبن المختلفة على تجبن اللبن بالمنفحة.

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أجري هذا البحث بغرض تطوير طريقة جديدة لتتبع تأثير معاملات اللبن المختلفة على المراحل المختلفة لتجبن اللبن بالمنفحة. من المعروف أن التجبن الإنزيمي يتم من خلال مرحلتين أساسيتين الأولى مرحلة إنزيمية فقط يتم فيها تحليل *k*-casein يليها المرحلة الثانية التي يتدخل فيها أيونات الكالسيوم الحرة باللبن لتسبب تجمع لميسيلات الكازين و هكذا حتى يشاهد التجبن. من الناحية النظرية لا يمكن الفصل بين المرحلتين و كذلك لا يمكن عمليا تحديد الزمن اللازم لإنهاء كل مرحلة و بالتالي كان من الصعب معرفة تأثير المعاملات المختلفة للبن على مرحلتي التجبن فهل المعاملات التي تسرع من تجبن اللبن بالمنفحة سببها الأسراع في المرحلة الأولى أم الثانية؟ و بالمثل للمعاملات التي تطيل من زمن التجبن بالمنفحة. تم في هذا البحث تطوير طريقة تعتمد على قياس التغير في لزوجة اللبن مباشرة من بعد إضافة المنفحة للبن و حتى حدوث التجبن ثم رسمت هذه التغيرات الحادثة في لزوجة اللبن ضد الزمن منذ إضافة المنفحة لتكون منحنى العلاقة بين اللزوجة-الزمن و الذي من خلاله أمكن التعرف على زمن كل مرحلة و بالتالي درست العوامل التي تؤثر على تجبن اللبن بالمنفحة بنفس الأسلوب مما ساعد على الإجابة على بعض الأسئلة السابقة.

تم دراسة تأثير كل من المعاملات الحرارية المختلفة (بسترة سريعة و بطيئة ، التسخين على 85°C و كذلك تأثير غلي اللبن) و كذلك درس تأثير تخزين اللبن تحت ظروف التبريد و التجميد. و درس أيضا تأثير أضافة تركيزات مختلفة من أيونات الـ Ca^{2+} و كذلك من المنفحة و من الـ NaCl أيضا. و درس أيضا تأثير التجنيس و تأثير إجراء عملية Cross-linking لبروتينات اللبن باستخدام إنزيم الـ Transglutaminase – و ذلك على مراحل تجبن اللبن بالمنفحة من خلال منحنى العلاقة بين اللزوجة-الزمن السابق ذكرة.

أظهرت النتائج ان هناك أربع مراحل أساسية في التغير للزوجة أثناء تفاعل اللبن مع المنفحة و هي مرحلة الـ lag phase او المرحلة التحضيرية و هذة لم يشاهد فيها أي تغير في اللزوجة (T_1) و هذة تمثل المرحلة الأولى من التجبن حيث يتم فيها فقط تحليل الـ *k*-casein و أمكن معرفة الزمن اللازم لها (T_1) و وجد انة هو الأساس في تأخير أو إسراع زمن التجبن عند معاملة اللبن بالحرارة و وجد ان هناك أرتباط كبير و معنوي ($r = 0.98$, $p \leq 0.01$) بين درجة الحرارة التي تم تسخين اللبن عليها و زمن الـ (T_1)و تم حساب معادلة الإنحدار التي يمكن من خلالها حساب درجة الحرارة التي تم تسخين اللبن عليها بمعلومية الـ (T_1). و جد أن إضافة أيونات الكالسيوم أدت لإختصار هذة المرحلة و كلما زادت النسبة المضافة كلما زاد مقدار الإختصار في هذة الفترة و بالتالي إنخفاض زمن التجبن الكلي. و نفس السلوك مع زيادة تركيز المنفحة فأمكن علاج التأخير في (T_1) بكلا الطريقتين. و كذلك عالج تجنيس اللبن التأخر الحادث بدرجة ممتازة. أما تخزين اللبن تحت تبريد أو تجميد فأطال جدا من زمن الـ (T_1) و وجد أن هذا التغير ليس عكسيا. كذلك إضافة الملح أطالت جدا من زمن (T_1) و كان مقدار الإطالة متناسب تناسبا طرديا مع نسبة الملح المضافة و لكن عالج تجنيس اللبن هذة الإطالة بدرجة كبيرة. اللبن المعامل بانزيم Transglutaminase لم يتجبن تماما لأن تفاعلة مع بروتين اللبن أدى إلى إخفاء الروابط التي يعمل عليها إنزيم المنفحة.

تلي هذة المرحلة مرحلة أخرى و هي زيادة سريعة في لزوجة اللبن و هي تمثل عن بدأ المرحلة الثانية و هذا يعني انة تم تحليل كمية كافية من الـ *k*-casein لتبدأ أيونات الكالسيوم في عمل Ca-bridging و ادت هذة التجمعات لزيادة اللزوجة و هكذا حتى وصلت إلى القمة الأولى للمنحنى و سمي الوقت عندها بـ (T_2) و أختلف هذا الزمن ايضا اختلافا معنويا بمعاملات اللبن المختلفة.

عقب ذلك إنخفاض سريع للزوجة حتى وصلت لأقل إنخفاض minimum و سمي الزمن الذي وصلت بعدة اللزوجة للـ minimum بـ (T_3) ثم ازدادت الزوجة بعدها سريعا حتى وصلت لأعلى قيمة و حدث بها ثبات بمرور الوقت و هذة سميت بـ (T_4) و اختلفت كل من الـ T_3 and T_4 ايضا اختلافا معنويا ما بين المعاملات المختلفة للبن.

أظهرت النتائج أن هذة الطريقة البسيطة كانت فعالة في تتبع تفاعل إنزيم المنفحة مع اللبن وكذلك في الأجابة عن الكثير من التساؤلات عن تأثير المعاملات المختلفة للبن على التجبن الإنزيمي لة.