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Effect of Thermal Treatments on the Determination of Bovine Milk Added to Ovine or Caprine Milk*

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ABSTRACT

Heat treatment of bovine, caprine and ovine milks at 74°C for 15 or 30 s caused negligible denaturation of whey proteins. After heat treatment at 90°C for 15 or 30 s, denaturation of β-lactoglobulin (β-lg) increased in the order bovine < caprine < ovine milk and denaturation of blood serum albumin (BSA) was in the order caprine < bovine < ovine milk. Mixing of bovine milk with caprine or ovine milk resulted in a 15–20% decrease in thermal stability of bovine β-lg when heated at 90°C for 30 s.

Heat treatment at 74°C for 30 s did not affect the quantitative determination of bovine milk in caprine or ovine milk using immunodiffusion or electrophoretic analysis of the whey protein fraction. Heat treatment at 90°C for 30 s allowed the determination of bovine milk by electrophoretic analysis but not by the immunodiffusion method.

INTRODUCTION

The determination of bovine milk in mixtures with milks of other species is difficult and most of the proposed methods have considerable limitations. Analysis of the protein fraction has been considered a promising method of study. In the case of fermented dairy products, such as cheeses, the enzymatic degradation of caseins renders the electrophoretic study of the casein

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fraction unsuitable as a method of analysis (Ramos & Juarez, 1984). Therefore, several authors have suggested study of the whey protein fraction to determine mixtures of different species in milk samples (Foissy, 1967; Ruiz Martinez & Santillana Lopez, 1986; Amigo *et al.*, 1987) as well as in several types of cheeses (Amigo *et al.*, 1986; Ramos & Juarez, 1986; Ruiz *et al.*, 1986).

During the industrial manufacture of cheese, milk is submitted to pasteurization. This thermal treatment can cause a partial denaturation of the whey proteins which may alter the results of the determination based on the electrophoretic or immunological analysis of this protein fraction. Although factors which influence the heat stability of bovine milk have been the subject of intensive research (Fox, 1982), and the heat stability characteristics of milks of other species have been studied (Fox & Hoynes, 1976; Ganguli, 1979; Zadow *et al.*, 1983; Sirry *et al.*, 1984), the thermal behaviour of the whey proteins of caprine and ovine milk has received little attention.

The aim of this work was to study the effect of thermal treatment on bovine, caprine and ovine whey proteins, as well as its influence on the quantitative determination of bovine milk in mixtures with milks of the other species.

MATERIALS AND METHODS

Samples

Bulk samples of raw ovine, caprine and bovine milk from herds in the Central Region of Spain (La Mancha) were used.

Samples of ovine and caprine milk containing 10, 20, 30, 40% of bovine milk were also assayed. Bovine milk with modified salts composition was obtained by dialysing for one day at 2°C with stirring against 30 vol bulk ovine or caprine milk.

Heat treatments

Portions (1 ml) of skim-milks were heated in a silicone oil bath at 74°C or 90°C for 15 or 30 s periods in sealed glass-capillary, thin-walled tubes (1 m long, 1·31 mm i.d. and 1·80 mm o.d.). These were coiled as chromatographic columns and could be introduced into a silicone bath, attaining the desired temperature within a few seconds. Heat treatments were carried out in duplicate.

Heated samples were precipitated at pH 4·6 with 10% (v/v) acetic acid and 1M sodium acetate and then centrifuged before analysis.

Analytical methods

Polyacrylamide gel electrophoresis (PAGE)
Quantitative PAGE was performed by the method of Hillier (1976) in cylindrical gel rods using β-lactoglobulin A (β-lg A) as internal standard. This was achieved by loading 3·31 μg of β-lg A on the gel and running for 15 min at 5 mA/gel before application of the sample. In every run, two gels containing known amounts of β-lg (3·34 μg), α-lactalbumin (α-la) (1·20 μg) and blood serum albumin (BSA) (0·19 μg) were included.

Gels were stained with Coomassie Blue G-250 without further decoloration (Blakesley & Boezi, 1977). Quantification was based on the measurement of the height of the peaks of densitograms obtained with a Chromoscan MKII.

Quantitative determination of bovine milk in ovine or caprine milk was achieved by measuring the amount of bovine β-lg in samples. Quantitative determinations were performed in triplicate.

Radial immunodiffusion
Analyses were performed by the method of Levieux (1978) using commercial CV Test II (Bioser S.A.) agar plates containing specific antibodies for immunoglobulins of bovine milk.

Statistical treatment
Analysis of variance was applied using the BMDP2V program (1981) with a CDC Cyber 180/855 Computer, in order to test the influence of the temperature, time and species factors on the denaturation of milk samples.

Linear regression analysis of the determined percentage of bovine milk in ovine or caprine milk on the real percentage was carried out.

RESULTS

Thermal denaturation of bovine, ovine and caprine whey proteins

The electrophoretic method allowed the quantitative determination of whey proteins in bovine, ovine and caprine milk. Figures 1 and 2 show the electrophoretic densitograms of whey protein fractions of mixtures of bovine/caprine milk and bovine/ovine milk, respectively.

In bovine/ovine milk mixtures bovine β-lg A and B showed higher electrophoretic mobilities than those of ovine milk but bovine α-la overlapped with that of ovine β-lg. In bovine/caprine milk mixtures, bovine β-lg A and B and α-la showed higher electrophoretic mobilities than the

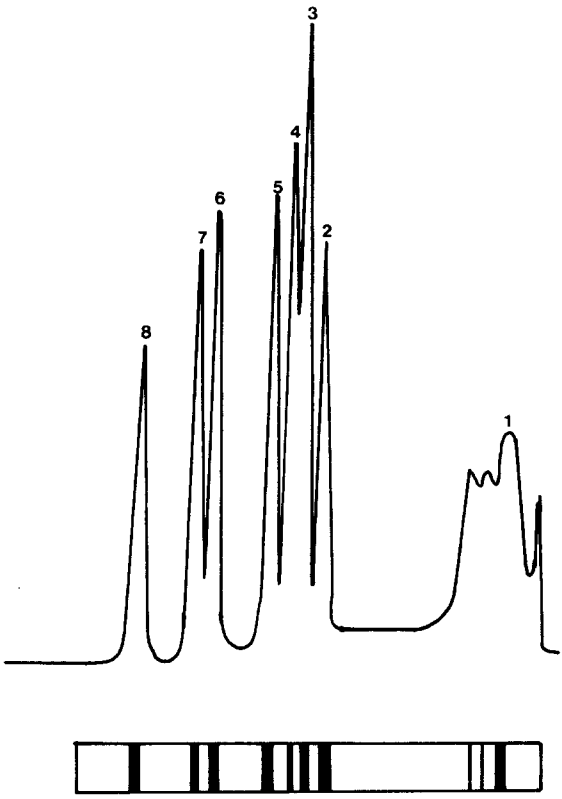

Fig. 1. Densitogram of an electrophoretogram of the whey protein fraction of a mixture of 40% bovine milk and 60% caprine milk. 1-immunoglobulin; 2-blood serum albumin; 3-caprine β-lactoglobulin; 4-caprine α-lactalbumin; 5-bovine α-lactalbumin; 6-bovine β-lactoglobulin B; 7-bovine β-lactoglobulin A; 8-β-lactoglobulin A (internal standard).

corresponding proteins of caprine milk. Similar results were found in a previous study on quantitative determination of bovine milk in caprine and ovine milk products (Amigo *et al.*, 1987).

Table 1 shows the percentage of undenatured whey proteins of bovine, ovine and caprine milk samples submitted to different thermal treatments. Analysis of variance demonstrated the dependence of blood serum albumin (BSA) and β-lg denaturation on the three studied factors (time, temperature and species). In the case of α-la, significant differences due to species only were observed. From the results obtained at 74°C it can be concluded that no denaturation took place under the conditions studied. In the case of heat treatment at 90°C, time and species influenced the denaturation of β-lg, increasing in the order bovine < caprine < ovine milk. BSA denaturation was in the order caprine < bovine < ovine milk.

Bovine milk samples from three different herds showed similar thermal

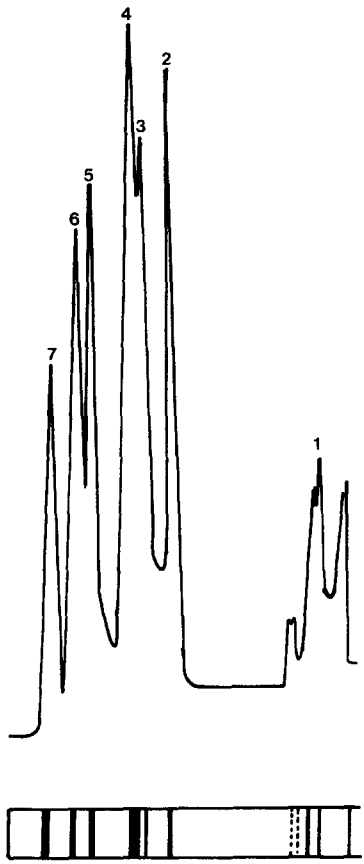

Fig. 2. Densitogram of an electrophoretogram of the whey protein fraction of a mixture of 40% bovine milk and 60% ovine milk. 1-immunoglobulin; 2-blood serum albumin; 3-ovine α-lactalbumin; 4-bovine α-lactalbumin and ovine β-lactoglobulin; 5-bovine β-lactoglobulin B; 6-bovine β-lactoglobulin A; 7-β-lactoglobulin A (internal standard).

behaviour and mixtures of bovine milk with caprine or ovine milk resulted in a 15–20% decrease in the thermal stability of bovine β-lg (see Table 2). This decrease may be due to the noticeable effect of the medium on the heat denaturation of whey proteins (Li-Chan, 1983; Varunsatian *et al.*, 1983).

In order to establish the effect of the milk salt solution on the thermal behaviour of whey proteins, samples of bovine milk were dialysed against ovine or caprine milk and then treated at 90°C for 30 s. Denaturation of β-lg increased about 10% in both cases as shown in Table 2. This indicates that the decrease in the thermal stability of bovine β-lg in mixtures of bovine milk with milks of other species can be in part attributed to the saline composition of the medium.

TABLE 1
Percentage of Undenatured Bovine, Caprine and Ovine Whey Protein during Heat Treatment

Heat treatment	*Type of milk*	*Undenaturated protein (%)*		
		BSA $\bar{X}$ (*CV*)[a]	*α-la* $\bar{X}$ (*CV*)[a]	*β-lg* $\bar{X}$ (*CV*)[a]
74°C/15 s	bovine	101·0 (3·4)	102·0 (3·7)	102·0 (1·8)
	ovine	102·0 (8·1)	96·2 (3·6)	103·0 (9·9)
	caprine	99·8 (2·5)	97·2 (2·1)	99·7 (4·5)
74°C/30 s	bovine	102·0 (4·4)	97·9 (7·5)	103·0 (3·2)
	ovine	95·2 (3·7)	91·9 (9·9)	101·0 (13·5)
	caprine	98·9 (4·7)	104·0 (8·4)	98·2 (12·9)
90°C/15 s	bovine	82·0 (8·9)	95·3 (7·5)	95·6 (9·5)
	ovine	74·9 (6·2)	92·2 (5·2)	87·7 (2·6)
	caprine	94·0 (4·9)	98·1 (3·2)	96·9 (14·6)
90°C/30 s	bovine	59·9 (7·6)	97·1 (2·7)	81·7 (5·8)
	ovine	53·2 (9·3)	91·3 (6·7)	73·5 (6·9)
	caprine	84·9 (2·0)	103·0 (7·3)	79·6 (6·3)

[a] CV = coefficient of variation.

TABLE 2
Effect of the Medium on the Denaturation of Whey Proteins during Heat Treatment at 90°C for 30 s

Sample	*Type of milk*	*Undenaturated protein (%)*		
		BSA $\bar{X}$ (*CV*)[a]	*α-la* $\bar{X}$ (*CV*)[a]	*β-lg* $\bar{X}$ (*CV*)[a]
1		60·0 (7·6)	97·1 (2·1)	81·7 (5·8)
2	bovine	66·9 (10·7)	94·3 (8·2)	84·1 (4·8)
3		57·4 (7·1)	96·4 (5·8)	79·8 (1·6)
4	bovine/ovine 40/60	68·2 (23·5)	—	65·0 (6·0) bovine
5	bovine/caprine 40/60	64·5 (27·4)	96·5 (3·0) bovine 91·9 (1·6) caprine	57·5 (3·7) bovine 79·4 (14·8) caprine
6	bovine milk dialysed against ovine milk	52·9 (16·4)	93·5 (7·1)	69·2 (8·7)
7	bovine milk dialysed against caprine milk	57·6 (13·6)	92·2 (1·4)	70·3 (3·4)

[a] CV = coefficient of variation.

Quantitative determination of bovine milk in thermally treated ovine or caprine milk samples

Ovine and caprine milk samples with known amounts of bovine milk in the range 10–40% were submitted to thermal treatments at 74°C or 90°C for 15 or 30 s and the percentage of bovine milk in the heated samples determined by immunodiffusion and electrophoresis. Linear regression analysis of the values found on the real percentages was carried out with the following samples: (a) heated samples at 74°C, (b) heated samples at 74°C and heated samples at 90°C for 15 s, and (c) heated samples at 74 and 90°C. The *R*-squared (R^2) estimated standard deviation (ESD) and coefficient of variation (CV) values are shown in Table 3. In all cases, the 95% confidence interval for intercept included the zero value. The linear regression lines and the 95% confidence bands for a predicted value of an individual observation are shown in Figs 3 and 4.

In samples submitted to 74°C for 15 or 30 s (group (a)), the CV was lower than 10% for the immunological and electrophoretic methods. This error

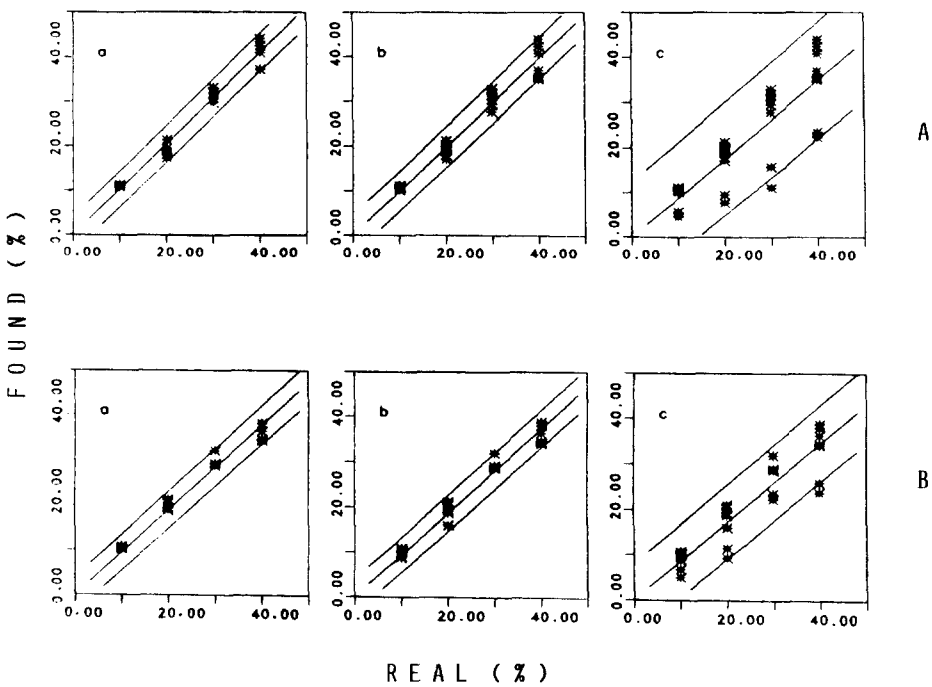

Fig. 3. Percentage of bovine milk in caprine (A) or ovine milk (B) (electrophoretically determined) vs. true percentage. Experimental data, fitted straight lines and the 95% confidence interval for a new observation. (a) 74°C for 15 or 30 s; (b) 74°C for 15 or 30 s and 90°C for 15 s; (c) 74°C for 15 or 30 s and 90°C for 15 or 30 s.

TABLE 3
Linear Regression Analysis of the Determined Percentage of Bovine Milk on the Real Percentage in Heated Milk Samples

Analytical method	*Type of milk*		*Heat treatment*		
			a	*b*	*c*
Electrophoresis		R^2	0·995	0·995	0·973
	bovine/ovine	ESD	1·878	1·898	3·977
		CV	7·820	8·060	18·34
		R^2	0·996	0·994	0·940
	bovine/caprine	ESD	1·904	2·177	6·108
		CV	7·450	8·750	28·010
Immunodiffusion,		R^2	0·997	0·988	0·741
	bovine/ovine	ESD	1·610	3·016	11·950
		CV	6·280	12·540	66·260
		R^2	0·993	0·973	0·730
	bovine/caprine	ESD	2·424	4·278	11·630
		CV	9·680	18·520	67·110

R^2 = The determination coefficient.
ESD = Estimated standard deviation.
CV = Coefficient of variation.
(Heat treatments: (a) heated samples at 74°C, (b) heated samples at 74°C and heated samples at 90°C 15 s, (c) heated samples at 74°C and heated samples at 90°C.)

was similar to that found for these analytical methods. When the heated samples at 90°C for 15 s were added (group (b)), the CV for the immunological determinations was in the range of 12·5–18·5%, i.e. was higher than that of the electrophoretic determinations (8·06–8·75%). When all heated samples (group (c)) were considered, the CV of the electrophoretic determinations increased to 18·34–28·01%, being higher than 66% of that of the immunological determinations.

From these results it can be concluded that pasteurization at 74°C for 15 or 30 s, used in practice in the high-temperature short time (HTST) system, does not affect the determination of the percentage of bovine milk in caprine or ovine milk using electrophoretic or immunological methods. Heat treatments at 90°C for 30 s makes the use of the immunological method unsuitable.

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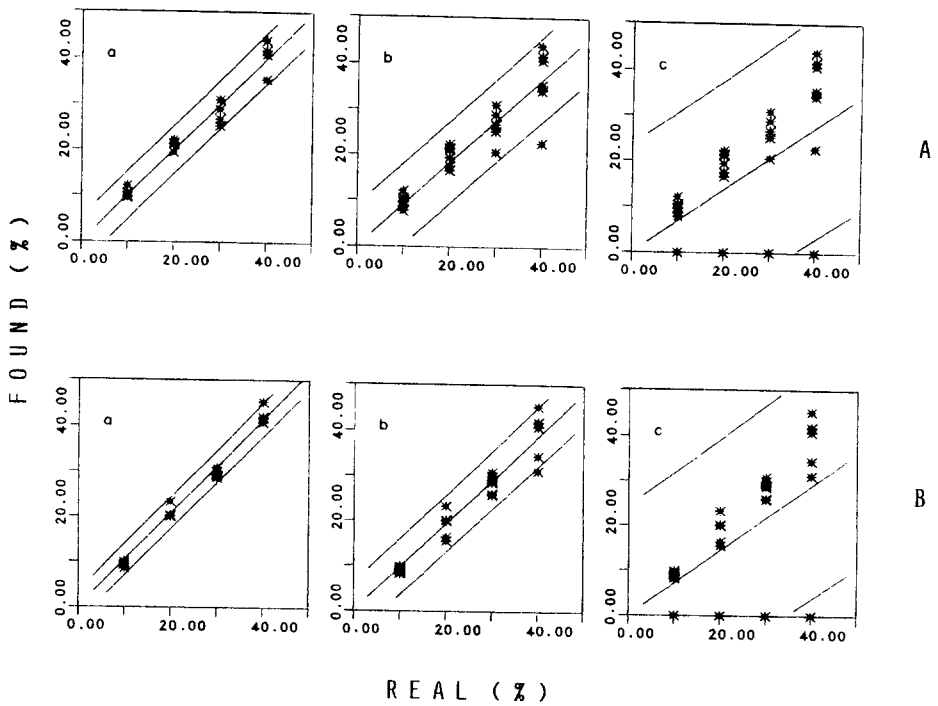

Fig. 4. Percentage of bovine milk in caprine (A) or ovine milk (B) (immunologically determined) vs. true percentage. Experimental data, fitted straight lines and the 95% confidence interval for a new observation. (a) 74°C for 15 or 30 s; (b) 74°C for 15 or 30 s and 90°C for 15 s; (c) 74°C for 15 or 30 s and 90°C for 15 or 30 s.

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