Effect of Thermal Treatment on the Protein Profile of Whey from Ovine and Caprine Milk throughout Lactation

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ABSTRACT

The independent and combined effects of temperature, heating period and stirring rate on the extent of precipitation of each of the major proteins in ovine and caprine acid wheys at various stages of lactation were studied using two-level, replicated, complete factorial designs. Evaluation of the extent of precipitation was based on the quantitative results obtained by gel permeation chromatography. Statistical analyses indicated that temperature is the most important factor in the precipitation of α-lactalbumin and β-lactoglobulin from acid whey from both ovine and caprine milks. The magnitude of the independent effects of heating time and stirring rate on the precipitation of β-lactoglobulin and α-lactalbumin from ovine whey reached local maxima as lactation elapsed, but such effects were rather small for caprine whey.

INTRODUCTION

Whey proteins possess excellent physicochemical, gelation and binding properties and, therefore, are used widely as functional ingredients in many formulated bakery, dairy and sausage products (Kinsella & Whitehead, 1989). Recovery of whey proteins for food uses may be achieved based on differences of molecular size (e.g. ultrafiltration, reverse osmosis and gel filtration), surface charge (e.g. ion exchange) or solubility (e.g. complexing agents and thermal denaturation). Whey proteins precipitated by heat treatments have found applications as additives in the manufacture of Cheddar cheese to improve its yield (Banks & Muir, 1985), in the manufacture of Quarg and Cottage cheeses to improve their consistency (Modler *et al.*, 1985) and in the manufacture of yogurt to increase its

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viscosity and water-holding capacity (Nielsen, 1976; Guirguis *et al.*, 1984). The thermally precipitated proteins also may be consumed directly, after pressing and moulding, as whey cheeses, which are designated by a variety of names, e.g. Serac, Brousse, Briocio or Grueil in France and Requeijão in Portugal (Roseiro & Wilbey, 1991).

The kinetics and thermodynamics associated with thermal treatments of bovine whey have been studied comprehensively (e.g. deWit & Klarenbeek, 1984; Hill & Irvine, 1988; Donovan & Mulvihill, 1987; Gough & Jenness, 1962; Guy *et al.*, 1967; Hill *et al.*, 1982, 1985; Hillier & Lyster, 1979; Larson & Rolled, 1955; Mulvihill & Donovan, 1987; Robinson, 1974). Although the protein and polypeptide content of ovine whey has been characterized extensively, both qualitatively and quantitatively (Bell & MacKenzie, 1967; Nielson, 1976; Hillier & Lyster, 1979; Jindal & Grandison, 1993; Barbosa, 1993), only a few workers (Ramos, 1978; Calvo *et al.*, 1989; Hill & Kakuda, 1990) have attempted to address the establishment of causal effects relating the operating parameters involved in thermal processing to the soluble protein profile in ovine and caprine whey. This situation may be due, partly, to the fact that ovine and caprine wheys are a very small fraction of the total whey produced in the developed world (Mills, 1986); however, in Portugal, the manufacture of cheese from milk from small ruminants has expanded considerably in recent years (Barbosa, 1993). Hence, studies pertaining to caprine and ovine wheys are timely in view of the increasing quantities of such dairy by-products. On the other hand, the proteins of ovine and caprine wheys possess a comparatively higher nutritional value than those of bovine whey (Moulin & Galzy, 1984), so a dietary impetus also exists for a fairly detailed knowledge of such wheys. An improved understanding of how the most common, and more easily manipulated, operating variables associated with thermal treatment affect the rate and extent of protein precipitation from acid whey, may be particularly useful for the controlled and optimized production of Requeijão on the farm level. This is so because Requeijão possesses a good market image and represents an extra source of income for artisan cheese makers via utilizing a by-product from the manufacture of traditional ewe's milk cheese.

The empirical conditions used in the manufacture of Requeijão are a temperature between 85 and 100°C, a heating time of about 30 min and moderate stirring. The main objective of the present study was to quantify, in the vicinity of such operating levels, the independent and combined effects of temperature, heating time and degree of stirring on the rate and extent of precipitation of the major proteins in ovine whey and in caprine whey obtained from milks collected throughout the normal lactation season.

MATERIALS AND METHODS

Reagents

Purified proteins from bovine milk (α-LA, β-LG, BSA, IgG and IgM) were purchased from Sigma (St Louis, MO, USA). The remaining MW standards (aldolase, ovalbumin and ribonuclease) and blue dextran were purchased from

Pharmacia (Uppsala, Sweden). Orotic acid was obtained from Sigma and uric acid from Biotrol (France). The NaCl, Na_2HPO_4, NaH_2PO_4, HCl, trichloroacetic acid (TCA), acetone and NaN_3 were purchased from Merck (Germany). All water used was deionized and distilled. Ovine milk was obtained throughout the lactation season from a single flock of Bordaleira ewes, caprine milk from a single flock of Alpina goats and bovine milk from a single herd of Friesian cows.

Equipment

The filter paper (pore size of 0.22 μm) was purchased from Nucleopore (USA). The dialysis tubing (MW cut-off of 2000 Da) was purchased from Sigma. Heat treatment was performed in a shaking water bath from Julabo (Germany). Protein precipitates were recovered in a RC5C centrifuge from DuPont (USA). The FPLC system (Pharmacia) used consisted of two P-500 positive displacement pumps, an electrically powered MV-7 motorized valve, a gel filtration column pre-packed with Superose 12 HR 10/30, a UV1 single-path spectrophotometer, a REC-102 double-channel recorder and an LCC-500 controller. Samples were lyophilized using an Alpha 1-4 apparatus (Martin Christ, Germany).

Preparation of whey

Thirteen batches of ovine milk were obtained periodically during the normal lactation season, from November to June. Seven batches of caprine milk were obtained periodically during the normal lactation season, from April to October. One batch of bovine milk was obtained in November. In order to obtain a standard whey during the whole lactation season, the following standard procedure for whey separation was used: each sample of milk was, upon receipt, acidified to pH 4.6 at 4°C using 1 M HCl and heated at *ca* 46°C for 10 min; after cooling, the aqueous whey was separated from the precipitate and the upper layer of milk fat by centrifugation at 8000*g* and 4°C for 15 min.

Heat treatments

Aliquots (10 ml) of clear whey were placed in test tubes (length = 16 cm; diameter = 16 mm) and subjected, using a shaking water bath, to heat treatments corresponding to the eight combinations of two temperatures (85 and 95°C), two heating periods (30 and 60 min) and two agitation rates (0 and 100 rpm). The aforementioned range of temperatures was chosen because precipitation would not start before *ca* 85°C, whereas boiling would prevent further temperature increases in the close vicinity of 95°C. The number of experimental conditions for each variable studied was dictated by the number of pre-screened variables with technological interest and the expected availability of material resources given the total number of experiments to be run (even using the most informative experimental layout). The experiments were performed in duplicate and in random order. Following heat treatment, each tube was cooled to room temperature (*ca* 20°C) in tap water. The precipitate thus formed was separated from the supernatant by centrifugation at 8000*g* and 4°C for 15 min and the supernatant was kept refrigerated until further analysis.

Preparation of heat-stable fraction

Aliquots of the ovine and caprine wheys collected in June and October, respectively, prepared using the standard procedure described above, were heated at 95°C for 60 min. The supernatants were separated from the precipitate by centrifugation at 1500*g* for 30 min. Following addition of TCA to 12%, the precipitates were separated from the supernatant by centrifugation at 1500*g* for 30 min and treated as described by Andrews (1978a) to obtain the acid-stable, heat-stable polypeptide fraction [often denoted as the proteose peptone (PP) fraction]. The supernatant remaining after precipitation by TCA was dialysed for 24 h against distilled water and the retentate was lyophilized.

Protein separation

Aliquots (100 μL) of the supernatant wheys following acid and heat treatment, or just acid treatment, were injected onto the column of the FPLC system and eluted using 0.05 M phosphate buffer, pH 7.0, containing 0.15 M NaCl (to correct ionic strength) and 0.2 g L^{-1} of NaN_3 (as preservative). The flow rate of the eluent was 0.4 mL min^{-1}. Prior to chromatographic analysis, the sample and buffer were filtered through paper filter and de-gassed. Detection of the proteins in the eluate was by absorbance at 280 nm. The void volume of the column was determined using blue dextran. The column was calibrated for MW using an aqueous solution containing 2 mg mL^{-1} of aldolase (MW: 158,000 Da), 7 mg mL^{-1} of bovine albumin (MW: 67,000 Da), 7 mg mL^{-1} of ovalbumin (MW: 43,000 Da), 2.8 mg mL^{-1} of bovine β-LG (MW: 36,000 Da), 5.8 mg mL^{-1} of bovine α-LA (MW: 14,400 Da) and 6 mg mL^{-1} of ribonuclease (MW: 13,700 Da). Quantitative calibration of the column in terms of bovine whey proteins was performed using various dilutions of an aqueous solution containing 9.10 mg mL^{-1} of bovine α-LA, 4.10 mg mL^{-1} of bovine β-LG, 4.9 mg ML^{-1} of bovine IgG, 6.8 mg mL^{-1} of bovine BSA, 0.13 mg mL^{-1} of orotic acid, 0.018 mg mL^{-1} of uric acid, 3 mg mL^{-1} of the acid-stable, heat-stable fraction from ovine or caprine whey, and 13 mg mL^{-1} of the lyophilized fraction obtained upon removal of this acid-stable, heat-stable fraction from ovine or caprine whey, respectively.

Data reporting and statistical analyses

The data to be statistically treated, P_i, corresponded to the percentage removal of each soluble protein in the whey by precipitation, *viz.*:

$$P_i = \frac{C_{i,0} - C_i}{C_{i,0} - C_{i,\infty}} \times 100 \quad (1)$$

where $C_{i,0}$ and C_i are the concentrations of protein i in acid whey before and after thermal treatment, respectively, and $C_{i,\infty}$ is the concentration of the same protein after thermal treatment for 60 min at 95°C (these conditions were considered to be sufficiently severe that all proteinaceous material will eventually precipitate; hence, any response remaining should result from absorbance by such interfering materials as acid-stable, heat-stable components). When true standards were not available (as was the case for ovine and caprine wheys), equation (1) was replaced by:

$$P_i = \frac{A_{i,0} - A_i}{A_{i,0} - A_{i,\infty}} \times 100 \tag{2}$$

which gives the same estimate of P_i as equation (1) provided that (as checked experimentally) the chromatogram areas are linear functions of the dilutions of the solutions under investigation (i.e. $A_i = a + bC_i$, where a and b are constants).

Statistical analysis of the results followed the classical two-level, replicated full factorial design (Box *et al.*, 1978). The tentative empirical model to be fitted to the data generated for ovine and caprine wheys is of the form:

$$\begin{aligned}\hat{P} = \omega_{avg} &+ \omega_T\left(\frac{T-90}{5}\right) + \omega_t\left(\frac{t-45}{15}\right) + \omega_s\left(\frac{s-50}{50}\right) + \omega_{Tt}\left(\frac{T-90}{5}\right)\left(\frac{t-45}{15}\right) \\ &+ \omega_{Ts}\left(\frac{T-90}{5}\right)\left(\frac{s-50}{50}\right) + \omega_{ts}\left(\frac{t-45}{15}\right)\left(\frac{s-50}{50}\right) \\ &+ \omega_{Tts}\left(\frac{T-90}{5}\right)\left(\frac{t-45}{15}\right)\left(\frac{s-50}{50}\right)\end{aligned} \tag{3}$$

where ω_{avg} is the grand average of the data; ω_T, ω_t and ω_s are the independent effects of temperature, heating time and agitation rate, respectively; ω_{Tt}, ω_{Ts} and ω_{ts} are the double interactions of effects involving the operating variables; and ω_{Tts} is the triple interaction of effects involving the operating variables. [In equation (3), temperature, T, must be expressed in °C; heating time, t, in min and stirring rate, s, in rotations per min; all three processing parameters enter the model in dimensionless and normalized form.] Utilization of equation (3) instead of a classical ANOVA table for statistical analysis of the data has the advantage that actual modelling (and thus educated predicting) is possible in addition to concluding which effects are statistically significant.

Estimation of the ω values using linear regression analysis is valid provided that the experimental errors are independent from one another, normally distributed, and show a constant variance (Box *et al.*, 1978). According to preliminary analyses using the original data, the plot of the residuals vs the average of each set of two replicates (not shown) and the plot of residuals vs the percentiles of a normal distribution (not shown) were not consistent with those assumptions for several data sets. Therefore, the data in question had to be transformed to:

$$y_{ij}^{(\lambda)} \equiv \frac{y_{ij}^{\lambda} - 1}{\lambda \dot{y}^{\lambda - 1}} \tag{4}$$

(where i denotes the experiment in question and j the replicate associated with those experimental conditions) in order to stabilize the variance and to normalize the residuals prior to further statistical analysis. In equation (4), $\dot{y}$ is the geometric mean of all untransformed data (i.e. $\dot{y} \equiv \sqrt[IJ]{\prod_{i=1}^{I}\prod_{j=1}^{J} y_{ij}}$, where I is the total number of experiments and J the total number of replicates performed for each set of experimental conditions). Parameter λ is determined by the likelihood estimation method; according to this method (Box *et al.*, 1978), λ is the value for

which the residual sum of squares of the transformed replicates (i.e. $y_{ij}^{(\lambda)}$) with respect to their average (i.e. $y_i^{(\lambda)}$), *viz.* $\sum_{i=1}^{I}\sum_{j=1}^{J}(y_{ij}^{(\lambda)} - y_i^{(\lambda)})^2$ is minimized.

RESULTS AND DISCUSSION

Identification and quantitative measurement of whey proteins

It can be argued that analysis of the protein profile by reverse-phase HPLC might have separated the protein fractions better; however, gel permeation chromatography, while relatively unaffected by the changes in charge or hydrophobicity that can occur in whey proteins during heat treatment (Law *et al.*, 1993) and exhibiting a rather low variability of retention times (Llano *et al.*, 1990), has met with a high degree of success in previous studies of denaturation and aggregation of proteins (Manji & Kakuda, 1986; Law *et al.*, 1994).

Fig. 1(a) is an FPLC chromatogram of bovine whey protein standards. Comparison of the retention times for the peaks corresponding to every protein in several independent runs (data not shown) indicated that the chromatographic method used was qualitatively and quantitatively reproducible, and may thus, in principle, be used safely for assays of ovine and caprine wheys.

Typical protein chromatograms of wheys obtained from bovine, ovine and caprine milks are shown in Fig. 1(b), (c) and (d), respectively. Typical chromatograms of the acid-stable, heat-stable whey fraction of ovine whey and the supernatant obtained after removal of this stable fraction are shown in Fig. 1(e) and (f), respectively. It is interesting to note that the retention times of the major peaks of the three whey protein chromatograms were similar. Further comparison of Fig. 1(a) with Fig. 1(b), (c) and (d) indicated that peaks 2, 3, 4 and 5 (with retention times of 26.3, 28.4, 31.2 and 34.8 min, respectively) corresponded to IgG, BSA, β-LG and α-LA, respectively, based on their retention times. Therefore, in the absence of reliable commercial standards for ovine and caprine whey proteins, the bovine whey protein standards were used directly hereafter to identify and, where indicated, quantify ovine and caprine whey proteins. The chromatograms obtained in this research work resembled those obtained by several researchers working with bovine whey (Andrews *et al.*, 1985; Hill *et al.*, 1987) and caprine whey (Hill & Kakuda, 1990) who used similar techniques of size exclusion.

The retention time of blue dextran (which is virtually equivalent to the void volume of the gel filtration column) was *ca* 19.5 min. The peaks in Fig. 1(b), (c) and (d) in the neighbourhood of 19.5 min can be accounted for by IgM, which has a MW of 900,000 (Dannenberg & Kessler, 1988), high MW impurities accompanying IgG (Andrews *et al.*, 1985), or other proteins of high MW which belong to the Ig family (in all cases the MW was close to the upper limit of the MW cut-off of the column used).

Peaks 6 and 7 in Fig. 1(c) and (d) were probably acid-stable, heat-stable compounds not included in the PP fraction, because the areas of these peaks remained essentially unchanged upon heating at 95°C for 60 min; this observation, which agrees with conclusions reported elsewhere (Andrews, 1978a, b;

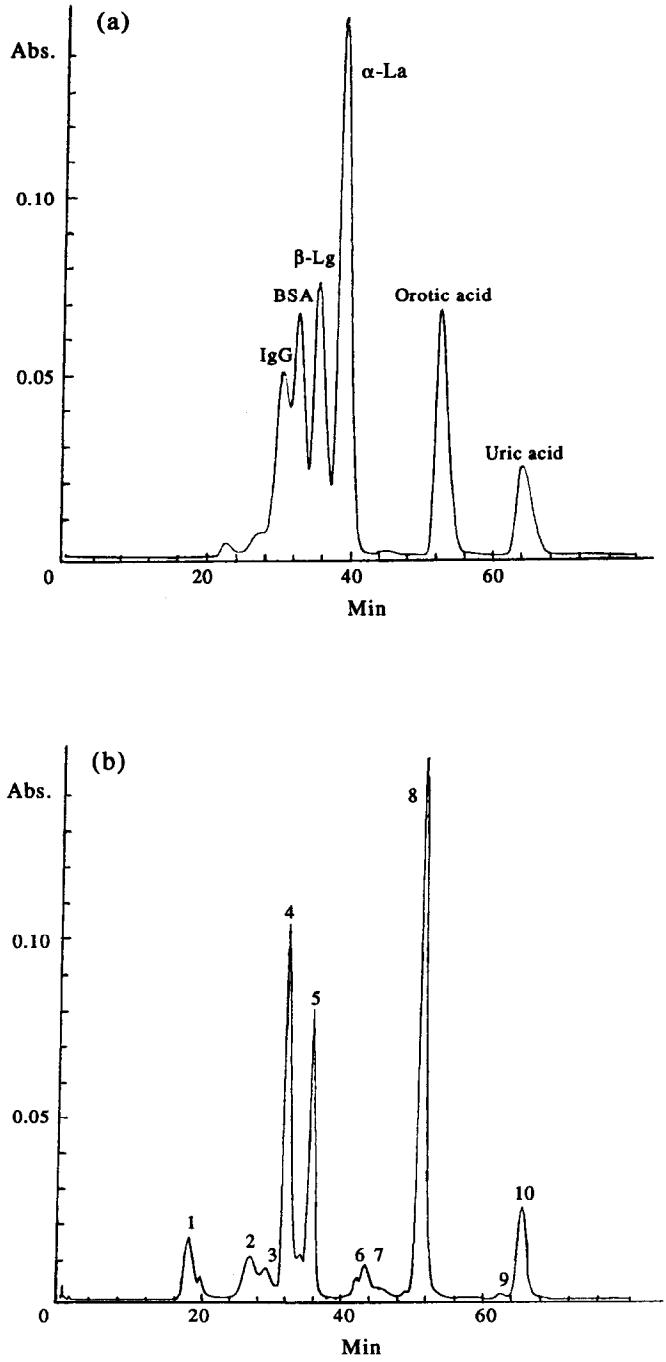


Fig. 1. FPLC chromatograms obtained for (a) standard bovine whey proteins (IgG, BSA, β-LG and α-LA) and orotic and uric acids; (b) a typical sample of bovine whey;

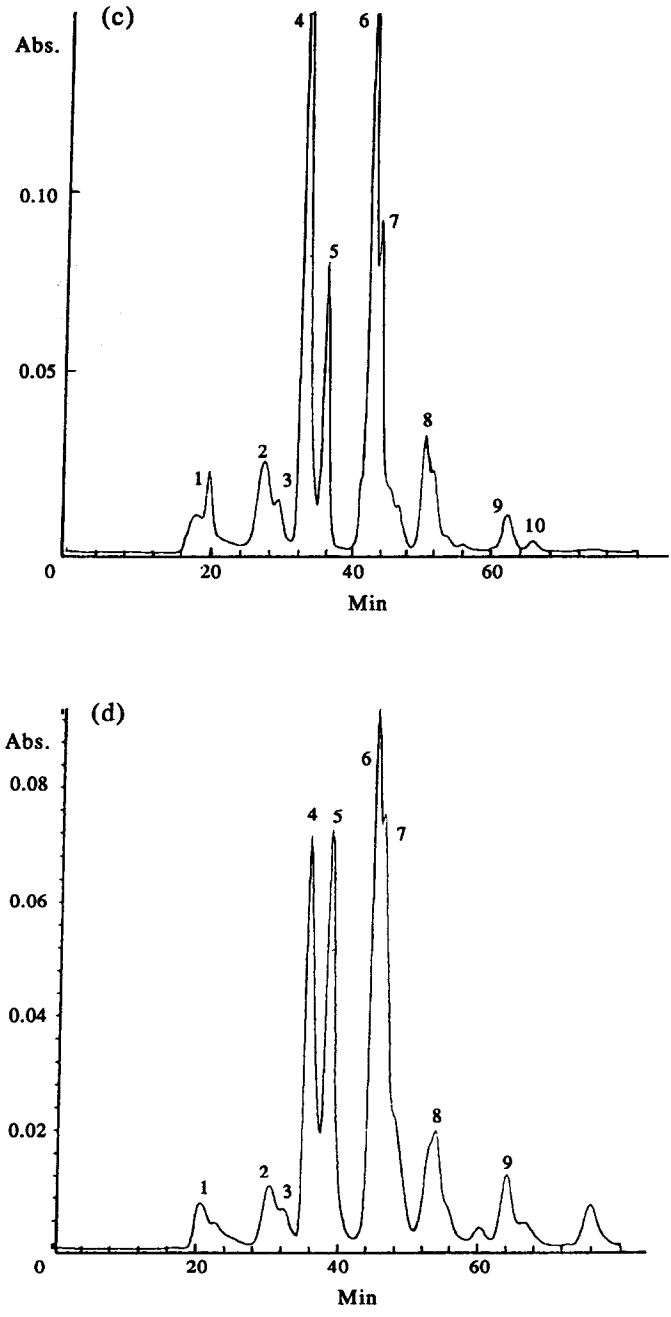
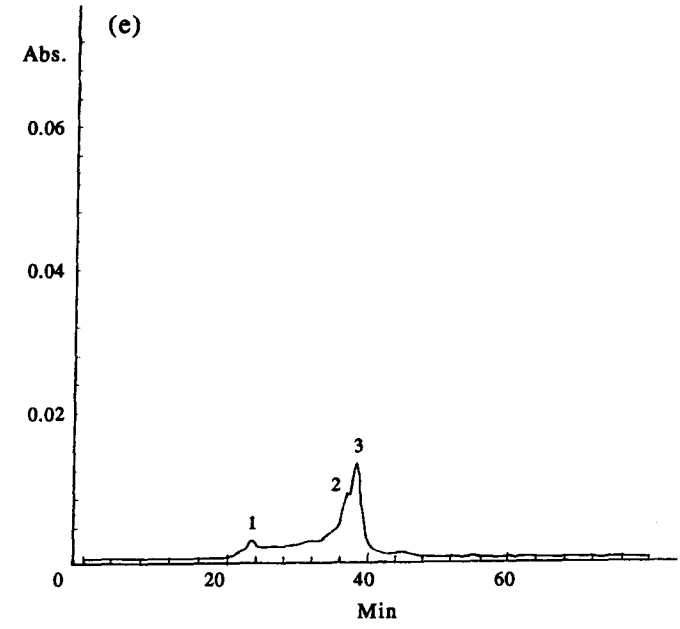


Fig. 1. *continued* (c) a typical sample of ovine whey; (d) a typical sample of caprine whey;



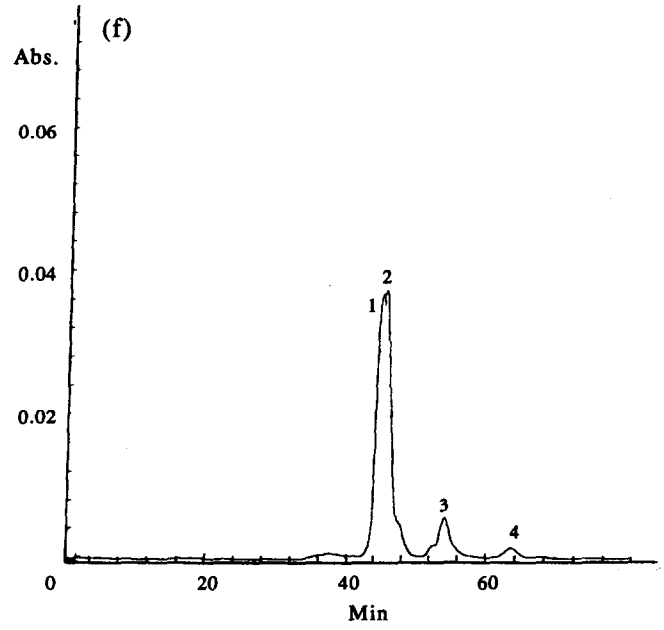


Fig. 1. *continued* (e) the acid-stable, heat-stable fraction in a typical sample of ovine whey; (f) the supernatant remaining upon removal of this acid-stable, heat-stable fraction in a typical sample of ovine whey.

Pearce, 1980; Ramos *et al.*, 1988), was confirmed by the fact that they were not present in the isolated PP fraction [see Fig. 1(e)]. Peaks 2 and 3 in Fig. 1(e) probably corresponded to the PP-3 and PP-5 fractions, respectively (Maubois *et al.*, 1965; Jindal & Grandison, 1993). It is clear that this fraction was masked in Fig. 1(b), (c) and (d) by peaks 4 and 5. The components of the supernatant after removal of the PP fraction, after concentration by lyophilization [see Fig. 1(f)], may correspond to peaks 6 and 7, which had similar retention times. Further work will be required to identify these peaks. Peaks 6 and 7 in Fig. 1(c) represented a fractional area lower than the equivalent peaks in Fig. 1(d), but higher than the equivalent peaks in Fig. 1(b). However, a final conclusion on the fractional concentrations thereof cannot be reached until the response factors for all components which appeared in the chromatograms of ovine and caprine whey have been determined; in fact, the area percentage of peak 4 was *ca* 25% of the total peak area of Fig. 1(c), but β-LG represents 55% of the proteins in ovine whey (Moulin & Galzy, 1984).

Peaks 8 and 9 in Fig. 1(c) and (d) may represent low MW nitrogenous compounds in ruminant wheys; this view is substantiated by the observation that orotic and uric acids, in this order, had the same retention times as these peaks.

Using the bovine standards to quantity the proteins in bovine, caprine and ovine wheys, the following average values were obtained: 3.27, 3.43 and 7.28 mg mL^{-1} for β-LG in bovine, caprine and ovine whey, respectively; 2.20, 3.16 and 3.06 for α-LA; 0.58, 0.44 and 1.19 for BSA; and 0.61, 0.51 and 1.19 for IgG. If the response factors of similar proteins in the three types of whey were approximately the same, the aforementioned values indicate that the concentrations of all proteins in ovine whey were approximately twice the concentration of their counterparts in bovine whey, except for α-LA; these ratios were expected because ovine milk and whey contain about twice as much total protein as bovine whey (Moulin & Galzy, 1984). If the percentages of the four major proteins are calculated, the values of *ca* 50, 33, 8 and 9% for β-LG, α-LA, BSA and IgG in bovine whey, *ca* 45, 42, 6 and 7% in caprine whey, and *ca* 57, 24, 9.5 and 9.5% in ovine whey, respectively, were obtained. Such values agree closely with those reported by Anifantakis (1986) for ovine whey, but disagree with data reported by Cossedu & Pidanu (1979) who found higher values for minor proteins and smaller values for α-LA in caprine whey from Sardinia.

When the chromatograms obtained for the three ruminant wheys were compared, several differences were apparent. One difference was in peak 8, which appeared as a single peak in bovine whey [see Fig. 1(b)] but was eluted in two partially unresolved peaks in ovine whey [see Fig. 1(c)]. Another major difference was associated with peaks 6 and 7, which, unlike ovine and caprine wheys [see Fig. 1(c) and (d)], were virtually absent from bovine whey [see Fig. 1(b)].

The variations of the peak areas corresponding to IgG, BSA, β-LG and α-LA in ovine whey were followed throughout lactation [see Fig. 2(a)]. Remarkably, the peak areas for β-LG and α-LA in ovine whey correlated linearly with one another (correlation coefficient > 0.98) throughout lactation and, as reported elsewhere (Regester & Smithers, 1991), they are likely to be a function essentially of the seasonal characteristics of the pasture used for feeding. The BSA showed slight variation, but IgG showed greater variability throughout lactation. From the variations of the corresponding peaks for caprine whey [see Fig. 2(b)], it was found that the lowest peak areas for all proteins occurred in April. The β-LG

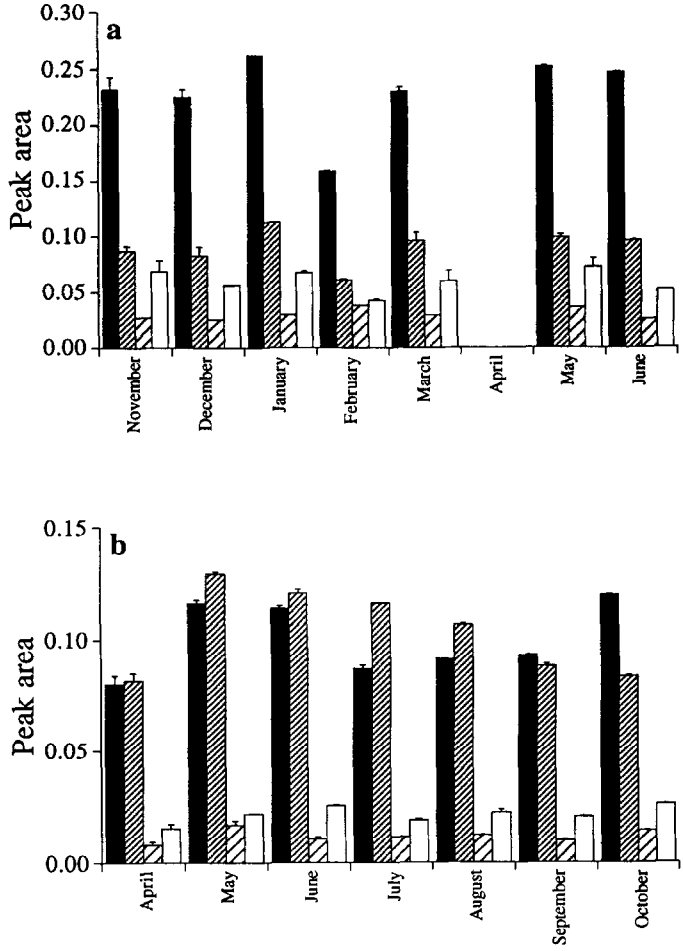


Fig. 2. Variation of the chromatogram peak areas for β-LG, α-LA, BSA and IgG throughout the lactation period (and associated 95% confidence intervals) in untreated (a) ovine whey and (b) caprine whey (■ = β-LG; ▨ = α-LA; ▨ = BSA; □ = IgG).

showed variability throughout lactation (which is, in a sense, similar to the trend for the same protein in ovine whey), but α-LA reached a maximum in May, and decreased slightly until October. The changes in the concentrations of the various proteins as the lactation season progressed were consistent with the results reported by Zygoyiannis & Katsaounis (1991) for Greek goats, who concluded that the nitrogen distribution is affected by the stage of lactation.

Thermal denaturation of whey proteins

The susceptibility to temperature, measured by the differences in the extent of heat precipitation upon treatments at 85 and 95°C for 60 min without agitation

TABLE 1

Average Experimental Values of Per Cent Precipitation of the Two Major Proteins in Ovine and Caprine Wheys Throughout the Lactation Season at the Various Experimental Conditions

Month	*Type of whey*	*Type of protein*	$T^* = -1$ $t^* = -1$ $s^* = -1$	$T^* = -1$ $t^* = -1$ $s^* = +1$	$T^* = -1$ $t^* = +1$ $s^* = -1$	$T^* = -1$ $t^* = +1$ $s^* = +1$	$T^* = +1$ $t^* = -1$ $s^* = -1$	$T^* = +1$ $t^* = -1$ $s^* = +1$	$T^* = +1$ $t^* = +1$ $s^* = -1$	$T^* = +1$ $t^* = +1$ $s^* = +1$
November	Ovine	α-LA	34.4	46.1	67.3	61.5	86.7	98.0	99.8	98.2
		β-LG	14.6	18.8	38.0	33.5	96.4	95.1	98.1	100.0
December	Ovine	α-LA	33.4	53.8	62.1	77.6	85.6	84.0	89.8	99.5
		β-LG	8.3	19.1	26.2	48.6	98.6	98.7	95.9	99.8
January	Ovine	α-LA	56.9	56.7	79.9	90.5	88.9	84.3	99.6	93.0
		β-LG	30.5	33.2	58.3	65.9	93.8	99.2	99.5	99.9
February	Ovine	α-LA	30.2	29.7	67.7	66.6	88.8	77.0	84.2	99.4
		β-LG	27.3	13.4	31.9	38.3	99.5	87.8	99.3	99.8
March	Ovine	WLA	68.2	57.7	91.3	74.1	85.2	84.6	99.5	90.4
		β-LG	50.7	38.8	68.1	42.5	98.2	99.3	99.8	96.8
	Caprine	β-LA	34.0	38.3	48.9	47.4	84.4	84.1	97.0	99.7
		β-LG	22.6	18.9	24.4	33.7	98.9	93.6	99.9	99.5
April	Caprine	α-LA	27.4	59.2	52.8	19.1	88.5	85.5	91.5	99.6
		β-LG	20.7	69.5	18.4	1.85	89.5	98.2	92.3	99.8
May	Ovine	α-LA	62.2	71.5	92.7	94.6	93.6	96.6	99.5	99.1
		β-LG	32.8	40.6	62.1	61.5	98.1	99.6	99.9	99.7
	Caprine	α-LA	59.8	61.3	66.6	85.3	93.6	89.8	99.8	99.2
		β-LG	32.2	27.2	32.9	58.2	84.7	94.3	96.4	99.8

June	Ovine	α-LA	54.2	55.3	83.4	82.0	94.6	96.6	100.0	97.6
		β-LG	35.8	34.5	56.5	57.5	97.0	97.1	96.5	97.0
	Caprine	α-LA	31.3	26.9	45.8	46.3	86.2	73.5	93.7	99.7
		β-LG	20.7	16.7	24.4	33.5	96.5	86.4	95.6	100
July	Caprine	α-LA	15.9	47.9	25.8	31.0	88.2	88.9	99.7	93.2
		β-LG	2.3	39.2	3.0	13.2	92.7	89.9	97.3	99.7
August	Caprine	β-LA	49.3	30.7	47.8	46.9	89.2	80.8	93.6	99.6
		β-LG	10.9	2.05	26.7	17.8	96.9	97.2	98.8	99.9
September	Caprine	α-LA	31.6	27.8	42.2	38.6	79.0	99.2	99.1	93.9
		β-LG	10.9	2.05	26.7	17.8	96.9	97.2	98.8	99.9
October	Caprine	α-LA	19.8	23.8	36.2	36.8	88.4	81.7	99.5	87.9
		β-LG	0.85	11.3	15.0	1.1	94.4	95.2	99.6	99.9

Note $T^* = (T - 90)/5$, where T is expressed in °C; $t^* = (t - 45)/15$, where t is expressed min; $s^* = (s - 50)/50$, where s is expressed in min^{-1}.

(results not shown) indicated that α-LA and β-LG remained soluble upon heating to 85°C, but not to 95°C, whereas BSA and IgG disappear rapidly from solution. These results agree with those by Ramos (1978) who reported that β-LG remains soluble upon heating ovine whey to 95°C, but that α-LA an β-LG disappear on heating to 95°C, and with those by Robinson (1974) who reported that complete precipitation of α-LA and β-LG occurs only on heating to above 90°C.

From the experimental results obtained for the degree of precipitation, P (see Table 1), it is interesting to note that the per cent removal of α-LA was higher than that of β-LG in most situations corresponding to 85°C for both ovine and caprine wheys. This observation, which suggests a higher heat sensitivity of α-LA, agrees with other published studies (Ruegg *et al.*, 1977; Dannenberg & Kessler, 1988) which indicated that α-LA shows a lower denaturation temperature than β-LG. (The extent to which this comparison is valid is limited, however, because denaturation of a protein does not necessarily lead to precipitation thereof, and

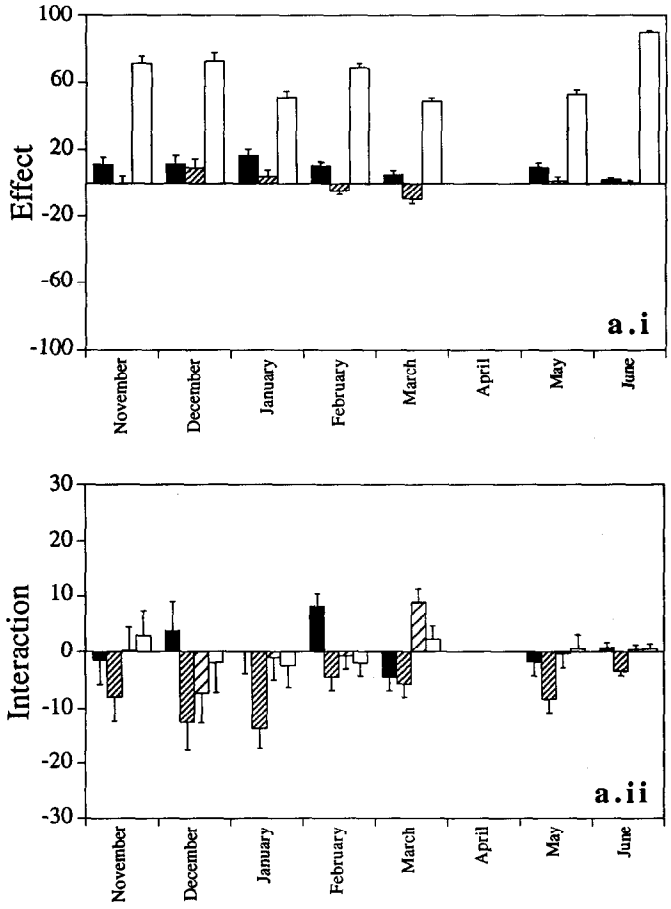


Fig. 3. (legend opposite).

differential scanning calorimetry results for whey proteins at a given concentration may not be strictly comparable with those at natural whey concentrations because whey protein interactions are concentration dependent.)

It was observed that, for the same months, the amounts of α-LA and β-LG precipitated were higher for ovine than for caprine whey. These results, which are in agreement with those obtained by Ramos (1979) who concluded that ovine milk is more heat labile than caprine milk in terms of soluble protein, could be attributed partly to differences in fat content.

The fitted values for all effects (using the transformed data where appropriate) following the general form of equation (3) and encompassing the precipitation of β-LG and α-LA are shown in Figs 3 and 4 for ovine and caprine wheys, respec-

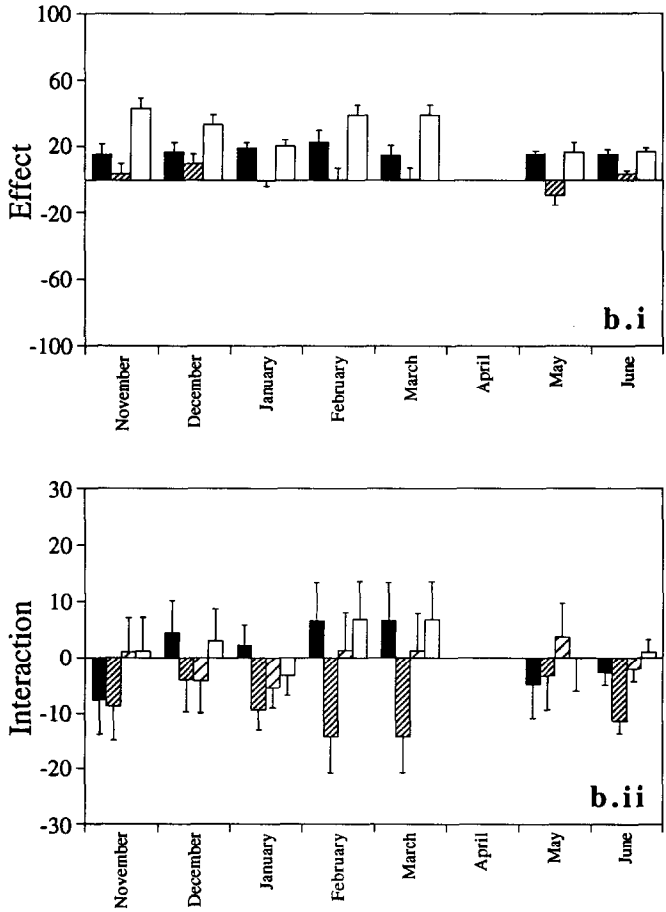


Fig. 3. Estimated values (and associated 95% confidence intervals) of (i) the independent effects (ω_T, ω_t and ω_s for the operating variables temperature, heating time and agitation rate, respectively) and their (ii) double interactions (ω_{Tt}, ω_{Ts} and ω_{ts}) and triple interaction (ω_{Tts}), for (a) β-LG; (b) α-LA in ovine whey. (i) ■ = t; ▨ = s; □ = T; (ii) ■ = t/T; ▨ = t/s; ▨ = s/T; □ = t/s/T.

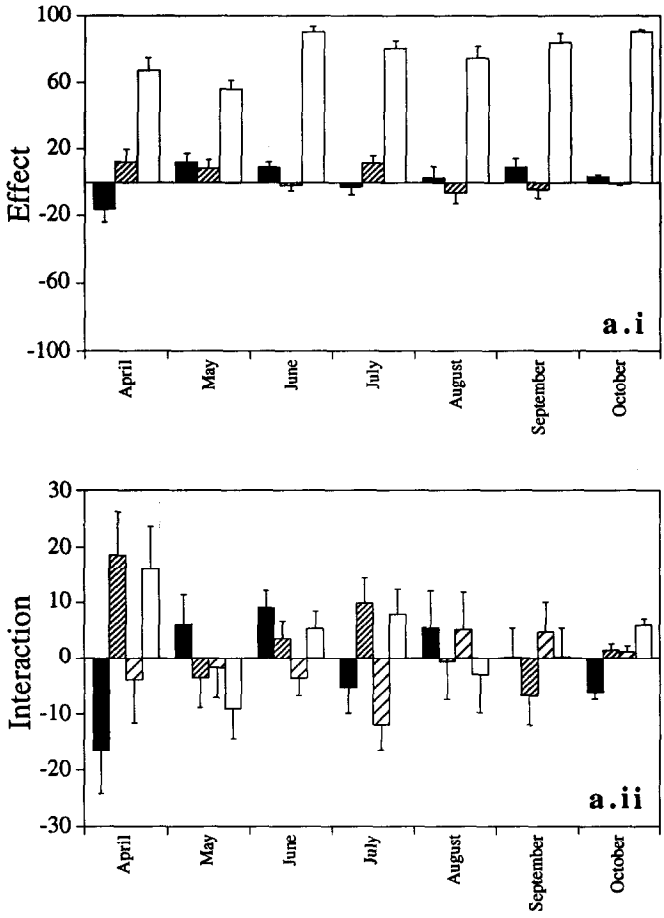


Fig. 4. (legend Opposite).

tively. Inspection of those figures indicates that the null hypothesis (i.e. the conclusion that the parameter in question is not statistically significant) is accepted for several of the effects at the 5% level of significance (i.e. the probability that the small value obtained for the effect in question is due to pure chance is above 95%). Those effects which were found to be statistically significant at the 5% significance level are tabulated in Tables 2 and 3. As expected, most double and triple interactions were not statistically significant, and those which were, in general, had values well below those of the independent effects. It is worthy of note that the stirring rate had a statistically negligible effect for most months for wheys from both types of small ruminant, whereas temperature had a statistically significant effect for all months and for both types of whey.

Figures 3 and 4 indicate that the most important effect (measured by the *t*-ratio, or the ratio of the parameter value in question to its confidence interval) for protein precipitation was temperature for both species in the case of β-LG and α-LA; this conclusion agrees with the work of Pearce

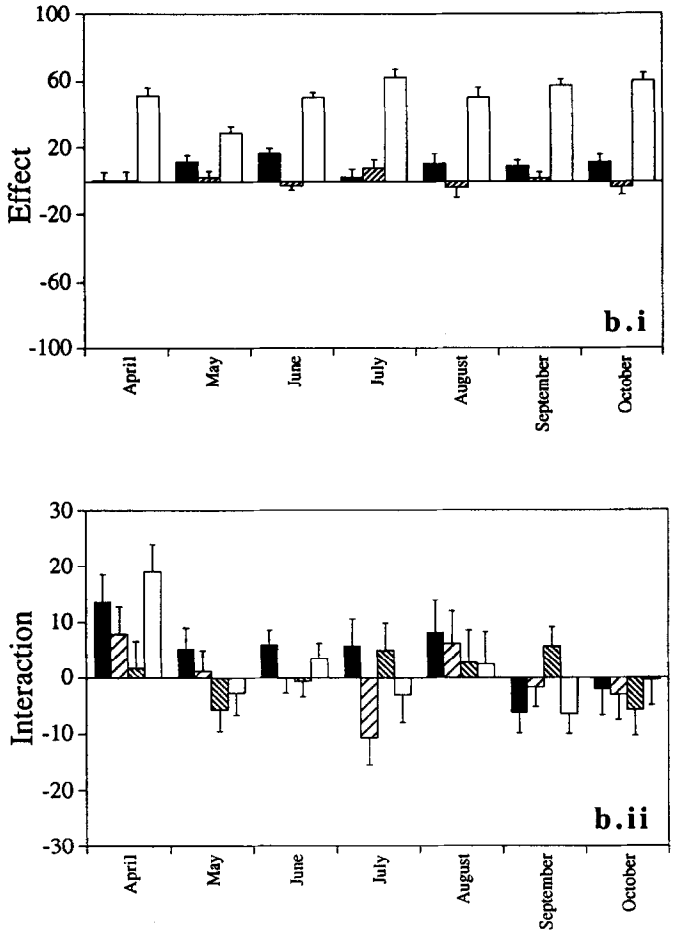


Fig. 4. Estimated values (and associated 95% confidence intervals) of (i) the independent effects (ω_T, ω_t and ω_s, for the operating variables temperature, heating time and agitation rate, respectively) and their (ii) double interactions (ω_{Tt}, ω_{Ts} and ω_{ts}) and triple interaction (ω_{Tts}), for (a) β-LG; (b) α-LA in caprine whey. (i) ■ = t; ▨ = s; □ = T; (ii) ■ = t/T; ▨ = t/s; ▨ = s/T; □ = t/s/T.

(1983) which showed that the extent of precipitation increased with temperature. The effect was more pronounced for β-LG than for α-LA, probably because α-LA undergoes reversible denaturation, while β-LG undergoes irreversible thermodenaturation due to aggregation (Ruegg *et al.*, 1977). It is interesting that the temperature effect was positive throughout the lactation season, and that for β-LG and α-LA from caprine whey there was a maximum and a minimum for the temperature around October and May, respectively.

In the case of precipitation of β-LG and α-LA, the time effect showed a maximum value for ovine whey around January and February. For caprine whey, the

TABLE 2

Estimates (and Corresponding 95% Confidence Intervals) of Linear Effects and Interactions Significant at the 5% Level for β-LG and α-LA in Ovine Whey

Month	*Protein*	ω_t	ω_s	ω_T	ω_{ts}	ω_{Tt}	ω_{Ts}	ω_{Tts}
November	β-LG	11.2 ± 4.3		71.2 ± 4.3		−7.9 ± 4.3		
	α-LA	15.4 ± 6.1		43.3 ± 6.1				
December	β-LG	11.4 ± 5.2	9.3 ± 5.2	72.7 ± 5.2		−12.3 ± 5.2		
	α-LA[a]	17.0 ± 5.7	9.8 ± 5.7	33.2 ± 5.7				
January	β-LG	16.7 ± 3.8		51.1 ± 3.8				
	α-LA[b]	19.1 ± 3.6		20.4 ± 3.6		−9.4 ± 3.6		
February	β-LG	10.3 ± 2.3	−4.5 ± 2.3	68.8 ± 2.3	8.2 ± 2.3	−4.4 ± 2.3		
	α-LA	38.8 ± 6.7		38.8 ± 6.7		−14.1 ± 6.7		
March	β-LG	5.1 ± 2.4	−9.8 ± 2.4	48.5 ± 2.4	−4.5 ± 2.4	−5.6 ± 2.4	8.9 ± 2.4	
	α-LA[a]	14.8 ± 6.1	−9.1 ± 6.1	16.6 ± 6.1				
May	β-LG[b]	9.7 ± 2.5		52.9 ± 2.5		8.3 ± 2.5		
	α-LA	17.5 ± 2.2		17.0 ± 2.2		−11.3 ± 2.2		
June	β-LG[b]	2.6 ± 0.9		90.0 ± 0.9		−3.4 ± 0.9		
	α-LA	15.6 ± 2.5		28.5 ± 2.5		−12.3 ± 2.5		

[a]Transformed value with $\lambda = 2$.

[b]Transformed value with $\lambda = 5$.

Note: ω_T, ω_t and ω_s are the independent effects of temperature, heating time and agitation rate, respectively; ω_{Tt}, ω_{Ts} and ω_{ts} are the corresponding double interactions and ω_{Tts} is the corresponding triple interaction.

TABLE 3

Estimates (and Corresponding 95% Confidence Intervals) of Linear Effects and Interactions Significant at the 5% Level for β-LG and α-LA in Caprine Whey

Month	*Protein*	ω_t	ω_s	ω_T	ω_{ts}	ω_{Tt}	ω_{Ts}	ω_{Tts}
April	β-LG	−16.4 ± 7.6	12.1 ± 7.6	67.3 ± 7.6	−16.6 ± 7.6	18.6 ± 7.6		16.0 ± 7.6
	α-LA[a]			51.7 ± 4.9	−13.6 ± 4.9	7.9 ± 4.9		19.1 ± 4.9
May	β-LG	12.2 ± 5.3	8.3 ± 5.3	56.2 ± 5.3				−9.1 ± 5.3
	α-LA[a]	11.9 ± 3.8		28.8 ± 3.8	5.2 ± 3.8		−5.7 ± 3.8	
June	β-LG[b]	9.1 ± 3.1		90.2 ± 3.1	9.1 ± 3.1			5.4 ± 3.1
	α-LA	16.9 ± 2.7		50.7 ± 2.7	5.9 ± 2.7			
July	β-LG		11.6 ± 4.5	80.5 ± 4.5		9.9 ± 4.5	−11.9 ± 4.5	7.9 ± 4.5
	α-LA		−7.8 ± 4.9	62.3 ± 4.9	−8.5 ± 4.9		−10.8 ± 4.9	
August	β-LG			74.7 ± 6.7				
	α-LA[b]	10.7 ± 5.9		50.7 ± 5.9	8.1 ± 5.9			
September	β-LG	9.0 ± 5.3		83.8 ± 5.3		−6.7 ± 5.3		
	α-LA	9.0 ± 3.5		57.7 ± 3.5	−6.3 ± 3.5		5.6 ± 3.5	−6.4 ± 3.5
October	β-LG	3.5 ± 1.1		90.2 ± 1.1	−6.2 ± 1.1			5.9 ± 1.1
	α-LA	11.7 ± 4.5		60.2 ± 4.5			−5.8 ± 4.5	

[a]Transformed value with $\lambda = 4$.

[b]Transformed value with $\lambda = 2$.

Note: ω_T, ω_t and ω_s are the independent effects of temperature, heating time and agitation rate, respectively; ω_{Tt}, ω_{Ts} and ω_{ts} are the corresponding double interactions and ω_{Tts} is the corresponding triple interaction.

effect of heating time was quite small. The stirring rate had effects on the fractional amounts of β-LG and α-LA precipitated from ovine whey that passed through maxima (centred, however, at different months depending on the peak in question); in caprine whey such effects were again of negligible importance in most months. The magnitude of the combined effect of time and temperature for ovine whey (which is a relevant effect for the precipitation of both β-LG and α-LA) may be due to the largely nonlinear nature of the precipitation process with time at any given temperature. In general, the significance of seasonal changes of the sensitivity of individual proteins to heating is difficult to substantiate from a physicochemical point of view. However, natural changes in milk composition, especially fat, salt and overall protein, throughout the lactation season (due to alteration of the metabolic rates probably triggered by changes in the nutritional value of the feed and in the outer weather conditions) are prone to affect the microenvironment of individual proteins in whey and thus alter their response to heating.

CONCLUSIONS

The percentage of the four major proteins, β-LG, α-LA, BSA and IgG, were *ca* 50, 33, 8 and 9%, respectively in bovine whey; *ca* 45, 42, 6 and 7% in caprine whey; and *ca* 57, 24, 9.5 and 9.5% in ovine whey. The variations of the amounts of β-LG and α-LA in ovine whey throughout lactation correlated linearly with one another. The statistical analyses showed that, although temperature had the most important effect in the precipitation of all major proteins in ovine and caprine acid wheys, the thermal stability increased from α-LA to β-LG in both ovine and caprine wheys. The magnitude of the effect of heating time and stirring rate on the extent of precipitation of β-LG and α-LA passed through maxima located in mid-lactation for ovine whey. The magnitude of the effect of heating time and stirring rate on the extent of precipitation of the major proteins of caprine whey was very small. The combined effect of heating time and temperature was also important for the precipitation of α-LA and β-LG from ovine whey. The empirical polynomial model considered (which was not applied previously to similar data) was particularly useful because the manipulated variables responsible for statistically significant changes in the protein profile could be identified in a simple way. These variables should be those selected for manipulation during manufacture of Requeijão if the maximum extent of precipitation of either protein is defined as the objective function.

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