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Effect of heat treatment on the rennet clotting time of goat and cow milk

Vincenzo Alloggio [a,*], Francesco Caponio [a], Antonella Pasqualone [a], Tommaso Gomes [b]

[a]*Istituto di Industrie Agrarie, Università degli Studi di Bari, Via Amendola 165/a, I-70126 Bari, Italy*
[b]*Istituto di Produzioni e Preparazioni Alimentani, Università degli Studi di Bari Via Napoli 25, I-71100 Foggia, Italy*

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Abstract

The effect of heat treatment (70, 80 and 95°C, for 1, 3 and 10 min) on the rennet clotting time (RCT) has been evaluated in cow milk (*Bruna Italiana*) and in the milks of five goat breeds (*Saanen*, *Camosciata*, *Ionica*, *Garganica* and *Maltese*). The RCT of raw cow milk ranked at an intermediate level compared to that of raw milk of goat breeds examined. With respect to the raw milks, the RCTs of cow milk became progressively longer as a function of both heating temperature and time, while all the goat milks' RCTs decreased. Significant ($P<0.05$ or <0.001) decreases have been registered in the RCTs of the *Camosciata*, *Ionica*, *Garganica* and *Maltese* goat milks even after mild heating (to 70°C for 1 min), while significant ($P<0.05$) variations have been observed in the *Saanen* goat milk only with intense heating (95°C for 1, 3 and 10 min).

Keywords: Rennet clotting time; Heat treatment; Goat milk

1. Introduction

It has been long-established that heat treatment has a detrimental effect on the clotting properties of cow milk. At temperatures higher than 70°C, both the enzymatic and non-enzymatic phases of rennet clotting are delayed and the RCT is longer than that of unheated milk (Dalgleish, 1990; Ustunol & Brown, 1985). The causes have been broadly investigated even though they have not yet been fully understood (Dalgleish, 1990; Fox, 1989; Haque, Kristjansson & Kinsella, 1987; Hermier & Cerf, 1987; Hofmann & Van Mil, 1997; Marshall, 1986; Wilson & Wheelock, 1972). This has been mainly ascribed to the following two mechanisms: the complex formed between β-lactoglobulin and k-casein which hampers the action of chymosin by hindering it sterically; a reduced concentration of the soluble calcium useful for clot formation during micelle aggregation.

For goat milk, on the other hand, the literature on this topic suggests that RCT remains unchanged by heat treatment at temperatures ranging from 65 to 85°C for 5–35 min (Montilla, Balcones, Olano & Calvo, 1995).

During some preliminary tests, on a product obtained from the milk of certain goat breeds whose processing requires prior heating of the milk at a minimum of 70°C for different length of time, we observed that the RCT did not remain constant but that it diminished as the heat treatment increased.

For these reasons, it would be interesting to determine the effects of heat treatment, at different temperatures for different lengths of time, on the RCT of milks from the most common goat breeds present in southern Italy and to compare them with those obtained in cow milk under the same conditions.

2. Materials and methods

2.1. Samples

Samples were collected at the time of morning milking from the bulk milk of *Bruna Italiana* cows of a region in southern Italy and of *Saanen*, *Camosciata*, *Ionica*, *Garganica* and *Maltese* goat breeds. Six different samples were taken from each of these breeds during the whole lactation cycle, starting from summer 1998. The pH of each sample was usually equal or close to 6.70. When

* Corresponding author.

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necessary, this value was reached by using a 0.1 N solution of HCl or of NaOH, before heat treatment.

2.2. Reagent

A purified rennin solution (Fabre, Sirtori, Italy, rennin strength 1:10,000), containing 80% chymosin and 20% bovine pepsin, was used.

2.3. Milk composition

Fat was determined by the Gerber method, and total protein (N×6.38) by the Kjeldahl technique. Total solids were determined by drying 3 g of the sample at 102±1°C and ash after burning at 550°C for 12 h.

2.4. Heat treatments

Aliquots (10 ml) of each whole milk sample set in glass test tubes (16×150 mm) were heated at 70, 80 and 95°C in a water bath for 1, 3 and 10 min. Heated aliquots were rapidly cooled at 20°C in an ice-water bath and kept at this temperature for 1 h before analysis.

2.5. Clotting times

Experiments on the measurement of RCT in whole milk were carried out following the method described by Berridge (1952). Heated and unheated aliquots were equilibrated to 38°C for 30 min and the same amount of rennet was added to each of them (50 μl of a freshly prepared 1:10 dilution of rennin strength 1:10,000). This quantity was preliminarily selected so that when it was added to the aliquots of unheated milk it yielded a clotting time that was never shorter than about 10 min. A stop watch was used to take the time from mixing of each aliquot and rennin to the first sign of sudden breakdown of the film on the test tube wall. All experiments were replicated four times using different aliquots of each milk sample.

2.6. Statistical analysis

One-way analysis of variance (ANOVA) was used to test the influence of the time and temperature on the RCT of both cow and goat milks.

3. Results and discussion

Table 1 shows the results obtained in determining some chemical parameters of all milk samples examined. All the figures are similar to those reported in the literature on the subject (Castagnetti, Chiavari & Losi, 1984; Storry, Grandison, Millard, Owen & Ford, 1983). The pH values ranged from 6.64 to 6.76 and required, to reach the pH value of 6.70, adjustments smaller than 0.1 units. With reference to goat milks, pH mean values higher than 6.70 were found for *Saanen* and *Camosciata*, while lower mean values were observed for *Ionica*, *Garganica* and *Maltese*. As far as the other analytical determinations are concerned, *Saanen* and *Camosciata* showed lower mean values than *Ionica*, *Garganica* and *Maltese*.

Examination of the effect of heat treatment on the RCT immediately shows that escalating values were registered only in cow milk (Fig. 1A) as a function of the time for which the milk was heated and that more substantial increases were obtained at the higher temperatures. This is in agreement with the findings of other authors for similar heat treatments (Montilla et al., 1995; Singh, Shalabi, Fox, Flynn, & Barry, 1988; Ustonol & Brown, 1985; Wilson & Wheelock, 1972). Conversely, the RCT values decreased in the milk from the different goat breeds (Fig. 1B–D). In almost all cases the decreases were greater at higher temperatures and with heating times of 1 min, while they were attenuated as the heating times became longer.

Table 2 shows the mean values and standard deviations of the RCTs measured for the raw milk (heating time 0) of each breed and for the milks heated at 70, 80 and 95°C for 1, 3 and 10 min, as well as the statistical analysis carried out to assess the significance of the changes highlighted in Fig. 1. The significance of the effect that the increases in temperature (lines) and in heating time (columns) had on the RCTs is indicated as a–c and A–D, respectively. As regards the time, the assessment was made considering the same value obtained for the raw milk of each breed for the temperatures tested.

The RCTs of the raw milk were particularly differentiated, *Bruna Italiana*, *Saanen*, *Camosciata* and *Ionica* showed values in line with those found by other authors (Storry et al., 1983), with the mean value for cow milk (17.4 min) slightly higher than that of the three breeds of goat cited (13.6, 12.9 and 11.7 min respectively); *Garganica* and *Maltese*, instead, ranged at a decidedly high level (34.7 and 42.3 min, respectively). Such behaviour still remains unexplained, as also reported by other authors (Montilla et al., 1995).

The changes in RCT values obtained for cow milk, after each time of heating and as a function of the increases in temperature (lines), were always highly significant ($P<0.001$). The RCTs ranged from 18.8 (1 min, 70°C) to 60.7 (10 min, 95°C). When the values were examined as a function of the heating time from 0 to 10 min (columns), the decreases were always significant at each temperature, with $P<0.05$ at 70°C (range 17.4–21.0 min), $P<0.01$ at 80°C (range 17.4–35.2 min), and $P<0.001$ at 95°C (range 17.4–60.7 min). These results are clearly consistent with the mechanisms reported in the literature given that the clotting times for the cow

Table 1
Composition of the cow milk (*Bruna Italiana*) and of the milks from the five goat breeds (mean values with standard deviation for six samplings)

Milk	pH	Fat (%)	Protein (%)	Ash (%)	Total solids (%)
Bruna Italiana	6.66 ± 0.03	3.62 ± 0.45	3.31 ± 0.21	0.68 ± 0.05	12.50 ± 0.58
Saanen	6.76 ± 0.02	2.93 ± 0.33	2.72 ± 0.39	0.76 ± 0.03	12.02 ± 0.59
Camosciata	6.75 ± 0.03	2.85 ± 0.35	2.62 ± 0.30	0.71 ± 0.04	11.95 ± 0.62
Ionica	6.64 ± 0.03	3.55 ± 0.67	2.95 ± 0.43	0.90 ± 0.08	12.65 ± 0.65
Garganica	6.69 ± 0.04	3.72 ± 0.51	3.21 ± 0.27	0.95 ± 0.06	13.80 ± 0.81
Maltese	6.65 ± 0.03	3.66 ± 0.46	3.02 ± 0.28	0.88 ± 0.04	13.85 ± 0.44

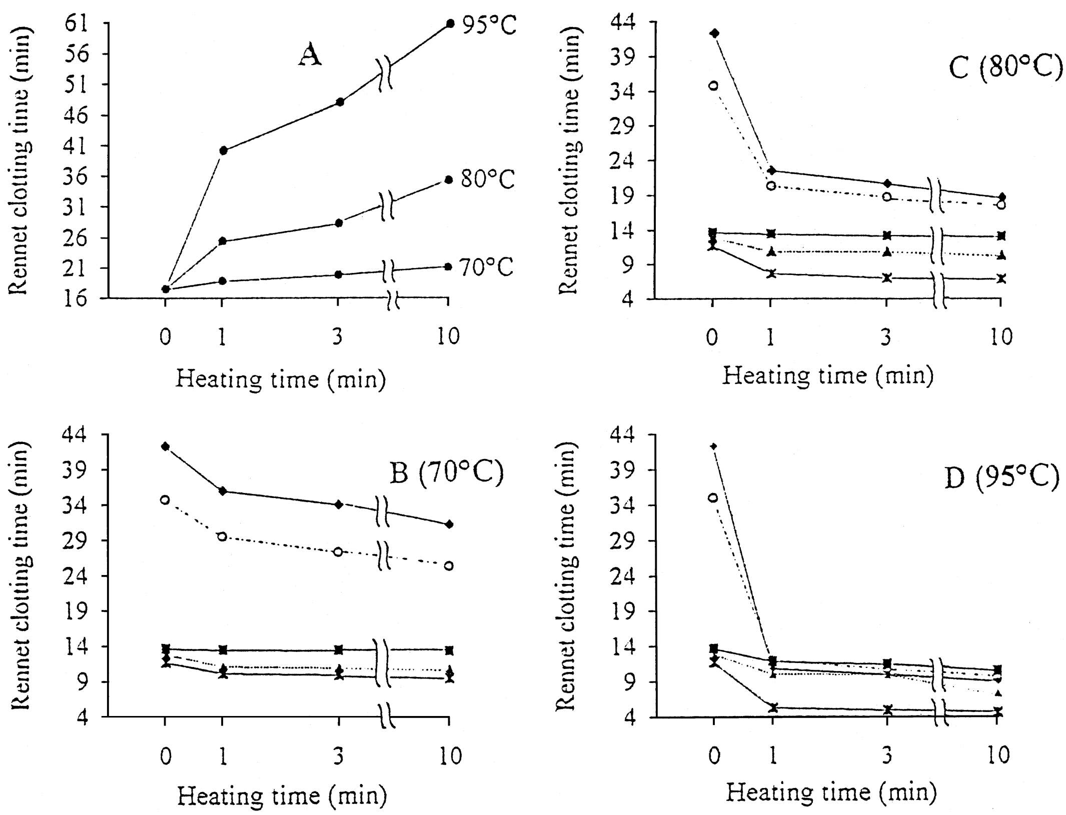

Fig. 1. RCT as a function of heating time and temperature of cow milk from *Bruna Italiana* (A) and of the goat milks from the five breeds considered- *Saanen* (-■-), *Camosciata* (···▲···), *Ionica* (-★-), *Garganica* (-···○···-), *Maltese* (-◆-)-(B,C,D).

milk (*Bruna Italiana*) were progressively longer as the intensity of heating increased.

Among the single goat breeds, the *Saanen* milk displayed only mild, statistically insignificant decreases in RCT when comparing values obtained at 70 and 80°C for each time, thus confirming the renneting behaviour reported by other authors under analogous conditions (Montilla et al., 1995). Heating at 95°C, instead, produced significant decreases ($P<0.05$) of the RCT at each of the times tested.

For the *Camosciata* milk, the values registered at each temperature examined as a function of time (columns) showed highly significant decreases ($P<0.001$) only at 1 min, as compared to raw milk. Prolonging heat treatment, however, yielded lower values that were significant ($P<0.001$) only at 95°C for 10 min. The decreases observed as a function of temperature for each time (lines) differed significantly ($P<0.001$) only when comparing the RCTs obtained at 70 and 80°C to those determined at 95°C.

The RCT values measured for the *Ionica*, *Garganica* and *Maltese* goat milks revealed the same trend when examined as a function of the increases in heating temperature (lines). The RCT values always decreased significantly ($P<0.001$) from 70 to 95°C. At the highest temperature (95°C) they were about 50, 60 and 70%, on average (*Ionica*, *Garganica* and *Maltese*, respectively), lower than the figures obtained at 70°C. The examination of RCT values as a function of the heating times at each temperature (columns), allows the conclusion that *Ionica* RCT showed a highly significant decrease, with respect to raw milk, only for 1 min heating ($P<0.01$ at

Table 2
Rennet clotting time (RCT) values either in raw milk of cow (*Bruna Italiana*) and five goat breeds or as function of heat treatment at 70, 80 and 95°C for 1, 3 and 10 min (mean values with standard deviation for six samplings)[ab]

Milk	Heating time (min)	RCT values (min)			
		Raw milk	Milk heated at		
			70°C	80°C	95°C
Bruna Italiana	0	17.4 ± 0.6A			
	1		18.8 ± 0.5a,B	25.3 ± 0.9b,B	40.1 ± 1.5c,B
	3		19.7 ± 0.5a,C	28.2 ± 1.1b,C	47.9 ± 1.7c,C
	10		21.0 ± 0.4a,D	35.2 ± 1.3b,D	60.7 ± 1.5c,D
Saanen	0	13.6 ± 0.3A			
	1		13.5 ± 0.3a,A	13.3 ± 0.2a,A	11.8 ± 0.7b,B
	3		13.4 ± 0.4a,A	13.1 ± 0.3a,A	11.4 ± 0.3b,B
	10		13.3 ± 0.4a,A	13.0 ± 0.3a,A	10.5 ± 0.5b,C
Camosciata	0	12.9 ± 0.7A			
	1		11.2 ± 0.4a,B	10.8 ± 0.5a,B	10.0 ± 0.6b,B
	3		10.9 ± 0.5a,B	10.8 ± 0.3a,B	9.9 ± 0.5b,B
	10		10.8 ± 0.5a,B	10.3 ± 0.7a,B	7.4 ± 0.5b,C
Ionica	0	11.7 ± 0.6A			
	1		10.2 ± 0.6a,B	7.6 ± 0.5b,B	5.2 ± 0.3c,B
	3		9.9 ± 0.5a,B	7.0 ± 0.3b,B	4.9 ± 0.3c,B
	10		9.5 ± 0.3a,B	6.8 ± 0.3b,B	4.6 ± 0.3c,B
Garganica	0	34.7 ± 0.9A			
	1		29.5 ± 0.9a,B	20.2 ± 0.5b,B	11.9 ± 0.8c,B
	3		27.3 ± 0.8a,C	18.7 ± 0.6b,C	10.7 ± 0.6c,C
	10		25.3 ± 0.9a,D	17.5 ± 0.8b,D	9.5 ± 0.7c,D
Maltese	0	42.3 ± 0.9A			
	1		36.0 ± 0.8a,B	22.4 ± 0.8b,B	10.8 ± 0.4c,B
	3		34.0 ± 0.9a,C	20.6 ± 0.8b,C	9.9 ± 0.2c,C
	10		31.2 ± 0.8a,D	18.7 ± 0.6b,D	9.0 ± 0.3c,D

[a] One common letter indicates no significance. a–c, Indicates the influence of the temperature at which the milk was heated. A–D, Indicates the influence of the time for which each milk was heated (the same rennet clotting time value of the raw milk was considered for each temperature).
[b] The significance level is indicated in Results and discussion.

70°C and $P < 0.001$ at the other temperatures). *Garganica* and *Maltese* milks, instead, always presented significant decreases ($P < 0.05$) for the former and ($P < 0.01$) for the latter.

The behaviour noticed in the goat milk was different from that of cow milk and in many cases the extent of this difference was higher than has ever been reported before. The unexpected results obtained, different from one breed to another, might be related to the characteristic mineral and protein composition of goat milk, whey proteins included (O'Connor & Fox, 1977; Jenness, 1980; Remeuf, Lenoir & Duby, 1989; Storry et al., 1983). In addition, the high genetic variability of nature and quantity of caseins in goat milk should be considered, affecting both micelle size and casein micelle composition (Pierre, Michel, Le Graët, & Zahoute, 1998), and consequently influencing the renneting properties (Storry et al.). It has been reported that, in goat milks with a different genotype for α_{s1}-casein, the average micelle diameter is smaller in micelles with a high level of α_{s1}-casein and greater in micelles without α_{s1}-casein (Pierre, Michel & Le Graët, 1995), and that when the milk has a low level of α_{51}-casein it has a faster coagulation time (Ambrosoli, Di Stasio & Mazzocco, 1988).

Considering the results obtained in our study, it would be desirable to investigate the specific behaviour of goat milk further, both before and after heating, since a better knowledge of the molecular mechanisms existing between principal components of goat milk could improve processing strategies.

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