Influence of Heat Treatment on Rennet Clotting Properties of Mixtures of Cow's, Ewe's, and Goat's Milk and on Cheese Yield

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A study of the influence of milk species on milk mixtures and heat treatment on rennet clotting properties (RCP) of milk has been performed. The effect of heating on cheese yield from milk of different species was also studied. The results obtained showed that the RCP of the milk mixtures of goat/cow milk were different from those of ewe/cow milk. These differences could be due to the different milk protein and salt composition of the milk of the three species as well as differences in the structure and composition of the micelles. Analysis of the milk mixtures containing different percentages of the milk of the three species showed that there were significant ($P < 0.05$) differences in the RCP of the milk mixtures studied. It is possible to increase the cheese yield by heating the milk from different species. However, the yield of goat's or ewe's milk only increased until a specific intensity of heat treatment; at higher heat treatment this parameter had no influence on cheese yield.

Keywords: *Rennet properties; cow, goat, ewe milk mixtures; cheese yield*

INTRODUCTION

Cheese from ewe's or goat's milk or mixtures of milk from these species with cow's milk has been traditionally manufactured in the Mediterranean countries, and their importance in the world market is well-known.

Although some cheeses are produced from raw milk, usually during the industrial manufacture of cheese, milk is submitted to heat treatments. It is well established that cow's milk submitted to temperatures >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 because the nature of the clot formed determines, in part, the quality of the final product because some characteristics such as syneresis and whey drainage depend on the coagulum characteristics.

Although most previous research on the effects of heating on renneting have been conducted on cow's milk, limited information is available on the effect of heat treatment on the rennet clotting properties (RCP) of ewe's or goat's milk or mixtures of milk from different species.

As mentioned previously, heat treatment influences the RCT of cow's milk. Ustunol and Brown (1985) observed that when cow's milk was submitted to heat treatment at 75 °C for 30 min, the milk did not coagulate. However, Montilla et al. (1995) found that the RCT of goat's milk was not affected by heat treatment at 85 °C for 35 min; similar results were obtained in our laboratory with ewe's milk (Balcones et al., 1996).

There has been a recent upsurge in the development of methods by which whey proteins can be incorporated into cheese. Not only does successful incorporation of whey protein increase the yield of cheese from a given volume of milk, but also the nutritional status of the cheese is enhanced (Banks and Muir, 1985). A number of workers studied the incorporation of whey protein by ultrafiltration of cheese milk before manufacture (Maubois and Mocquot, 1974; Brule et al., 1975; Matthews et al., 1976; Covacevich and Kosikowski, 1977; Mann, 1982; Mahaut and Korolczuk, 1992). Another proposed method is the incorporation of heat-denatured whey proteins in curd; this method is based on the use of extensively heated milk, which contains significant amounts of heat-denatured whey protein (Brown and Ernstrom, 1982; Banks and Muir, 1985; Marshall, 1986; Banks et al., 1987).

Since the RCT of goat's and ewe's milk is not affected at high temperatures, the milk from these species could be submitted to high heat treatment without changes in the RCP and the cheese yield could be increased by the incorporation of heat-denatured whey proteins into curd.

The aim of this study is to assess the influence of the concentration of cow's, ewe's, and goat's milk in mixtures of milk of the three species submitted to heat treatment on the RCP as well as its influence on cheese yield.

MATERIALS AND METHODS

Milk Samples. Raw bulk milk from herds in the Central Region of Spain were used. Different mixtures of milk from goat's, ewe's, and cow's milk were studied; in all cases the pH was adjusted to 6.7 before heat treatments.

All experiments were repeated four times using different milk samples.

A rennet solution was obtained by dissolving 300 mg of rennet powder containing 85% of chymosin and 15% of bovine pepsin (Hansen, Copenhaguen, Denmark; rennet strength 1:100000) in 100 mL of a 0.01 M sodium citrate buffer at pH 5.2.

Heat Treatment. Portions (10 mL) of milk samples were submitted to different heat treatments in a water bath in tightly sealed Pyrex glass tubes (16 × 162 mm). The temper-

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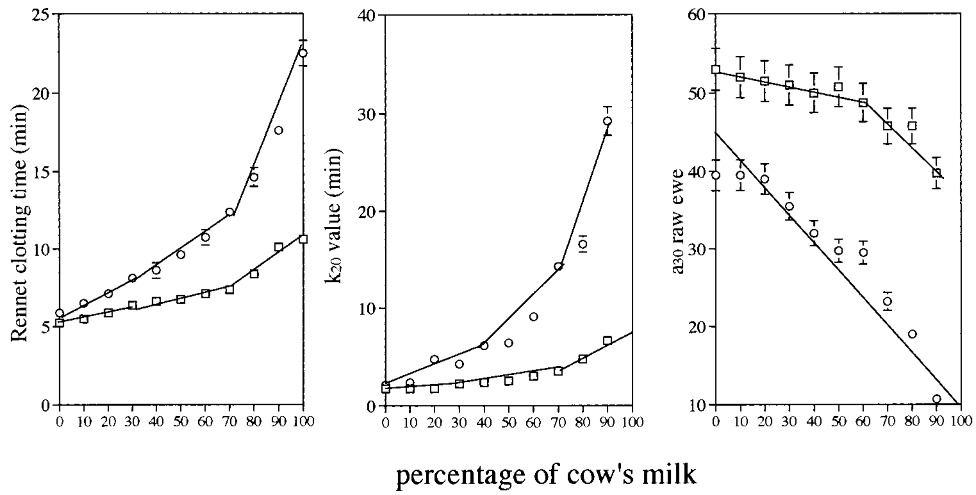

Figure 1. RCP of raw (squares) or heated (circles) cow/goat milk mixtures.

Table 1. Slopes of the Lineal Regression Calculated for RCP of Cow/Goat Milk Mixtures as a Function of Different Cow's Milk Percentage

RCP	heat treatment	% cow's milk in milk mixture[a] 0−40%	40−70%	70−100%
RCT	unheated	0.057 (0.0080)[aA]	0.034 (0.0130)[bA]	0.087 (0.0080)[aA]
	unheated	0.013 (0.0001)[aC]	0.069 (0.0047)[bC]	0.308 (0.0550)[cB]
k_{20}	unheated	0.013 (0.0001)[aC]	0.069 (0.0047)[bC]	0.090 (0.0320)[bC]
	75 °C, 20 min	0.087 (0.0061)[aD]	0.181 (0.0190)[bD]	0.479 (0.1100)[cD]

[a] Slope value of the equation (standard deviation). Means in the same rows without a common superscript lower case letter differ ($P < 0.05$). Means in the same column, to rennet clotting time, without a common superscript capital letter differ ($P < 0.05$), and means in the same column, to k_{20}, without a common superscript capital letter differ ($P < 0.05$).

ature was continuously controlled by a thermocouple. In all cases the desired temperature was reached in <1.5 min. The come-up time was used 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.

RCP. The renneting properties were determined by the Formagraph apparatus (McMahon and Brown, 1982); 10 mL of milk was allowed to equilibrate to 30 °C for 30 min, then 200 μL of the rennet solution was added to each sample, and the RCP were determined. This apparatus draws a "firmness versus time" diagram as RC occurs. The RCT is the time from the addition of enzyme until the point at which the lines diverge. The time in minutes, from RCT until the two lines are 20 mm apart, represents the rate of curd firming (k_{20}). The curd at this time is considered to be firm enough for cutting. The a_{30} value is the width of the graph 30 min after the enzyme is added.

Cheese Yield. A laboratory scale method was used to manufacture Iberico cheese (cheese made with mixtures of cow's, ewe's, and goat's milk). Two hundred milliliters of milk was added to 4% (p/v) of 50 units (Lacto Labo, France) of starter solution containing *Lactococcus lactis* subsp. *lactis* and *Lactococcus lactis* subsp. *cremoris* (70:30); after 30 min at 30 °C, 2 mL of 1 M $CaCl_2$ was added, and after 5 min, a solution at 2% (v/v) of the rennet solution was added. The coagulum was cut 0.5 h later, kept at 30 °C for 30 min, and then centrifuged.

Yield was determined by weighing the pellet obtained by centrifugation of the curd at 13000*g* for 15 min (Calvo et al., 1993).

Statistical Analysis. A polynomial lineal regression analysis was performed to study the relationship between the RCP of the mixtures and the percentage of cow's milk in the mixtures. From the slopes and their standard deviations the differences were studied. A variance analysis was used to study the differences between the RCP of milk mixtures containing milk from the three analyzed species and to analyze the differences between the cheese yield obtained in the different experiments.

The regression and the variance analysis were performed using the Stat View 4.01 program of Macintosh.

RESULTS AND DISCUSSION

Goat's Milk Supplemented with Cow's Milk. Goat's milk was supplemented with 10−90% (v/v) of cow's milk. Cow's and goat's milk and milk mixtures were heated at 75 °C for 20 min; the RCP of the raw and heated samples were determined.

All the experiments were performed using three different milk samples. The mean values and confidence intervals are shown in Figure 1. As can be observed, the representation of the RCT as a function of the cow's milk concentration could be fit to a polynomial equation; because it is very difficult to try to find the possible influence of the cow's milk concentration in the RCT from this equation, taking into account the figures form three different intervals of cow's milk concentration were fixed (0−40, 40−70, and 70−100% of cow's milk) calculating the simple lineal regression of the defined intervals.

The slopes and standard deviations of the calculated straight lines are shown in Table 1. In all of the calculated equations the coefficient of correlation was >0.960.

In raw milk mixtures the slopes of the straight lines of the intervals 0−40 and 40−70% were significantly ($P < 0.05$) different; however, no differences were found between the slopes of the 0−40 and 70−100% cow's milk intervals. The obtained results could indicate a low influence of the percentage of cow's milk in the RCT of raw mixtures.

A significant ($P < 0.05$) increase of the slope values was found in the three intervals of heated samples. Heated samples showed a slope significantly higher than those of raw samples in the intervals 40−70 and 70−100%, whereas in the interval 0−40% the heated samples showed a lower slope than the raw one.

The curd firmness was also determined by measurement of the k_{20} (time required for the curd to attain the consistency necessary to be cut) and a_{30} (measurement of the consistency of the coagulum) values. As can be observed in the Figure 1, the k_{20} value as a function of the cow's milk concentration can be fit to a polynomial equation. The intervals of cow's milk concentration to calculate the polynomial lineal regression were the same as used in the RCT (the values of the 100% of cow's milk were not used in heated samples, because the coagulum had not the consistency necessary to be cut).

As can be observed in Table 1 the slopes of the k_{20} values for the different raw milk mixtures had behaviors different from that described for the RCT. The slope value was significantly lower in the interval 0−40% of cow's milk than in the other two; no significant differences were found in the slopes of the other two assayed intervals. A significant increase in the slopes with increasing cow's milk percentage was found in heated samples. All of the slopes were significantly higher in the heated than in the raw samples when the same intervals were compared.

As can be observed in Figure 1, the a_{30} value as a function of the cow's milk concentration of the mixture can be fit to a polynomial equation in raw samples and to a polynomial lineal regression in the heated samples (the coefficient of correlation was 0.915). The results obtained in heated samples could indicate that the a_{30} value is directly affected by the cow's milk percentage in all of the analyzed samples.

The lineal regression analysis to raw milk samples did not show a good fit to a polynomial lineal regression to the intervals fixed above. However, the a_{30} values can be fit to polynomial lineal regression in the intervals 0−70 and 70−90% cow's milk. The calculated slopes and their standard deviations were −0.088 (0.015) and −0.450 (0.087) to the two intervals, respectively. The obtained results indicate that the cow's milk concentration in the mixture has a high influence on the a_{30} value.

As has been indicated previously (Remeuf and Lenoir, 1986; Dinesh and Gupta, 1988; Montilla et al., 1995), there are significant differences between the RCP of cow's and goat's milk. On the other hand, Montilla et al. (1995) found that heat treatment has a different effect in the RCP of the milk of the two species.

The possible causes of these differences have been discussed previously by Montilla et al. (1995). The salt concentration, the micelle composition and size, the influence of heat treatment in the aggregation of denatured whey proteins with the κ-casein, and the influence of this aggregation on the enzymatic action of rennet could affect in a different way the RCP of cow's and goat's milk. However, it is very difficult to know exactly the parameters that can influence the RCP of milk from different species, as well as the physicochemical changes during heating that can affect the mentioned properties.

The difficulty in determining the factors influencing the RCP of milk is greater when milk mixtures from different species are analyzed. However, some conclusions can be obtained from the results described above.

Montilla et al. (1995) found that the RCT of raw cow's milk increased approximately 4 times when it was heated at 75 °C for 20 min. We found that the RCT increased when raw mixtures were submitted to the same heat treatment; however, this value increased by only 3 times when a milk mixture containing 90% cow's milk was heated and decreased to double in heated mixtures containing 70% cow's milk.

The changes in the RCT of goat's milk submitted to high heating are slight (Montilla et al., 1995), which could influence the RCT of the different milk mixtures. The lower values found in the milk mixtures analyzed, when they were compared with cow's milk, could be due to a higher action of rennet to the κ-casein of goat's milk due to the formation of aggregates between the denatured whey proteins and the κ-casein in heated cow's milk; the different sizes of the micelles from the two species could also influence the approach of the micelles sufficient for coagulation to occur.

Some differences have been reported in the saline composition of cow's and goat's milk; calcium content is higher in goat's milk (Morand-Fher and Flamant, 1983). Addition of calcium is known to decrease the RCT and increase the rate of firming of rennet milk gels in cow's milk samples (Lucey and Fox, 1993) and to have little effect on goat's milk (Montilla et al., 1995). The higher amount of calcium in goat's milk could also influence the decrease of RCT on milk mixtures containing cow's milk.

When the slopes obtained for raw milk samples of the two first intervals were compared, a significant, although slight, decrease of the slope was found in the interval of 40−70% cow's milk. However, the slope values are similar in the other two intervals analyzed. This could indicated that the influence of the percentage of the milk in the mixture does not have a high influence in RCT of raw samples, although, as has been indicated, the influence in heated samples is noticeable (see the increase in the slopes with increasing percentage of cow's milk).

The milk mixture composition influences also the coagulum strength. As in the other mixtures analyzed, the coagulum formed from a heated mixture of goat's and cow's milk containing 90% cow's milk has the consistency necessary to be cut; however, the k_{20} value is also influenced by the heat treatments.

The slope of the k_{20} value increased with increasing percentage of cow's milk in the three intervals. However, in raw milk samples no significant differences were found between the slopes of the 40−70 and 70−100% intervals; this could indicate that there is a great influence of cow's milk composition on the coagulum formation.

The obtained results show that it is possible to decrease the RCT in heated samples of cow's milk by adding goat's milk prior to the heat treatment. However, the addition of goat's milk affects slightly the rennet strength.

Ewe's Milk Supplemented with Cow's Milk. A study similar to that performed with goat's milk supplemented with cow's milk was carried out with ewe's milk supplemented with different percentages of cow's milk. The sample preparation and analysis were similar to those of the previous study. The obtained results are shown in Figure 2 and Table 2.

As can be observed, the representation of the RCT as a function of the cow's concentration could, as in cow/goat milk mixtures, be fit to a polynomial equation (see Figure 2). The same intervals described above were used to calculate the simple polynomial lineal regres-

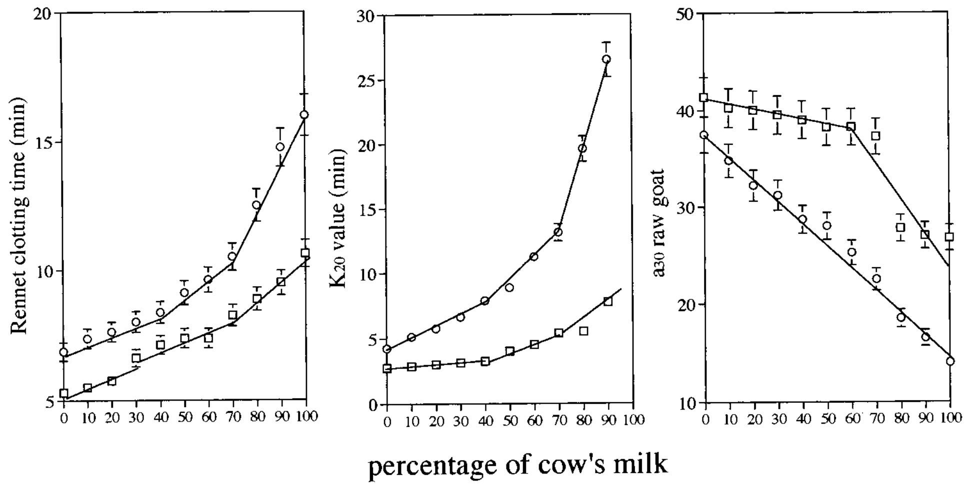

Figure 2. RCP of raw (squares) or heated (circles) cow/ewe milk mixtures.

Table 2. Slopes of the Lineal Regression Calculated for RCP of Cow/Ewe Milk Mixtures as a Function of Different Cow's Milk Percentage

		% cow's milk in milk mixture[a]		
RCP	heat treatment	0−40%	40−70%	70−100%
RCT	unheated	0.039 (0.0033)[aA]	0.026 (0.0032)[bA]	0.115 (0.0080)[cA]
	75 °C, 20 min	0.074 (0.0065)[aB]	0.124 (0.0100)[bB]	0.334 (0.0420)[cB]
k_{20}	unheated	0.017 (0.0059)[aC]	0.039 (0.0064)[bC]	0.120 (0.0220)[cC]
	75 °C, 20 min	0.099 (0.0230)[aD]	0.270 (0.0760)[bD]	0.752 (0.3130)[cD]

[a] Slope value of the equation (standard deviation). Means in the same rows without a common superscript lower case letters differ ($P < 0.05$). Means in the same column, to rennet clotting time, without a common superscript differ ($P < 0.05$); means in the same column, to k_{20}, without a common superscript capital letter differ ($P < 0.05$).

sion. The slopes and their standard deviations of the calculated straight lines are shown in Table 2. In all of the calculated equations the coefficient of correlation was >0.900.

In raw milk mixtures the slopes of the straight lines of the three intervals were significantly ($P < 0.05$) different; the slopes showed also a significant difference in heated samples. In raw and heated mixtures the higher slope was found in the interval 70−100% cow's milk, indicating a high influence of cow's milk on the RCT.

The difference between the slopes of the straight lines of the two first intervals of raw milk samples, although significant, was not very high; however, this difference was higher in the same intervals of the heated milk samples. These results indicate a higher influence of cow's milk concentration in heated than in raw milk mixtures. These results are corroborated when the slopes of the straight lines obtained from raw and heated mixtures for each interval are compared; in all cases the heated samples showed a higher slope than the raw one.

The k_{20} and a_{30} values were also determined. As can be observed in Figure 2, as the RCT, the k_{20} value as a function of the cow's milk concentration can be fit to a polynomial equation. The intervals of cow's milk concentration to calculate the polynomial lineal regression were the same used in the RCT.

As can be observed in Table 2, the slopes of the k_{20} values for the different milk mixtures had a behavior similar to that described for the RCT.

As in the goat/cow milk mixtures the a_{30} value can be fit to a polynomial lineal regression in the heated samples (the coefficient of correlation was 0.985). The results obtained in heated samples could indicate that the a_{30} value is directly affected by the cow's milk percentage in all of the analyzed samples.

The same intervals indicated above to the other milk mixture analyzed were used to determine the slopes of the a_{30} values as a function of the cow's milk concentration. The calculated slopes and their standard deviations were −0.052 (0.005) and −0.050 (0.014) to the two intervals, respectively. The obtained results indicate no significant differences in the two studied intervals.

There are few data in the literature about the factors influencing the RCP of ewe's milk. Pellegrini et al. (1994) reported that RCT rises while the gel griming rate becomes slower with advancing lactation. Balcones et al. (1996) did not find a great influence of high heat treatments on RCT and curd firmness.

The possible causes of the differences in RCP of raw or heated cow's and ewe's milk could be differences in milk composition and micelle composition and conformation. It has been described that the protein and ash concentrations are higher in ewe's than in cow's milk. Ewe's milk contains a higher protein concentration than the other two milk species studied (Anifantakis, 1985). O'Connor and Fox (1977) reported a higher concentration of calcium and phosphorus in ewe's than in cow's milk. Anifantakis (1985) did not report great differences in the β-lactoglobulin concentration of the two species studied; differences between composition and size distribution of micelle have also been reported (Storry et al., 1983; Anifantakis, 1985).

The differences in milk composition of ewe's and cow's milk could also influence other RCP. Some differences in the casein concentrations of the two species have been reported by different authors (Storry et al., 1983; Anifantakis, 1985). Pagnacco and Caroli (1987) and Marziali and Ng-Kwai-Hang (1986), studying the effect

Table 3. Influence of the Milk Mixture Composition on the Rennet Clotting Properties of Raw or Heated Samples

sample[b]	RCT[a] (min)		k_{20}[a] (min)		a_{30}[a] (min)	
	raw	heated	raw	heated	raw	heated
1	7.5^{1aA} (0.40)	8.1^{bA} (0.25)	2.5^{dA} (0.00)	6.9^{eA} (0.25)	49.0^{gA} (0.82)	30.5^{hA} (1.00)
2	5.8^{aB} (0.47)	7.8^{bA} (0.25)	1.6^{dB} (0.25)	4.4^{eB} (0.25)	47.8^{gA} (2.10)	34.0^{hB} (0.80)
3	7.3^{aA} (0.25)	9.3^{bB} (0.64)	2.5^{dA} (0.00)	6.8^{eA} (0.28)	48.0^{gA} (1.42)	34.0^{hB} (1.25)

[a] Mean (standard deviation). Means in the same rows, from RCT, without a common superscript lower case letter differ ($P < 0.05$); means in the same rows, from k_{20}, without a common superscript lower case letter differ ($P < 0.05$); and means in the same rows, from a_{30}, without a common superscript lower case letter differ ($P < 0.05$). Means in the same column without a common superscript capital letters differ ($P < 0.05$). [b] Sample 1 was 25:25:50 cow/ewe/goat milk. Sample 2 was 25:50:25 cow/ewe/goat milk. Sample 3 was 50:25:25 cow/ewe/goat milk.

of genetic polymorphism on coagulation properties of milk, found that the amount and composition of κ-casein were the most significant factors affecting curd firmness.

As was indicated, it is very difficult try to explain some of the obtained results when the RCP of milk mixtures are analyzed.

The slopes obtained in the raw milk mixtures show differences among the three studied intervals; however, the slope was lower in the interval 40−70% than in the interval 0−40%. The difference in the two intervals was slight; this could indicate a low influence of the mixture concentration on the RCT when the concentration of cow's milk is <80%.

A high influence of cow's milk percentage on RCT was obtained in heated samples. The obtained slopes from the three analyzed intervals in heated mixtures increased with increasing cow's milk concentration, as was also observed in the mixture containing goat's milk.

It has been observed in our laboratory (Balcones et al., 1996) that high heat treatment affects slightly the RCT of ewe's milk. As was found for the mixtures of cow's and goat's milks, the RCT is lower in milk mixtures than in cow's milk submitted to same heat treatment. Because the aggregates between the denatured whey proteins and the κ-casein from cow's milk impede the rennet enzymatic action, it could be possible that the rennet attack preferentially the κ-casein from ewe's milk.

The higher amount of calcium in ewe's milk could also influence the decrease of RCT in milk mixtures containing cow's milk.

The milk mixture composition influences also the coagulum strength. Montilla et al. (1995) found that cow's milk heated at 75 °C for 20 min did not have the consistency necessary to be cut; however, heated milk mixtures containing 90% cow's milk coagulate approximately 6 times later than the raw mixtures. This could indicate that in milk mixtures the destabilized micelles are enough to form aggregates allowing the formation of a consistent coagulum; on the other hand, the increase in the calcium content due to the presence of ewe's milk improves the coagulum strength.

Mixtures of Cow's, Ewe's, and Goat's Milks. The influence of heat treatment and the milk mixture of the three species in different concentrations on the RCP of milk wase also studied. The RCP were determined for three different milk samples containing the following proportions of cow's, ewe's, and goat's milks: 25:25:50, 25:50:25, and 50:25:25, all of which were heated at 75 °C for 20 min. Analysis of variance was calculated to study the influence of the above-mentioned factors.

As can be observed in Table 3, all of the RCP were significantly ($P < 0.05$) affected by heating; the RCT and the k_{20} value increased for heated samples, whereas the a_{30} value decreased. The percentage of the different milk species also significantly affected ($P < 0.05$) the RCP of milk. Raw samples containing the highest ewe's milk concentration showed significantly lower RCT and k_{20} values.

Table 4. Cheese Yield of Cow's, Ewe's, and Goat's Milk Samples Submitted to Different Heat Treatments

heat treatment	cheese yield[a] (%)		
	cow's milk	ewe's milk	goat's milk
raw	100	100	100
75 °C, 5 min	88.7^{aA} (7.78)	94.8^{acA} (10.0)	107.2^{bcA} (14.9)
75 °C, 15 min	103.1^{aB} (1.40)	113.3^{bAC} (9.0)	124.0^{cB} (4.53)
75 °C, 30 min	103.1^{aB} (1.40)	113.3^{bAC} (9.0)	124.0^{cB} (4.53)
80 °C, 30 min	nd[b]	126.9^{aBC} (18.2)	122.5^{aB} (2.96)
90 °C, 5 min	nd	129.7^{aBC} (17.6)	125.6^{aB} (5.79)
90°C 15 min	nd	128.5^{aBC} (14.4)	128.6^{aB} (4.11)
90 °C, 30 min	nd	131.2^{aBC} (15.7)	132.1^{aBC} (3.07)

[a] Mean (standard deviation), $n = 4$. Means in the same rows without a common superscript lower case letter differ ($P < 0.05$); means in the same columns without a common superscript capital letter differ ($P < 0.05$). [b] nd, not determined; milk did not coagulate by rennet.

In general, although significant differences were found among the RCP of the three milk mixtures studied, the differences may not be very important in cheese manufacture. The results obtained could be used to increase the heat treatment of milk mixtures in an attempt to increase the cheese yield without changing the characteristics of the ripened cheese.

Cheese Yield. To study the influence of the intensity of the heat treatment on the cheese yield of cow's, ewe's, and goat's cheese milk, milk samples of these species were submitted to different heat treatments: 75 °C for 5, 15, or 30 min; 80 °C for 30 min; and 90 °C for 5, 15, or 30 min. The cheese yields were calculated (the results were expressed as percentages considering as 100% the cheese yield obtained from raw milk); the obtained results are shown in Table 4.

Cow's milk submitted to heat treatments at 80 °C for 30 min and at up to 90 °C for 5 min did not coagulate by rennet. Significant differences ($P < 0.05$) between the yields of the three species were obtained only in the samples heated at 75 °C for different times. The highest cheese yield was obtained from goat's milk. No significant differences ($P < 0.05$) were observed between ewe's and goat's milk samples submitted to higher heat treatments.

No significant differences were found between the yields of ewe's and goat's milk heated at 80 °C for 30 min or at 90 °C for different times with goat's milk samples. These results could be due to the intensity of the whey protein denaturation during the heat treatments studied.

Calvo et al. (1989) found that whey protein denaturation was significantly higher in goat's and ewe's milk than in cow's milk heated at temperatures >70 °C. These results are in agreement with those reported by Montilla et al. (1995), who found <5% of undenatured β-lactoglobulin when goat's milk was heated at 80 °C for 5 min, whereas >20% of β-lactoglobulin remained undenatured in cow's milk. Montilla et al. (1995) and Balcones et al. (1996) reported a total denaturation of β-lactoglobulin during heating at 80 °C for 30 min.

The lack of influence of high heat treatments on cheese yield of goat's and ewe's milk cheese could be due to the total denaturation of the whey proteins at heating temperatures lower than those applied in this study. On the other hand, there are few studies about the aggregation of the denatured whey proteins from ewe's or goat's milk. The possible differences in the denatured whey protein aggregation between them or with the caseins could also influence the cheese yield of heated ewe's or goat's milk.

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