Influence of Heat Treatments on Whey Protein Denaturation and Rennet Clotting Properties of Cow's and Goat's Milk

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Effect of heat treatments (65–85 °C for 5–35 min) on the rennet clotting properties and whey protein denaturation of cow's and goat's milk was investigated. The rennet clotting time (RCT) of raw cow's milk was longer than that of goat's milk. Although heat treatments increased the RCT of cow's milk samples, this parameter was not affected when goat's milk was heated up to 85 °C for 30 min. Heat treatments higher than 70 °C for 15 min rendered a very weak curd from cow's milk, however the consistency of curd from goat's milk was slightly affected by heat treatments. Whey proteins from goat's milk were more denatured than those from cow's milk on samples heated at temperatures higher than 75 °C. A good relationship was established between whey protein denaturation and RCT in cow's milk. Dialysis of cow's milk against an excess of goat's milk decrease the RCT of raw and heated samples; goat's milk was not affected by dialysis treatment against cow's milk. The influence of $CaCl_2$ concentration on RCT was higher on cow's than on goat's milk for heat treatments up to 80 °C for 30 min.

Keywords: *Goat's milk; rennet; clotting time*

It is well established that milk which has been heated at temperatures above 70 °C has a longer rennet clotting time (RCT) and forms a weaker curd than unheated milk (Moir, 1930; Ustunol and Brown, 1985; Dalgleish, 1990). Production of a satisfactory curd is important in the manufacture of cheese, since the nature of the clot formed, in part, determines the quality of the final product.

Rennet coagulation is a two-phase process. The initial phase, the enzymatic cleavage of κ-casein, is followed by the coagulation phase, during which individual micelles aggregate to form the curd. The effect of heating on these phases is still under discussion.

It is considered that the complex formed between denatured β-lactoglobulin (Wilson and Wheelock, 1972; Damicz and Dziuba, 1975; Reddy and Kinsella, 1990) or α-lactalbumin (Shalabi and Wheelock, 1976) and κ-casein when milk is heated increases the rennet clotting time. Dalgleish (1990) suggested that a maximum of 50% of the β-lactoglobulin could be incorporated to κ-casein before the rennet clotting time increased. Changes in the milk salts balance during heating can also influence clotting properties of milk (Kannan and Jenness, 1961; Amram et al., 1982).

The role of the complex formed between whey proteins and caseins on the rennet clotting behavior of milk is uncertain. The complex may alter the surface of micelles in such a way as to make it impossible for the renneted micelles to approach each other sufficiently close so as to coagulate. On the other hand, the complex may hinder the enzymatic action of rennet.

Goat's milk production and its use in the cheese industry has increased in recent years. Goat's milk cheeses have been the vehicles of sporadic illness outbreaks (Young and Suvamoparrat, 1975). To produce microbiologically safe goat's cheese, raw milk has to be heat treated.

Apart from the microbiological point of view, pasteurization of milk offers other advantages. Although most previous investigations on the effects of heating on renneting have been conducted on cow's milk, limited information is available on the effects of heat treatment on rennet clotting properties, whey protein denaturation, and complex formation with casein of goat's milk.

Ramos (1978) reported that in pasteurized milk (63 °C for 30 min) 2.3 and 0%, respectively, of the soluble proteins of cow's and goat's milk were denatured. Khandelwal and Gupta (1980) indicated that the whey proteins from goat's milk were denatured at temperatures higher than 60 °C and β-lactoglobulin was completely denatured at temperatures as low as 80 °C.

Our objective was to determine the effect of heat treatment on rennet clotting properties and whey protein denaturation of both cow's and goat's milk.

MATERIALS AND METHODS

Samples. Raw cow's and goat's milk from herds in the central region of Spain were used. Milk pH was adjusted to 6.68 before heat treatments. Cow's or goat's milk with modified salt composition was obtained by dialyzing for 2 days at 7 °C with stirring against goat's or cow's milk, respectively. Ca^{2+} in the milk was increased by adding 5 M $CaCl_2$ solution; the pH was readjusted to the initial pH before heat treatments.

A rennet solution was obtained by dissolving 300 mg of rennet containing 85% chymosin and 15% bovine pepsin (CHR Hansen Denmark) in 100 mL of 0.01 M sodium citrate buffer at pH 5.2.

Heat Treatments. Portions (15 mL) of milk were heated at 65, 70, 75, 80, and 85 °C in a water bath for 5–35 min in tightly sealed Pyrex glass tubes (16 × 162 mm). The temperature was continuously controlled by a thermocouple. In all cases less than 1.5 min was necessary to reach the desired temperature. The come-up time was taken into account to determine the effective holding time. After the heating period, samples were immediately cooled in an ice–water bath and kept at 7 °C until analysis.

All experiments were replicated four times using different milk samples.

Renneting Properties. The renneting properties were determined with the Formagraph instrument (McMahon and Brown, 1982). This instrument draws a "firmness versus time" diagram as clotting occurs. Rennet clotting time, represented by r, indicates time from addition of enzyme until two lines diverge. The time in minutes from r until the two lines are 20 mm apart represents the rate of curd firming (k_{20}). The curd at this time is firm enough for cutting.

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Ten milliliters of the different samples of heated and unheated milks was equilibrated to 36 °C for 30 min; then 200 μL of rennet solution was added to each sample, and the rennet clotting properties were determined.

Protein Analysis. The serum proteins and caseins were obtained by acidifying milk to pH 4.6 by adding 2 M HCl and then centrifuging the samples.

Caseins were analyzed by sodium dodecyl sulfate (SDS)–PAGE using Phast system electrophoresis equipment (Pharmacia, U.K.). SDS–PAGE was done on 20% homogeneous gels in accordance with the manufacturer's instructions.

Whey proteins soluble at pH 4.6 were determined by HPLC as described by Resmini et al. (1989). The level of denaturation of each protein was calculated as the decrease in area of each peak, expressed as a percentage of the area of the corresponding peak in acid filtrate from raw milk.

Statistical Treatment. Analysis of variance was applied using the BMDP2V program (1981), with a CDC Ciber 180/855 computer, to test the influence of the species, time, and temperature on whey protein denaturation in milk samples. Linear regression analysis of the results was also done.

RESULTS AND DISCUSSION

The rennet clotting time, which comprises time for both the enzymatic and aggregation phase, and the time a curd is firm enough for cutting (k_{20}) were determined. A low concentration of rennet was used (6 mg/100 mL of milk) compared to that (10 mg/100 mL of milk) normally used with a Formagraph to coagulate cow's milk. This low level was used to slow coagulation of goat's milk, so parameters could be accurately measured on the Formagraph chart and comparison between goat's and cow's milk could be made.

Comparison of the RCT revealed that the clotting time of raw cow's milk was longer than that of goat's milk (18.0 vs 4.1 min). Although this phenomenon has been previously observed (Remeuf and Lenoir, 1986), the reason for the difference is still unknown. The relative proportions of the major components of the casein are very different for both species. Goat's milk is poor in α_s-casein, and the proportions of κ- and β-casein are higher. The micelle structure also differs from that of cow's milk, the average diameter of the micelle being higher as is the degree of dispersion (Remeuf and Lenoir, 1986). However, more studies have to be done to clarify the relationship between the casein composition, the properties of the micelle, and the rennet clotting properties.

With increasing severity of heating of cow's milk, the RCT was progressively lengthened (see Figure 1a), as has been previously reported (Moir, 1930; Ustunol and Brown, 1985; Dalgleish, 1990). This parameter increased linearly with heating time in samples heated at up to 70 °C, and it increased markedly with heating time at temperatures higher than 70 °C. Cow's milk submitted to thermal treatments higher than 75 °C for 25 min, 80 °C for 20 min, or 85 °C for 15 min did not coagulate after 90 min of incubation.

The RCT of goat's milk was not affected by the different heat treatments assayed (see Figure 1b). Dinesh and Gupta (1988) did not observe an increase in RCT when goat's milk was heated at up to 90 °C for 10 min.

The rates of curd firming (k_{20}) of raw milks were 16.3 and 2.3 min for cow's and goat's milk, respectively. The k_{20} value was also determined on heated samples (see Table 1). The k_{20} value increased with heating time in cow's milk samples heated at up to 70 °C for 15 min; samples given higher heat treatments had a weak curd without enough consistency to be detected by the Formagraph. Only a slight increase in k_{20} value was observed when goat's milk was heated.

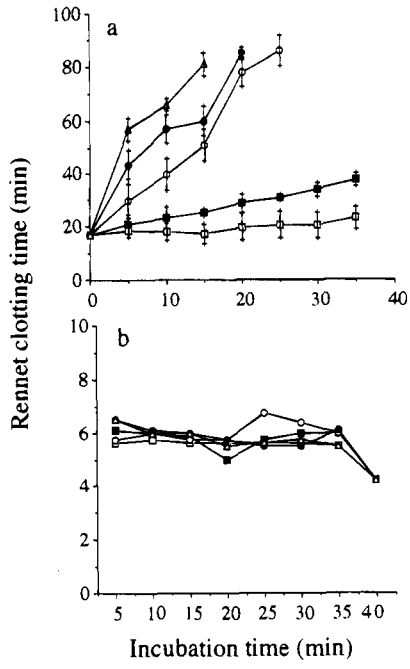

Figure 1. Influence of heat treatments on the rennet coagulation time of cow's (a) and goat's (b) milk samples heated at 65 (□), 70 (■), 75 (○), 80 (●), and 85 °C (△) for 5–35 min. Mean ± SEM.

Since whey protein denaturation (Dalgleish, 1990) and complex formation with κ-casein (Wilson and Wheelock, 1972; Damicz and Dziuba, 1975; Reddy and Kinsella, 1990) have been reported to influence RCT, whey protein denaturation of both milks was analyzed.

Figures 2 and 3 show the percentage of undenatured α-lactalbumin and β-lactoglobulin from cow's and goat's milk given different thermal treatments. Less than 20% denaturation was observed for both types of milk heated at up to 70 °C; no significant influence of heating time was detected. A marked influence of heating time was detected on samples heated at 75 °C. Samples heated at 80 °C for 5 min showed denaturation of more than 50% of α-lactalbumin and 75% of β-lactoglobulin. Whey protein denaturation was significantly higher in goat's than cow's milk heated at temperatures higher than 70 °C; these results are in agreement with those reported previously by Calvo et al. (1989). Less than 5% of undenatured β-lactoglobulin was found when goat's milk was heated at 80 °C for 5 min, whereas more than 20% of β-lactoglobulin remained undenatured in cow's milk. Khandelwal and Gupta (1980) reported complete denaturation of β-lactoglobulin when goat's milk was heated at 80 °C.

Linear regression analysis of the values of the percentage of undenatured α-lactalbumin or β-lactoglobulin and the rennet clotting time of cow's milk samples resulted in the following equations:

$$\text{RCT} = 71.08 - 0.55a, R^2 = 0.83$$

$$\text{RCT} = 94.73 - 0.77b, R^2 = 0.80$$

a is the percent of undenatured α-lactalbumin, and b is the percent of undenatured β-lactoglobulin. However, the RCT of goat's milk was not correlated with the percentage of whey protein denaturation.

To study the effect of the mineral fraction of milk on the clotting properties, goat's and cow's milk samples

Table 1. Effect of Heat Treatments on the Rate of Curd Firming (k_{20}, Minutes)

	heating temperature									
	65 °C		70 °C		75 °C		80 °C		85 °C	
heating time (min)	cow's milk	goat's milk	cow's milk	goat's milk	cow's milk	goat's milk	cow's milk	goat's milk	cow's milk	goat's milk
5	16 (2.4)[a]	3 (0.50)	15 (6.7)	3.6 (0.4)	nd[b]	5.3 (0.8)	nd	6.6 (1.7)	nd	6.3 (0.7)
10	16 (3.3)	3.0 (0.4)	23 (4.8)	4.0 (0.4)	nd	5.5 (1.2)	nd	7.1 (1.3)	nd	6.0 (0.8)
15	17 (3.9)	3.1 (0.1)	33 (10.1)	4.0 (0.5)	nd	5.5 (0.5)	nd	6.6 (1.8)	nd	6.0 (1.1)
20	19 (4.2)	3.3 (0.3)	nd	4.6 (0.7)	nd	6.2 (1.2)	nd	6.1 (1.0)	nd	6.3 (2.0)
25	20 (5.1)	3.1 (0.5)	nd	4.6 (0.7)	nd	6.5 (2.7)	nd	6.3 (1.6)	nd	6.5 (1.7)
30	24 (2.6)	3.5 (0.4)	nd	4.8 (0.9)	nd	7.3 (2.2)	nd	6.3 (1.3)	nd	7.0 (1.7)
35	25 (1.2)	3.3 (0.3)	nd	5.1 (1.3)	nd	7.6 (1.9)	nd	6.6 (1.6)	nd	6.5 (1.2)

[a] Mean (standard deviation), $n = 4$. [b] nd, not determined (not enough curd consistency to be measured by the Formagraph).

Table 2. Effect of Milk Salt Solution on the Rennet Clotting Properties of Cow's and Goat's Milk

		rennet clotting properties			
		r^a (min)		$k_{20}{}^b$ (min)	
sample	incubation at 7 °C (days)	cow's milk	goat's milk	cow's milk	goat's milk
control raw milk	0	11.1 (1.4)[c]	3.3 (1.5)	9.3 (0.6)	2.8 (0.3)
control heated[d] milk	0	18.6 (6.9)	4.3 (0.6)	24.2 (9.6)	4.0 (0.9)
control heated[d] milk	2	16.0 (0.7)	3.0 (0.7)	23.2 (1.1)	3.6 (1.0)
dialyzed[e] raw milk	2	3.8 (1.9)	4.4 (1.8)	4.0 (0.5)	2.8 (0.8)
heated[d] dialyzed[e] milk	2	5.8 (2.1)	6.5 (1.8)	17.7 (1.2)	5.3 (1.0)

[a] r = rennet clotting time. [b] k_{20} = rate of curd firming (the curd at this time is firm enough for cutting). [c] Mean (standard deviation), $n = 4$. [d] Heated at 70 °C for 30 min. [e] Milk dialyzed against milk of the other species.

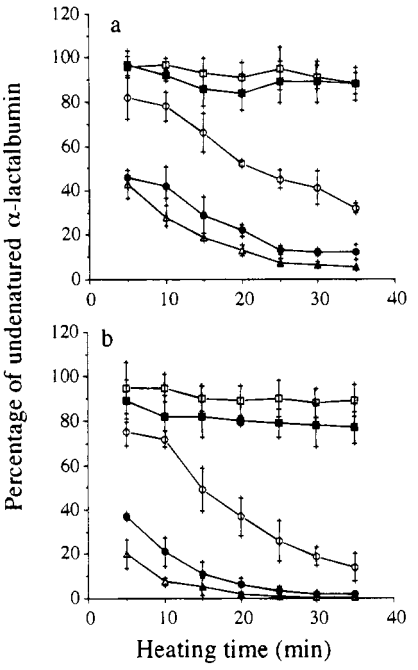

Figure 2. Influence of heat treatments on α-lactalbumin denaturation of cow's (a) and goat's (b) milk samples heated at 65 (□), 70 (■), 75 (○), 80 (●), and 85 °C (△) for 5–35 min. Mean ± SEM.

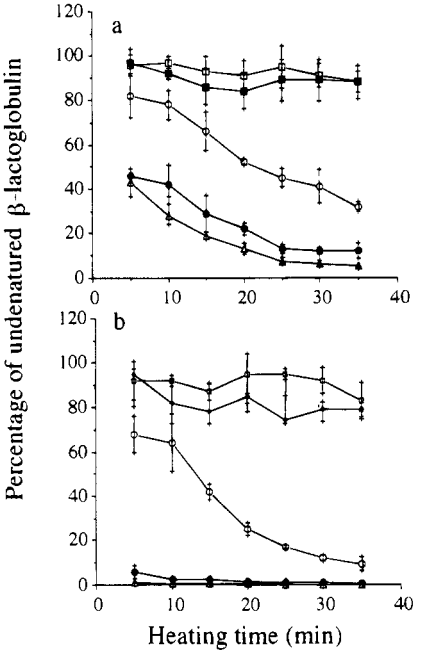

Figure 3. Influence of heat treatments on β-lactoglobulin denaturation of cow's (a) and goat's (b) milk samples heated at 65 (□), 70 (■), 75 (○), 80 (●), and 85 °C (△) for 5–35 min. Mean ± SEM.

were dialyzed against cow's and goat's milk, respectively, and then heated at 75 °C for 10 min.

RCT and k_{20} values of heated samples were not influenced by storage time (see Table 2). Dialysis of cow's milk resulted in a 60% decrease of RCT in both raw and heated samples; a decrease of k_{20} values was also observed. Some differences in the saline composition of cow's and goat's milk have been reported; calcium content is higher in goat's than cow's milk (Morand-Fehr and Flamant, 1983). It is known that the addition of calcium decreases the RCT and increases the rate of firming of rennet milk gels (Lucey and Fox, 1993). Rennet properties of goat's milk were not affected by dialysis.

To determine the influence of calcium content on the rennet clotting properties of goat's and cow's milk, different concentrations of $CaCl_2$ were added to raw milk samples which were then heated at 70 or 80 °C for 30 min. Addition of $CaCl_2$ above 7 mmol/L caused coagulation of goat's milk during heating at 80 °C for 30 min, whereas cow's milk remained stable in all assays. Previous studies have shown that goat's milk is more unstable to heating processes than is cow's milk (Zadow et al., 1983).

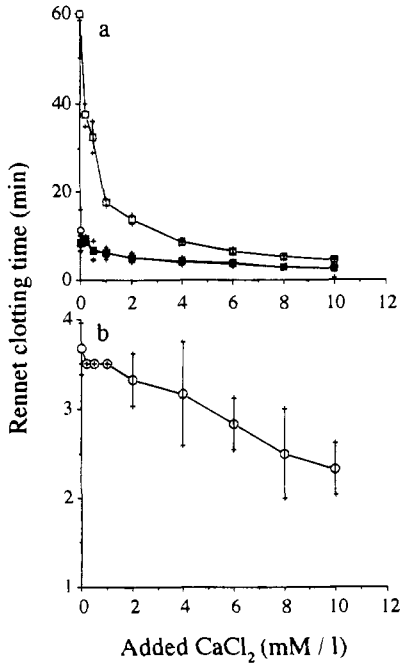

Figure 4. Effect of $CaCl_2$ concentration on the rennet clotting time of cow's (a) and goat's (b) milk samples: (■) raw milk; (○) milk heated at 70 °C for 30 min; (□) milk heated at 80 °C for 30 min. Mean ± SEM.

The effect of $CaCl_2$ concentration on the RCT of cow's milk is shown in Figure 4a. Addition of $CaCl_2$ decreased the RCT of raw milk and offset the adverse effects of heating. A slight decrease in RCT was observed for both raw milk and milk treated at 70 °C for 30 min. However, a noticeable effect was observed in cow's milk heated at 80 °C for 30 min even for $CaCl_2$ additions lower than 2 mmol/L.

The influence of $CaCl_2$ concentration on RCT was greater for cow's than goat's milk. No differences were observed between control and samples submitted to different heat treatments. Addition of $CaCl_2$ decreased the RCT from 4.8 to about 2.5 min.

The present results show a marked influence of the salt composition of milk on the RCT of cow's milk and a minimal effect on goat's milk. On the other hand, whey protein denaturation does not cause changes in the RCT of goat's milk. Since the composition and structure of casein micelles are relevant to the renneting process (Jakob and Puhan, 1992), the differences between the RCT values of heated cow's and goat's milk samples could be attributed to their differences in individual casein composition and dimensions of the casein micelles.

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