Rennet coagulation properties of heated milk

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LUCEY, J. A., GORRY, C. & FOX, P. F. 1993. **Rennet coagulation properties of heated milk.** Agric. Sci. Finl. 2: 361-369. (Teagasc, National Dairy Products Research Centre, Moorepark, Fermoy, Co. Cork, Ireland and Dept. Food Chem., National Food Biotechnology Centre, University College, Cork, Ireland.)

Heating impaired the rennet coagulation properties of milk which deteriorated further during storage, i.e. rennet hysteresis occurred. Acidification to pH values ≤ 6.2 or addition of low concentrations of $CaCl_2$ greatly improved the rennet coagulation properties of heated milk. Acidification of heated milk to pH values < 5.5 followed by neutralization to pH 6.6 to produce reformed micelles, resulted in greatly improved rennet coagulation properties except for severely heated milks (120°C for 10 min) which were not coagulable even after acidification/neutralization. Acidification of heated milk to pH values ≤ 5.5 and storage at the low pH for 24 h before neutralization resulted in a further improvement in the rennet coagulation properties. Dialysis of heated milk that had been acidified and reneutralized against an excess of normal milk resulted in a dramatic deterioration of its rennet coagulability. Reheating milk that had been heated, acidified and reneutralized resulted in little change in RCT or gel firmness. Addition of heated milk to raw milk resulted in an increase in RCT of the latter and a reduction in gel firmness.

Key words: rennet coagulation, heated milk, acidification and reneutralization

Introduction

Severe heating (> 75°C for > 30 sec) of milk, without pH adjustment or other treatment impairs its renneting properties and renders it difficult to coagulate (MORRISSEY 1969, VAN HOOYDONK et al. 1987, SINGH et al. 1988, LUCEY 1992). It has been established that when heated, β-lactoglobulin (β-lg) and κ-casein form a complex via sulphydryl - disulphide interchange (ZITTLE et al. 1962, SAWYER et al. 1963, SAWYER 1969), although hydrophobic interactions may play a role in the initial stages of complex formation (HAQUE and KINSELLA 1988). This is the most important factor affecting the rennet coagulability of heated milk (KANNAN and JENNESS 1956, 1961, VAN HOOYDONK et al. 1987, DALGLEISH 1990).

The rennet coagulation time (RCT) of milk increases with the severity of heat treatment (MORRISSEY 1969, SINGH et al. 1988, VAN HOOYDONK et al. 1987, DALGLEISH 1990, LUCEY 1992). However, there are conflicting reports as to whether the increased RCT is due to impairment of the enzymatic or aggregation reactions, or both. There have been several reports that the total amount of peptides released by chymosin is reduced when casein micelles are subjected to extremely high temperatures, e.g. 90°C for 1 h, in the presence of β-lg (WHEELOCK and KIRK 1974, SHALABI and WHEELOCK 1976) or α-lactalbumin (SHALABI and WHEE-

LOCK 1976). However, MARSHALL (1986) reported that the enzymatic stage is hardly affected by heating at 75 or 85°C for 30 min. VAN HOOYDONK et al. (1987) showed that heating decreased both the initial velocity of κ-casein hydrolysis and the amount of hydrolysable κ-casein in milk heated up to 120°C for 5 min. REDDY and KINSELLA (1990) reported that heating (85°C for 15 min) of β-lg with κ-casein or with casein micelles inhibited chymosin hydrolysis, resulting in a reduced initial rate of hydrolysis (37%) and a reduction (26%) in the amount of glycomacropeptide released; heating casein micelles alone did not affect hydrolysis by chymosin. REDDY and KINSELLA (1990) suggested that the conformation of the chymosin-susceptible bond of κ-casein may be somewhat different and probably less readily accessible to the enzyme after complex formation with β-lg.

It is generally agreed (PYNE 1945, MORRISSEY 1969, MARSHALL 1986, VAN HOOYDONK et al. 1987, SINGH et al. 1988) that the secondary phase of rennet coagulation is more adversely affected by heating than the enzymatic phase. Denatured whey proteins on the surface of casein micelles sterically hinder the aggregation of rennet-converted micelles resulting in prolonged RCT (VAN HOOYDONK et al. 1987).

Heated milks exhibit a phenomenon known as rennet hysteresis, i.e. the RCT increases when the heated sample is cooled and held following heating (MATTICK and HALLETT 1929, POWELL and PALMER 1935, PYNE 1945, MORRISSEY 1969, LUCEY 1992). Most of the hysteresis effect occurs within 5 h of heating (MATTICK and HALLETT 1929, MOIR 1930). Heating causes the precipitation of calcium phosphate with a concomitant reduction in soluble calcium (MATTICK and HALLETT 1929, PYNE 1945, HILGEMAN and JENNESS 1951, TESSIER and ROSE 1958, DEMOTT 1968, GEERTS et al. 1983) which inhibits the aggregation reaction which is sensitive to changes in [Ca^{2+}] (PYNE 1945). Some authors (PYNE 1945, MORRISSEY 1969, VAN HOOYDONK et al. 1987) have suggested that rennet hysteresis is caused mainly by the slow solubilization of heat-induced calcium phosphate during storage, while others (KANNAN and JENNESS 1961) consider that the heat-induced interaction between β-lg and κ-casein is the principal factor involved. LUCEY (1992) showed that heat-precipitated calcium phosphates did not solubilize during cold storage and was not responsible for rennet hysteresis. It is not clear exactly what causes rennet hysteresis but it is possible that during storage of heated milk, physicochemical changes may occur in the β-lg κ-casein complex (SAWYER 1969) which may provide additional steric hindrance and a further reduction in the rate of aggregation.

The strength of rennet gels (curd tension) is also adversely affected in heated milk (DILL and ROBERTS 1959, MAUK and DEMOTT 1959, KEOGH 1966, ASHWORTH and NEBE 1970, SINGH et al. 1988, LUCEY 1992, MCMAHON et al. 1993). This reduction in gel strength is presumably caused by the disruption of the continuity of the gel network caused by attachment of denatured whey proteins to the casein micelles. The denatured whey proteins may sterically hinder the close approach and contact between casein micelles, resulting in a weaker, looser network due to reduced crosslinking (MCMAHON et al. 1993). The reduced aggregation rate of heated milk means that its gel strength increases at a slower rate than in unheated milk. Thus, the strength of rennet-induced gels from heated milk, determined at a particular time after renneting, will be lower than from unheated milk.

The objectives of this study were to investigate the effects of various treatments, i.e. pH adjustment, addition of $CaCl_2$ and acidification/neutralization on the rennet coagulation properties of heated milk.

Material and methods

Heating

Fresh whole milk was heated at temperatures in the range 70 - 100°C for 10 min in an oil bath, while a direct steam autoclave (Astell Scientific, Sidcup, Kent), with a thermocouple mounted in the cap of the milk bottle to monitor temperature, was used for heat treatment at 120°C for 10 min. After heating, milks were cooled rapidly by immersion in ice.

Rennet coagulation properties

The rennet coagulation properties were determined by the Formagraph, as described previously (LUCEY and FOX 1992).

Acidification and reneutralization

Heated milk was cooled rapidly to 2°C before acidification to pH values in the range 6.5 - 4.6 and reneutralized immediately to pH 6.6 at 2°C or held overnight at 2°C before reneutralization. Milks were warmed to 20°C for 1 h, readjusted to pH 6.6, before renneting at 35°C. pH adjustments were performed with an Autotitrator (Mettler DL 21, Greifenese, Switzerland).

Effect of reheating acidified/reneutralized heated milk

Heated (90°C for 10 min) milk was acidified to pH 5.5 at 2°C and reneutralized to pH 6.6 at 2°C. Milks were warmed to 20°C and readjusted to pH 6.6 and then reheated to 90°C for 10 min. The rennet coagulation properties were determined at 35°C.

Effect of $CaCl_2$ on the renneting properties of heated milk

Calcium chloride (1 M) was added to unheated and heated milks at levels of 1 to 10 mM/L. The pH of the Ca-supplemented milks was adjusted to pH 6.6 and held for 1 h prior to rennet coagulation.

Effect of dialysis on the renneting properties of heated milk

Heated milk or heated milk that had been acidified and reneutralized were dialysed against 20 volumes of unheated milk for 2 days at 4°C.

Results and discussion

The RCT of milk increased (Fig. 1a) and the firmness of renneted milk gels decreased (Fig. 1b) as the severity of heat treatment was increased. Milks heated to very high temperatures (> 100° for 10 min) did not coagulate without pH adjustment. Storage of heated milk caused a further deterioration in the rennet coagulation properties, especially for high heat (> 80°C for 10 min) treatments, i.e. rennet hysteresis occurred (Fig. 1a).

Acidification of heated (100°C for 10 min) milk resulted in shorter RCTs (Fig. 2a) and increased gel firmness, with a maximum at pH 6.2 (Fig. 2b). Acidification can therefore offset some of the impaired rennet coagulation properties of heated milks, probably by reducing charge repulsion and increasing $[Ca^{2+}]$.

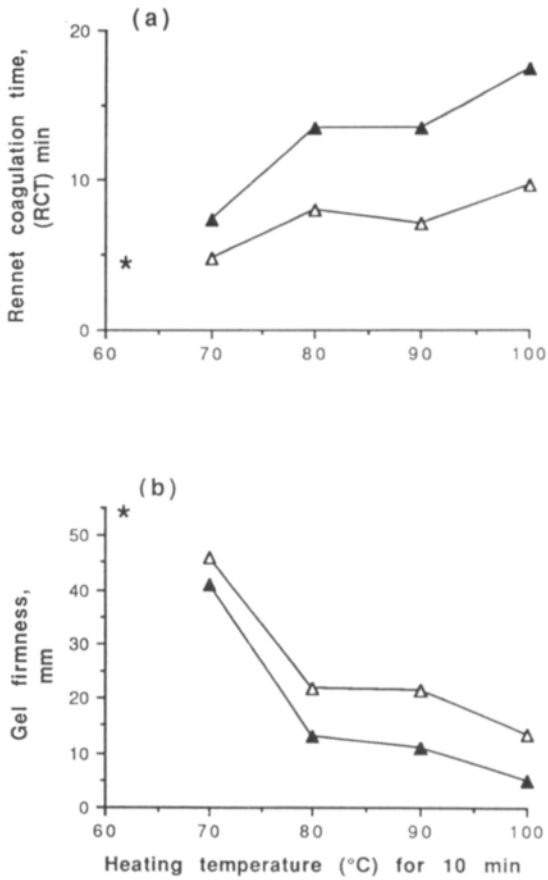

Fig. 1. Rennet coagulation time (RCT) (a) and the firmness (Formagraph reading after 30 min) of the resultant gels (b) of milks immediately after heating (Δ) and after holding at 4°C for 24 h (▲). Control milk (✱) had a coagulation time of 4.5 min and a gel firmness of 54 mm.

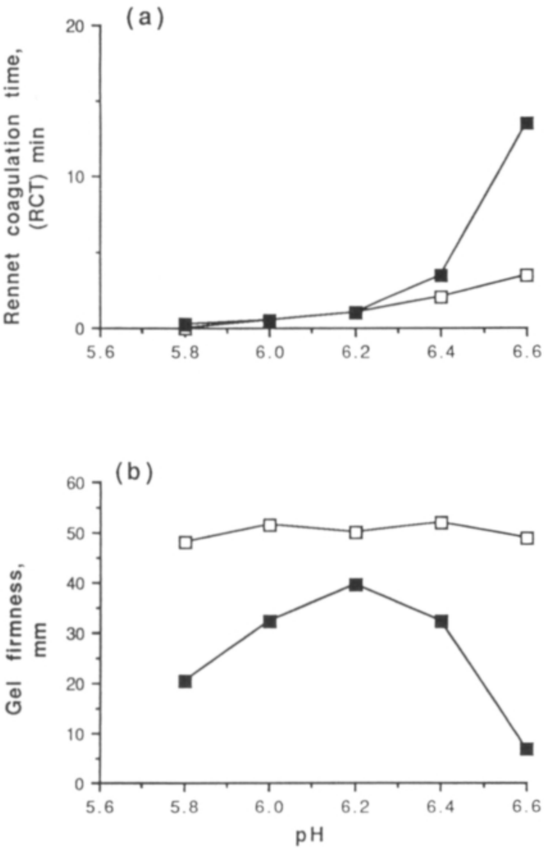

Fig. 2. Effect of pH on (a) rennet coagulation time and (b) the firmness of the resultant gels from unheated milk (□) or from milk heated at 100°C for 10 min (■).

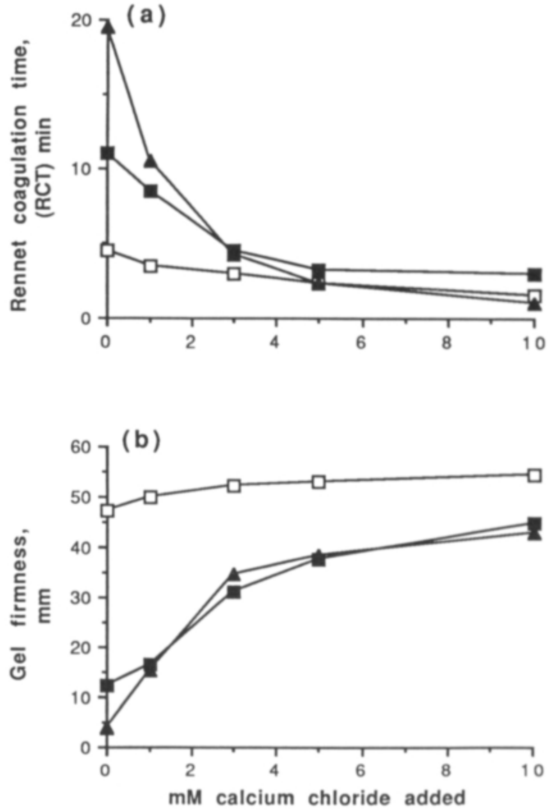

Fig. 3. Effect of $CaCl_2$ on (a) rennet coagulation time at pH 6.6 and (b) the firmness of the resultant gels from unheated milk (□) or milk heated at 100°C for 10 min measured immediately (■) or after 24 h at 4°C (▲).

The addition of low concentrations of $CaCl_2$ resulted in a reduction in RCT (Fig. 3a) and increased gel firmness (Fig. 3b). Heated milks to which ≥ 2.5 mM $CaCl_2$ were added did not exhibit rennet hysteresis or a deterioration in gel firmness during storage. This suggests that the addition of low concentrations of $CaCl_2$ compensates for the additional impairment of the aggregation reaction which apparently occurs during storage of heated milk. However, the addition of $CaCl_2$ did not completely restore the original gel firmness, suggesting that the incorporation of whey proteins adversely affects the gel network. VAN HOOYDONK et al. (1987) and SINGH et al. (1988) reported that the coagulability of high heat-treated milk could be restored by the addition of low concentrations of $CaCl_2$.

Acidification of heated milk (100°C for 10 min) to pH values ≤ 5.5 before immediate neutralization to pH 6.7 resulted in greatly improved RCT (Fig. 4) but gel firmness (Fig. 5) was not completely restored to that of raw milk. Acidification and holding at low pH values for 24 h before neutralization further improved the rennet coagulation properties. Acidified and reneutralized milks have a higher $[Ca^{2+}]$ than normal milk (SINGH et al 1988, TAN and FOX unpublished) which is probably responsible for its improved rennet coagulation properties. Dialysis of heated milk that had been acidified and

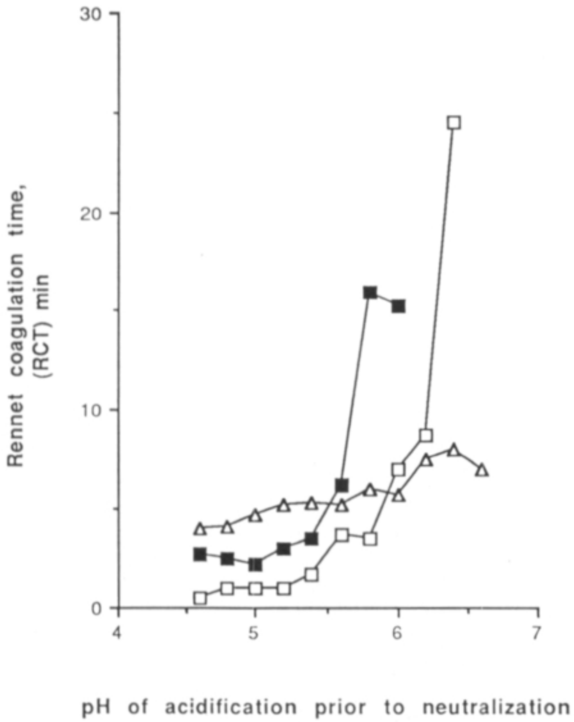

Fig. 4. Rennet coagulation time of unheated milk acidified to pH values in the range 6.5 to 4.6 and reneutralized immediately to pH 6.6 (Δ) or heated milk (100°C for 10 min) acidified to pH values in the range 6.5 to 4.6 and reneutralized immediately to pH 6.6 (■) or heated milk (100°C for 10 min) acidified to pH values in the range 6.5 to 4.6 and maintained at the low pH for 24 h at 4°C before reneutralization to pH 6.6 (□).

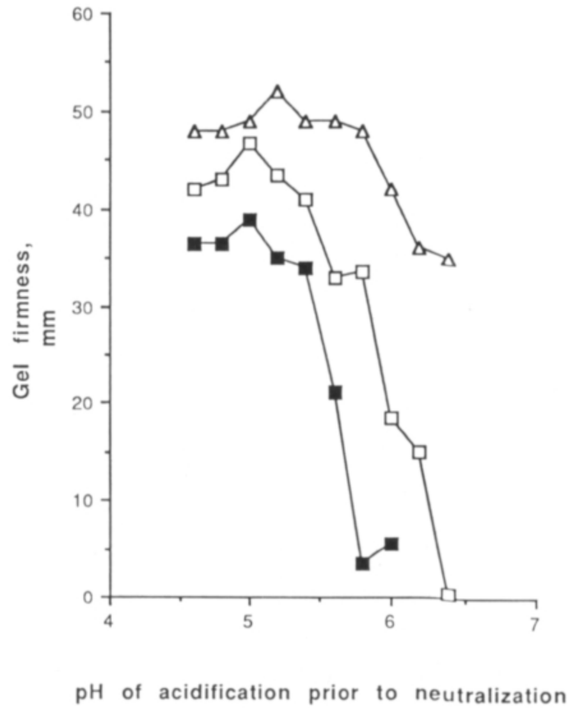

Fig. 5. Firmness of renneted milk gels from unheated milk acidified to pH values in the range 6.5 to 4.6 and reneutralized immediately to pH 6.6 (Δ) or heated milk (100°C for 10 min) acidified to pH values in the range 6.5 to 4.6 and reneutralized immediately to pH 6.6 (■) or heated milk (100°C for 10 min) acidified to pH values in the range 6.5 to 4.6 and maintained at the low pH for 24 h at 4°C before reneutralization to pH 6.6 (□).

reneutralized against an excess of raw milk resulted in a dramatic deterioration in its rennet coagulability (Table 1), probably because dialysis restores the [Ca^{2+}] to the original value of milk (TAN and FOX unpublished).

BANKS and MUIR (1985) reported that the casein in starter made from sterilized milk, which was acidified by lactic acid bacteria and subsequently neutralized on addition to the cheese milk, was incorporated into cheese curd. They suggested that this was due to the disruption of micelles at low pH values, making the buried/masked κ-casein available for hydrolysis and capable of participation in gel formation after renneting. However, VAN HOOYDONK et al. (1987) reported that acidification (to pH values ≥ 5.5) and neutralization to pH 6.7 did not increase the rate of hydrolysis of κ-casein. REDDY and KINSELLA (1990) reported that reducing the pH of heated and cooled mixtures of casein micelles with β-lg to pH 5.8 and immediately readjusting to pH 6.8 before the addition of chymosin increased the final amount of glycomacropeptide released by about 13%, although there was little change in the rate of hydrolysis.

VAN HOOYDONK et al. (1987) found that acidification of heated milk to pH values ≥ 5.5, followed immediately by neutralization, had little effect on the renneting properties of heated milks but storage of milk samples for 24 h at pH 5.5 before neutralization improved rennet coagulability. VAN HOOY-

Table 1. Effect of dialysis on the rennet coagulation properties of acidified and reneutralized milk.

Treatment	Rennet coagulation time (RCT) min	Gel firmness (a_{30}) mm
CONTROL		
Original milk	4.0	51.0
Acidified to pH 5.0 and readjusted to pH 6.6	1.5	53.0
Acidified and readjusted milk dialysed against control milk	15.0	8.0
HEATED MILK (100°C for 10 min)		
Heated milk, immediately	11.5	11.0
Heated milk, after storage for 2 days at 4°C	17.5	3.5
Heated milk, dialysed against control milk	7.5	22.0
Heated milk, acidified to pH 5.0 and readjusted to pH 6.6	4.5	28.5
Heated milk, acidified to pH 5.0 and readjusted to pH 6.6 and dialysed against control milk	N.C.	N.C.

N.C. No coagulation was observed

Table 2. Effect of reheating on the rennet coagulation properties of milk that had been previously heated and acidified/reneutralized.

Treatment	Rennet coagulation time (RCT) min	Gel firmness (a_{30}) mm
CONTROL		
Original milk	6.0	36.5
Acidified to pH 5.5, held for 2 h, readjusted to pH 6.6	4.5	48.0
HEATED MILK (90°C for 10 min)		
Original heated milk	11.0	12.5
Heated milk, reheated at 90°C for 10 min	11.0	10.0
Heated milk, acidified to pH 5.5 held for 2 h and readjusted to pH 6.6 (acidified and readjusted)	5.5	15.0
Heated milk, acidified and readjusted before reheating at 90°C for 10 min	6.0	20.0
Heated milk, acidified and readjusted before reheating at 100°C for 10 min	7.5	19.7

DONK et al. (1987) suggested that acidification and storage at low pH values was necessary to solubilize heat-precipitated calcium phosphate which was reprecipitated on neutralization in a form similar to indigenous CCP which they suggested may be the most important factor responsible for the improved rennetability of heated milks following acidification and neutralization. They also suggested that heat-precipitated calcium phosphates may solubilize more slowly than indigenous CCP on acidification and that the slow solubilization of heat-precipitated calcium phosphate during storage may contribute to rennet hysteresis.

However, VAN HOOYDONK et al. (1987) did not acidify heated milk to pH values < 5.5; the results of the present study demonstrate that acidification to pH values < 5.5 improved the rennet coagulation properties of heated milk, even when the samples were reneutralized immediately to pH 6.6.

Reheating milk resulted in little change in RCT or gel firmness (Table 2). Acidification and reneutralization prior to reheating resulted in an improvement in RCT and gel firmness. A similar trend was observed when heated milks were acidified to pH 5.0 and reneutralized prior to reheating (results not shown). After acidification and neutralization, milks should be carefully readjusted to pH 6.6 at 20°C before reheating or precipitation (heat instability) occurred at relatively low temperatures, e.g., 80°C for 10 min.

Addition of heated milk (100°C for 10 min) to raw milk resulted in an increase in RCT of the latter and a reduction in gel firmness (Fig. 6). The blending of low levels of heated milk with unheated milk may provide an acceptable method of utilisizing high heat-treated milk.

Severely heated milk (120°C for 10 min) did not coagulate even after acidification to low pH values

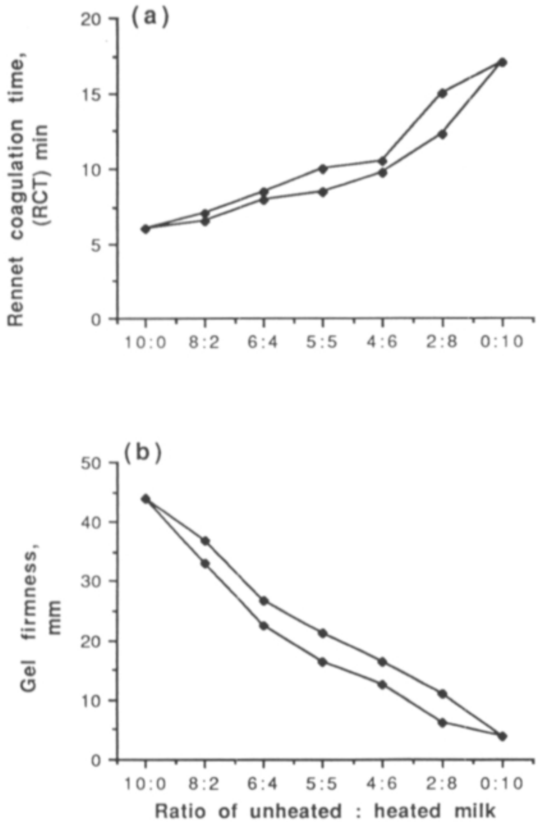

Fig. 6. Effect of blending heated and unheated milks on (a) the rennet coagulation time and (b) the firmness of rennet gels (results from two trials).

and reneutralization to pH 6.6. This suggests that other factors prevent coagulation, e.g., drastic physicochemical changes occur in casein micelles at such high temperatures, including dephosphorylation of protein residues (Howat and Wright 1934) and conversion of CCP to hydroxyapatite (Visser et al. 1986). Heat-induced changes in casein micelles have been reviewed (Singh 1988).

Conclusions

The RCT of milk increased with increasing severity of heat treatment probably due to inhibition of the aggregation of renneted micelles caused by complex formation between β-lg and κ-casein. The increased RCT can be offset by pH adjustment or addition of $CaCl_2$, although the original gel firmness was not completely restored. Acidification and neutralization resulted in greatly improved rennet coagulation properties probably due to increased $[Ca^{2+}]$.

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Manuscript received April 1993

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SELOSTUS

Kuumennetun maidon juoksettumisominaisuudet

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Kuumennus heikensi maidon juoksetteen saostumisominaisuuksia. Varastointi heikensi edelleen juoksettumista. Hapattaminen pH 6,2:een tai alle tai $CaCl_2$:n lisäys pieninä konsentraatioina paransivat huomattavasti juoksettumisominaisuuksia. Hapattaminen ensin alle pH 5,5:een ja sitä seurannut pH:n nosto 6,6:een johti juoksettumismiskyvyn huomattavaan paranemiseen, paitsi voimakkaasti kuumennetuissa maidoissa (120°C 10 min), joissa ei tapahtunut juoksettumista hapattamisen/neutraloinnin jälkeen.

Hapattaminen pH 5,5:een tai alle ja varastointi alhaisessa pH:ssa 24 h ennen neutralointia johti juoksetteen saostumisominaisuuksien paranemiseen. Kuumennetun maidon (hapatettu ja sitten neutraloitu) sekoittaminen normaalin maidon kanssa johti juoksettumiskyvyn huomattavaan heikkenemiseen. Aikaisemmin kuumennetun, hapatetun ja neutraloidun maidon uudelleen kuumentaminen aiheutti vain vähäisiä muutoksia juoksettumiskykyyn tai saostuman kiinteyteen. Kuummennetun maidon lisääminen raakamaitoon johti jälkimmäisen juoksettumiskyvyn paranemiseen mutta samalla saostuman kiinteys heikkeni.