Characterization of Requeijão and Technological Optimization of its Manufacturing Process

M. E. Pintado, J. A. Lopes da Silva & F. X. Malcata*

Escola Superior de Biotecnologia, Universidade Católica Portuguesa, Rua Dr. António Bernardino de Almeida, 4200 Porto, Portugal

ABSTRACT

In attempts to characterize Portuguese whey cheese (Requeijão) and optimize the manufacture thereof, 17 whey cheeses were produced according to a factorial design using heating time, heating temperature and fractional addition of ovine/caprine milk as manipulated technological variables. Chemical analyses were carried out for the 17 cheeses, whereas sensorial and rheological analyses were carried out for eight selected whey cheeses and a reference (i.e. a whey cheese produced locally according to traditional procedures). A true local maximum exists for moisture content (at a temperature of about 93°C, heating time of about 30 min and addition of about 17% ovine milk) which lies well within the range chosen for experimentation. Fat content of Requeijão was positively affected by heating temperature (especially via its quadratic effect) and, to a lesser extent, by heating time (especially via its linear effect); nitrogen content was especially affected by heating temperature (via its quadratic effect); and moisture content was affected especially by heating temperature (via its quadratic effect). The sensorial analyses showed that the eight whey cheeses produced were preferred with respect to the reference whey cheese. For rheological analyses the most significant observations pertain to the high strain dependence of the dynamic moduli, absence of a true equilibrium storage modulus, and relatively low difference between the loss and the storage modulus.

NOTATION

BSA	Bovine serum albumin
FPLC	Fast protein liquid chromatography
IgG	Immunoglobulin G
α-La	α-Lactalbumin

* Author to whom correspondence should be addressed.

β-Lg β-Lactoglobulin

INTRODUCTION

During recent years extensive efforts have been implemented in developing new methods, and extending existing ones, with the purpose of upgrading whey, the major by-product of cheese and casein manufacture industries (Mathur & Shahani, 1981). Most whey is still disposed of into the public sewage, but such option implies not only a loss of excellent nutrients for human consumption (e.g. whey protein, lactose and minerals) but also results in pollution of fresh water supplies (due to the high biochemical oxygen demand of whey).

Precipitation by heat processing still remains the simplest method to recover proteins from sweet and acid wheys. Such a process encompasses aggregation phenomena which are preceded by the unfolding and denaturation of protein (Hill *et al.*, 1982; Mangino, 1992; Mulvihill & Kinsella, 1987; Pearce, 1989). Ricotta is probably the oldest and the best known whey cheese in which protein is recovered by heat precipitation, and several processing parameters have been studied with respect to their effect on the final characteristics (Vodret, 1970; Mathur & Shahani, 1981; Weatherup, 1986; Modler, 1988; Pereira *et al.*, 1988). In Portugal there is a tradition regarding manufacture of a whey cheese called Requeijão, especially with ovine whey. The importance of dairy sheep is currently increasing in the Mediterranean Basin (Mills, 1986): although in the distant past Requeijão was sold door-to-door in limited regions of the interior of Portugal, nowadays it is an ubiquitous food speciality sold in plastic packages at virtually every supermarket (Santiago, 1993). Although there is a surplus of bovine milk in Europe and so its price is below that of small ruminants' milks, it should be emphasized that Requeijão manufactured with ovine milk whey is organoleptically much better than its bovine counterpart, and a concomitantly higher demand allows a higher final retail price which compensates for the higher cost of the feedstock.

The traditional method of manufacture of Requeijão consists in heating the starting material, i.e. ovine whey or mixtures thereof with ovine or caprine milk, at a temperature ranging from 90 to 100°C for about 30 min under smooth stirring conditions. The curd rises spontaneously to the surface and is scooped into plastic molds and allowed to drain and cool for several minutes. This manufacture protocol uses technological conditions determined empirically and no consistent scientific study attempting to quantitatively describe the influence of each processing parameter on the final product properties has appeared to date. The objective of this research effort was to characterize Requeijão so as to gain a deeper insight into such a product in terms of technological parameters, and consequently determine how to change its manufacturing conditions in order to optimize its (quality-related) characteristics.

MATERIALS AND METHODS

Reagents

Purified proteins from bovine milk (α-La, β-Lg, BSA and IgG) were purchased from Sigma (USA), whereas NaCl, Na_2HPO_4, NaH_2PO_4, HCl, NaN_3, H_2SO_4, NaOH and isoamilic acid were purchased from Merck (Germany). Ovine whey was obtained from bulk manufacture of Serra cheese (a traditional Portuguese ovine cheese prepared via enzymatic coagulation of raw ovine milk using a plant rennet). Ovine milk was obtained from a flock of Bordaleira sheep and caprine milk was obtained from a flock of Serrana goats.

Equipment

Filter paper (pore size 0·22 μm) was purchased from Nucleopore (USA). The FPLC system (Pharmacia) utilized for protein separation consisted of two P-500 positive displacement pumps, an electrically-powered MV-7 motorized valve, a gel filtration column prepacked with Superose 12 HR 10/30, a UV1 single path spectrophotometer monitor, a REC-102 double channel recorder, and an LCC-500 controller. Thermostatted centrifugation for the determination of fat content was achieved in a Norma Milk G centrifuge (Italy). Total nitrogen was determined using the Microkjeltec system from Tecator (Sweden). Moisture content was determined using an oven from Heraeus (Germany). Dynamic rheological characterization was performed using a controlled stress rheometer (Carri-Med CS-50, UK) fitted with a parallel plate device (gap, 3 mm and plate diameter, 2 cm).

Preparation of whey cheeses

Seventeen different whey cheeses were manufactured from a single, pooled batch of 40 litre of whey. Of these 17 whey cheeses, eight were produced according to all possible combinations of two levels of temperature (90 and 95°C), two levels of heating time (30 and 60 min), and two types of addition of milk (10%(v/v) of caprine milk and 10% (v/v) of ovine milk using initial volume of whey as reference); three were produced at 90°C, 45 min and without any addition of milk (thus corresponding to a central point); and the remaining six were produced as axially laid experimental counterparts (see Table 1).

The manufacture of each plain whey cheese required 1250 ml of whey. (Using experimental protocols detailed in the next subsection, one could conclude that the ovine whey used as feedstock had the following composition: 0·30%(w/w) nitrogen content, 1·25%(w/w) fat content and 91·4%(w/w) moisture content.) For whey cheeses where whey was blended with milk, 125 ml of whey were substituted by milk from the appropriate source. (Using experimental protocols detailed in the next subsection, one could conclude that the ovine and caprine milks used as feedstocks had the following composition: 0·95 and 0·75%(w/w) nitrogen content, 8·40 and 3·80%(w/w) fat content, and 83·3 and 88·4%(w/w) moisture content, respectively.) The glass vessel with the whey (or mixture of whey and milk) was heated to the appropriate temperature for the appropriate heating time (see Table 1) in a water bath. The proteinaceous coagulum was, upon formation, recovered from the upper layer of hot whey and poured into plastic molds, slightly pressed and left to drain and cool to room temperature for 2 h under a cover (in order to reduce contamination), and finally left to drain for 24 h at 4°C. Rheological and sensorial analyses were carried out on fresh whey cheese portions after this time period had elapsed and the remaining portions were kept frozen at -20°C until further chemical analyses. A whey cheese manufactured *in loco* using traditional procedures was used as a reference for assays of the experimental whey cheeses. [Using experimental proto-

TABLE 1

Second Order Experimental Design for Heating Time (t), Temperature (T) and Percentage of Milk Added (m); Experimental Data Obtained for the Fat, Nitrogen and Moisture Content of Whey Cheese, and Nitrogen Content, Concentration of β-Lg, and Concentration of α-La of the Remaining Deproteinized Whey; Values of Main Linear Effects (x_1, x_2 and x_3), Values of Main Quadratic Effects (x_1x_1, x_2x_2 and x_3x_3), and Values of Second-Order Interactions (x_1x_2, x_1x_3 and x_2x_3) Associated with 95% Confidence Intervals. (Runs were made in random order)

		E & I										Cheese whey			Deprot. whey		
	U	avg [$\bar{y}$]	x_1 [β_1]	x_2 [β_2]	x_3 [β_3]	x_1x_1 [β_{11}]	x_2x_2 [β_{22}]	x_3x_3 [β_{33}]	x_1x_2 [β_{12}]	x_1x_3 [β_{13}]	x_2x_3 [β_{23}]	Fat (%)	Nitrog. (%)	Moist. (%)	Nitrog. (%)	β-Lg (mg/mL)	α-La (mg/mL)
	1	+1	−1	−1	−1	+1	+1	+1	+1	+1	+1	18·25	1·459	67·26	0·175	1·208	1·521
	2	+1	−1	−1	+1	+1	+1	+1	+1	−1	−1	17·00	1·333	68·02	0·181	1·685	1·797
	3	+1	−1	+1	−1	+1	+1	+1	−1	+1	−1	18·00	1·161	69·62	0·204	1·648	1·903
	4	+1	−1	+1	+1	+1	+1	+1	−1	−1	+1	21·25	1·331	65·82	0·176	0·931	1·347
C	5	+1	+1	−1	−1	+1	+1	+1	−1	−1	+1	20·00	1·249	67·26	0·146	0·767	1·134
	6	+1	+1	−1	+1	+1	+1	+1	−1	+1	−1	20·00	1·260	65·98	0·174	0·581	0·929
	7	+1	+1	+1	−1	+1	+1	+1	+1	−1	−1	22·00	1·587	66·86	0·155	0·614	0·869
	8	+1	+1	+1	+1	+1	+1	+1	+1	+1	+1	26·75	1·288	64·16	0·139	0·416	0·756
	9	+1	0	0	0	0	0	0	0	0	0	18·50	1·165	69·16	0·193	1·599	1·730
c	10	+1	0	0	0	0	0	0	0	0	0	17·75	1·233	71·26	0·196	1·92	1·960
	11	+1	0	0	0	0	0	0	0	0	0	18·00	1·290	70·67	0·217	1·575	1·730
	12	+1	−1	0	0	+1	0	0	0	0	0	21·00	1·195	69·72	0·175	0·436	0·775
	13	+1	+1	0	0	+1	0	0	0	0	0	18·00	1·688	66·95	0·163	0·393	0·718
	14	+10	−1	0	0	+1	0	0	0	0	0	25·50	1·116	67·04	0·159	0·510	0·902
ax	15	+10	+1	0	0	+1	0	0	0	0	0	20·50	1·327	66·27	0·161	0·389	0·747
	16	+1	0	0	−1	0	0	+1	0	0	0	19·75	1·486	68·45	0·134	0·318	0·718
	17	+1	0	0	+1	0	0	+1	0	0	0	18·75	1·222	68·58	0·152	1·654	1·889
	Fat	19·2506	1·1250	0·7750	0·5750	-0·6248	2·8742	-0·8742	0·5938	0·3438	1·1563						
		±0·2984	±0·4324	±0·4324	±0·4324	±0·4324	±0·4324	±0·4324	±0·5175	±0·5175	±0·5175						
Whey cheese	Nitrog.	1·2800	0·0593	0·0277	-0·0508	0·1235	-0·0966	0·0359	0·0833	-0·0415	-0·0017						
		±0·0489	±0·0709	±0·0709	±0·0709	±0·0709	±0·0709	±0·0709	±0·0848	±0·0848	±0·0848						
	Moist.	69·4427	-0·9230	-0·2830	-0·6890	-0·4129	-2·0963	-0·2352	-0·2975	-0·1175	-0·7475						
		±0·8464	±1·2265	±1·2265	±1·2265	±1·2265	±1·2265	±1·2265	±1·4467	±1·4467	±1·4467						
	Nitrog.	0·179361	-0·0134	0·0000	0·0008	0·0067	-0·0023	0·0193	-0·0063	0·0043	-0·0098						
		±0·0102	±0·0148	±0·0148	±0·0148	±0·0148	±0·0148	±0·0148	±0·0177	±0·0177	±0·0177						
Deprot. whey	β-Lg	1·1285	-0·3137	-0·0753	0·0712	-0·2868	-0·2519	0·2846	-0·0005	-0·0180	-0·1508						
		±0·1505	±0·2181	±0·2181	±0·2181	±0·2181	±0·2181	±0·2181	±0·2610	±0·2610	±0·2610						
	α-La	1·3513	-0·2937	-0·0661	0·0573	-0·2632	-0·1853	0·2934	-0·0462	-0·0048	-0·0925						
		±0·1038	±0·1504	±0·1504	±0·1504	±0·1504	±0·1504	±0·1504	±0·1800	±1800	±1800						

Note: ax-axial points; c-Center points; C-Corner points; EE-Estimated Effects; E&I-Effects (linear and quadratic) and Interactions (second order); U-rUn; x_1-Normalized value of heating time, defined as (t-45)/15, where t is expressed in min; x_2-Normalized temperature, defined as (T-92·5)/2·5, where T is expressed in °C; x_3-Normalized percentage of goat's milk, defined as (m-0)/10, where m is expressed in %.

cols detailed in the next subsection, one could conclude that such reference whey cheese had the following composition: 59·0%(w/w) moisture content, 29·5% (w/w) fat content and 1·33%(w/w) nitrogen content.]

Chemical analyses

The moisture content of Requeijão was determined according to the IDF method (FIL/IDF 4: 1958). The fat content of whey was determined using Gerber's butyrometric determination (Portuguese standard NP 469). The fat content of whey cheese was determined using Van Gulik's butyrometric determination (Portuguese Standard NP 2105). The nitrogen contents were determined according to the IDF method (FIL/IDF 20B: 1993) adapted to micro conditions by using one tenth of all samples and reagents. The measurements of fat, nitrogen and moisture contents of whey cheese are shown in Table 1.

Determination of β-Lg and α-La concentrations was carried out by injecting 100 μl of deproteinized whey, eluting using a 0·05 M phosphate buffer at pH 7·0 containing 0·15 M NaCl (used to correct ionic strength) and 0·2 g/litre of NaN_3 (used as a preservative) at a flow rate of 0·4 ml/min and detecting by reading absorbance at 280 nm. Prior to chromatographic analysis, the sample and eluting buffer were filtered through filter paper and degassed. Quantitative calibration, done in terms of bovine whey proteins, was performed using various dilutions of an aqueous solution containing 9·10 mg/ml of bovine α-La, 4·10 mg/ml of bovine β-Lg, 4·9 mg/ml of bovine IgG, 6·8 mg/ml of bovine BSA, 0·13 mg/ml of orotic acid and 0·018 mg/ml of uric acid. The measures of nitrogen content and of the concentrations of α-La and β-Lg in the supernatant whey after protein precipitation (i.e. in the deproteinized whey) are depicted in Table 1.

Sensorial analyses

A sensorial panel was selected on the basis of the results of preliminary tests performed with the goal of recognition of basic tastes, ability to determine the intensity of basic tastes, recognition of odour and rating of texture (Stevens & Albright, 1990). Whey cheese samples were evaluated at room temperature by the 13 panelists that met the minimum qualifications for the sensorial criteria described above. Whey cheeses were graded for firmness and smoothness based on a multiple comparison test (Meilgaard *et al.*, 1988) using whey cheese produced according to the traditional way as a control; the scales used for both parameters ranged from 1 to 9 (1: 'extremely less firm and smooth than control', 5: 'equal to control' and 9: 'extremely firmer and smoother than control'). The panelists were also requested to indicate their relative overall preference on a scale of 1 to 5 (1: 'most preferred', 5: 'least preferred') according to a ranking test (Meilgaard *et al.*, 1988). Due to the large amount of samples to be tested (eight samples and one control) the taste panel met at two different periods separated by 3 h, each one including the control and four samples taken at random. The data obtained are depicted in Table 2.

Rheological analyses

In order to characterize the viscoelastic behaviour of Requeijão, the eight samples selected as indicated above were left at room temperature for 1 h and then cut using a cork borer so as to generate round discs with a diameter of 2·0 cm and a thickness of 3 mm. Slippage was prevented by adding two drops of cyanoacrylate bonding agent and spreading evenly over the lower plate of the rheometer and over the cheese disc. The sample was covered with light oil to prevent dehydration and allowed to stand for 30 min at 20°C before the experimental test was carried out. Each experiment consisted in characterizing the frequency dependence of both the elastic component G' (storage modulus) and the viscous component G'' (loss modulus) at a low strain amplitude (2%).

Mathematical analyses

The first empiric model to be tentatively fitted by linear regression to the experimental results listed in Table 1 for the first 11 runs has the form

$$\hat{y} = \bar{y} + \alpha_1 x_1 + \alpha_2 x_2 + \alpha_3 x_3 \tag{1}$$

where ŷ is the fitted response, ȳ is the average of all data and the αs are adjustable parameters. The x_is are the technological (manipulated) variables under scrutiny in coded, normalized form, and are defined as: $x_1 = (t\text{-}45)/15$ where t is the heating time expressed in min; $x_2 = (T\text{-}92{\cdot}5)/2{\cdot}5$ where T is the heating temperature, expressed in °C; and $x_3 = (m\text{-}0)/10$, where m is the percent addition of caprine milk (a negative value for m means addition of ovine milk in the same proportion instead

TABLE 2
Average Firmness and Smoothness Data Obtained from a Multiple Comparison Test and Overall Preference Data from a Ranking Test, for the Evaluation of Eight Different Whey Cheeses using a Traditional Whey Cheese as Control

Cheese Conditions	*Firmness*[a]	*Smoothness*[b]	*Overall Preference*[c]
Analysis 1			
10% CM-90°-30 min	3.6	6.3	4
10% CM-90°-60 min	2.8	5.4	1
10% CM-95°-60 min	3.8	5.4	2
10% OM-95°-30 min	3.7	6.3	3
Control	5.3	4.7	5
Analysis 2			
10% OM-95°-30 min	4.9	5.9	3
10% OM-95°-60 min	4.6	6.7	2
10% OM-90°-60 min	4.3	6.6	4
10% CM-95°-60 min	4.8	6.1	1
Control	5.2	5.1	5

[a] Scale 1–9: 1-extremely less firm than control, 5-as firm as control, 9-extremely firmer than the control.
[b] Scale 1–9: 1-extremely less smooth than control, 5-as smooth as control, 9-extremely smoother than control.
[c] Scale 1–5: 1-most preferred, 5-least preferred.
CM-Caprine milk.
OM-Ovine milk.

of caprine milk). Estimates of the sums of all quadratic effects (i.e. those including x^2) for all processing parameters following the method outlined in Box *et al.* (1978) have shown that second order effects were likely to be of importance; hence the experimental design had to be expanded accordingly. The experimental results for the six extra experimental points (laid out as axial points) are also depicted in Table 1. The model to be fitted by linear regression analysis to the data is then

$$\hat{y} = \bar{y} + \beta_1 x_1 + \beta_2 x_2 + \beta_3 x_3 + \beta_{11} x_1^2 + \beta_{22} x_2^2 + \beta_{33} x_3^2 + \beta_{12} x_1 x_2 + \beta_{13} x_1 x_3 + \beta_{23} x_2 x_3 \quad (2)$$

where the βs are adjustable parameters. The estimates obtained for all parameters are tabulated in Table 1 together with their 95% confidence interval.

Differentiating eqn (2) independently with respect to x_1, to x_2 and to x_3, and setting the result thus obtained equal to zero provides a necessary condition for a local optimum to exist. The resulting three linear equations can, in turn, be algebraically solved with respect to x_1, x_2 and x_3, respectively; the results in terms of loci of the optima when variables x_2 and x_3, or x_1 and x_3, or x_1 and x_2, respectively, are deliberately prefixed are depicted in Table 3. Only a true local maximum exists for moisture content (located at $x_1 = -0{\cdot}985$, $x_2 = 0{\cdot}306$ and $x_3 = -1{\cdot}705$), although local optima exist for combinations of every set of prefixed values of one or two processing variables.

Analyses of variance were implemented on the results obtained from the sensorial and rheological experiments. The results are shown in Table 4.

TABLE 3

Loci and Type of Optima with Respect to the Operating Variables Heating Time (x_1), Heating Temperature (x_2) and Percentage of Milk Added (x_3) Associated with the Quadratic Models Fitted to the Data Obtained for the Manufacture of Requeijão

		LO			*TO*		
		x_1	x_2	x_3	x_1	x_2	x_3
Whey cheese	Fat	$0{\cdot}901 + 0{\cdot}475x_2 + 0{\cdot}275x_3$	$-0{\cdot}135 - 0{\cdot}103x_1 - 0{\cdot}200x_3$	$0{\cdot}329 + 0{\cdot}196x_1 + 0{\cdot}657x_2$	max	min	max
	Nitrogen	$-0{\cdot}240 - 0{\cdot}337x_2 + 0{\cdot}168x_3$	$0{\cdot}144 - 0{\cdot}432x_1 - 0{\cdot}093x_3$	$0{\cdot}706 + 0{\cdot}576x_1 - 0{\cdot}025x_2$	min	max	min
	Moisture	$-1{\cdot}222 - 0{\cdot}361x_2 - 0{\cdot}143x_3$	$-0{\cdot}068 - 0{\cdot}071x_2 - 0{\cdot}178x_3$	$-1{\cdot}466 - 0{\cdot}251x_1 - 1{\cdot}591x_2$	max	max	max
Deproteinized whey	Nitrogen	$0{\cdot}996 + 0{\cdot}464x_2 - 0{\cdot}316x_3$	$0{\cdot}000 - 1{\cdot}377x_1 - 2{\cdot}150x_3$	$0{\cdot}0208 + 0{\cdot}117x_1 - 0{\cdot}253x_2$	min	max	max
	$\beta-$Lg	$-0{\cdot}547 + 0{\cdot}001x_2 - 0{\cdot}031x_3$	$-0{\cdot}149 - 0{\cdot}001x_1 - 0{\cdot}076x_3$	$-0{\cdot}125 + 0{\cdot}032x_1 + 0{\cdot}265x_2$	max	max	min
	$\alpha-$La	$0{\cdot}559 - 0{\cdot}088x_2 - 0{\cdot}009x_3$	$-0{\cdot}178 + 0{\cdot}125x_1 + 0{\cdot}250x_3$	$-0{\cdot}097 + 0{\cdot}008x_1 + 0{\cdot}157x_2$	max	max	min

Note: *LO*-Loci of Optima; *TO*-Type of Optima.

TABLE 4

ANOVA Table for Organoleptic Data Expressed as Firmness and Smoothness and for Rheological Data Expressed as Storage Moduli G' and G'' Obtained at $\omega = 0{\cdot}1$ rad/s

Source of variation	*Degrees of freedom*	*Sum of squares*	*Mean square*	*F-ratio*
Firmness				
Samples	9	75·66	8·41	5·71
Panelists	12	35·83	2·99	2·03
Error	108	158·94	1·47	
Smoothness				
Samples	9	52·27	5·81	3·18
Panelists	12	49·91	4·16	2·28
Error	108	197·32	1·83	
G'				
Treatments	1	2053689	2053689	1·76
Error	8	9350611	1168826	
G''				
Treatments	1	288800	288800	3·39
Error	8	681337	85167	

RESULTS AND DISCUSSION

During manufacture of whey cheeses one could observe that the amount of proteinaceous coagulum produced with whey previously blended with milk was higher and the curd was more cohesive than in the absence of milk addition, an observation that was somewhat expected in view of the coprecipitation of caseins with whey proteins; similar results were obtained by Mathur and Shahani (1981). A fine precipitate remained in the deproteinized whey because the manufacture of Requeijão did not encompass an acidification step after the heating process.

From the nitrogen content of deproteinized whey, it can be pointed out that the yield of nitrogen in the whey cheese ranged from 53·7% of the total nitrogen of the initial whey (when whey cheese was prepared by coagulation at 95°C for 60 min with addition of 10% of caprine milk) to 32·7% of the total nitrogen of the initial whey (when whey cheese was prepared by coagulation at 92·5°C for 45 min without milk adition). These results are in agreement with Hill *et al.* (1982), who reported that the theoretical maximum recovery of nitrogen from whey ranges from 55 to 65% since the heat stable proteose-peptone and non-protein nitrogen account for from 35 to 45% of the total nitrogen. However, Modler and Emmons (1989) have shown that the proteose-peptone content in the deproteinized whey decreases when whey is mixed with milk, which suggests that the proteose-peptone fractions could coprecipitate with caseins.

Comparison of the traditional whey cheese used as a reference in this study with the results obtained for the experimental whey cheeses indicates that although the nitrogen content is similar, a lower moisture content and a higher fat content are exhibited by the traditional whey cheese, probably due to the higher temperature used for its manufacture.

The experimental results tabulated in Table 1 are similar to those obtained for Ricotta cheeses produced with ovine whey in Sardinia (Vodret, 1970). Inspection of this table allows one to conclude that fat content of whey cheese is affected positively by heating time (via the linear effect) and heating temperature (via both linear and quadratic effects), and by addition of milk either positively (via the linear effect) or negatively (via the quadratic effect). In terms of interactions, they are significant at the 5% level except the interaction heating time/addition of milk. Pereira *et al.* (1988) concluded that increases in heating rate lead to a greater retention of fat, which agrees with our observations.

For fat there is not a true local maximum (see Table 3); nevertheless, if temperature is fixed at either 90 or 95°C optima are obtained at $t = 51$ min and $m = 2{\cdot}5\%$ ovine milk, and $t = 71$ min and $m = 13{\cdot}3\%$ caprine milk, respectively. In this case the first condition would be preferable because it corresponds to conditions that ensure a higher yield. It is interesting to note that when the temperature is 95°C, the fat content in whey cheese is higher if caprine milk rather than ovine milk is added, irrespective of the heating time, a quite unexpected observation in view of the fact that ovine milk is richer in fat than caprine milk. Different interaction of whey proteins with fatty acid residues depending on the animal species in question (Perez *et al.*, 1993) coupled with the fact that interaction between the fat globules and the gel matrix results from combination of the β-Lg fraction adsorbed onto fat globules with the κ-casein of the gel matrix via dissulphide bridges promoted by the heating step (Xu *et al.*, 1992) could tentatively explain why higher amounts of milk fat are apparently released in the manufacture of ovine whey cheese with addition of one type of milk than with addition of the other type.

No linear effect of any processing variable is important for the nitrogen content of whey cheese (see Table 1); however, the quadratic effects of heating temperature and heating time are significant at the 5% level. Kalab & Modler (1985) also observed that the amount of whey protein denatured increased considerably when the heating temperature was increased. Again there is no true local optimum for this property (see Table 3), but if one sets the heating temperature to 90 and 95°C, optima are obtained at $t = 48$ min and $m = 8{\cdot}2\%$ caprine milk, and $t = 37{\cdot}5$ and $m = 4{\cdot}4\%$ caprine milk, respectively.

The quadratic effect of temperature is the only effect that is statistically significant at the 5% level for the determination of the moisture content of whey cheese. Vodret (1970) reported that differences in the moisture content of whey cheeses could be consistently related to precipitation temperature. A true global maximum exists for this parameter in whey cheese (see Table 3), namely $t = 30{\cdot}2$ min, $T = 93{\cdot}3$°C and $m = 17\%$ ovine milk. The moisture content exhibited a significant negative correlation with fat ($r = 0{\cdot}910$, results not shown).

The fraction of nitrogen in deproteinized whey is significantly affected only by the quadratic effect of milk addition. This result is consistent with conclusions by Modler and Emmons (1989), namely that the addition of milk reduces the proteose-peptone content of deproteinized whey. A true local optimum could not be found once again (see Table 3), yet setting the heating time to 60 and 30 min it is possible to obtain maxima located at $T = 81{\cdot}8$°C and $m = 12{\cdot}2\%$ caprine milk, or $T = 99{\cdot}5$°C and $m = 6{\cdot}6\%$ ovine milk, respectively.

Although the major whey proteins would eventually precipitate upon sufficiently stressing thermal processing, small amounts of α-La and β-Lg may remain in the deproteinized whey together with the heat stable fractions depending on the conditions employed. Both β-Lg and α-La are affected by heating time via its linear effect, as well as by all tested technological variables via their quadratic effects. Inspection of Table 3 indicates that no true overall optimum exists. However, if addition of milk was preset at 10% caprine milk one would obtain $t = 36{\cdot}3$ min and $T = 91{\cdot}4$°C for β-Lg, and $t = 36{\cdot}9$ min and $T = 91{\cdot}6$°C for α-La, whereas if it were preset at 10% ovine milk one would have obtained $t = 37{\cdot}3$ min and $T = 92{\cdot}1$°C for β-Lg, and $t = 36{\cdot}6$ min and T = 92·9°C for α-La. The values obtained for the optima of either protein are close enough that it should be possible to reach conditions that yield maximum recovery of both proteins.

In the performance of the organoleptic tests, a traditional whey cheese was included as a control: this control was aimed at determining whether the evaluation was impartial and consistent. From the data depicted in Table 2, it can be concluded that the traditional whey cheese used as a control scored an average of 5·3 and 5·2 for the first and second analyses, respectively; therefore, the sensorial evaluation could be labelled as good because these controls are very close to one another, which means that blocking had no effect on the accuracy of the organoleptic evaluation. When one compares the results for the experimental whey cheeses with those for the traditional whey cheese (control) for firmness, it is obvious that all samples are less firm than the reference, although two samples (95°C, 30 min, 10% ovine milk and 95°C, 60 min, 10% caprine milk, respectively) are indeed quite close to 5. Analyses of the effects of moisture using fat-free and protein-free bases (namely using the ratios (%moisture)/(100 − %fat) and (%moisture)/(100 − %protein)) have shown a poor correlation between these corrected measures of moisture and firmness, thus suggesting that differences in moisture content should be caused by protein aggregation due to temperature effects. This poor correlation could be partially accounted for by realization that the water held in a gel can be either in bound form (via adsorption forces or capillary forces) or in free form, and thus a given amount of water may give rise to different degrees of firmness upon spontaneous shrinkage of whey proteins during heat treatments above the gelation temperature (de Wit, 1988). In general, the higher values for firmness are obtained for whey cheeses prepared at 95°C, and the control is also included in this list since it is prepared nearly at boiling conditions. The effect of higher temperatures in imparting a high firmness to whey cheese has been discussed elsewhere (Hill *et al.*, 1982).

The results for smoothness shown in Table 2 indicate that all cheeses are smoother than the control, although two of them (90°C, 60 min and 10% caprine milk, and 95°C, 30 min and 10% caprine milk, respectively) are rather close to the control.

Statistical analyses of the results for firmness and smoothness (see Table 4) were carried out with all samples including the control. Although for firmness a high *F*-ratio was found (i.e. there are differences among the firmness of the samples at the 1% level of significance), for smoothness a low *F*-ratio was found (i.e. there are no differences among the smoothness of the samples at the 5% level of significance).

The overall preference data displayed in Table 3 indicate that the least preferred whey cheese is the control, which suggests the existence of room for technological optimization of traditional whey cheese manufacture. Due to splitting of the organoleptic evaluation in two blocks, it is not possible to determine the most preferred of all eight whey cheeses, but it is possible to select the two most preferred whey cheeses from each group, namely those prepared at 90°C for the first analysis and 95°C for the second, 10% caprine milk, and heating for 60 min. If one compares the firmness and smoothness of these two whey cheeses, one concludes that for the whey cheese prepared at 90°C smoothness is close to the control and firmness is well below it, whereas for the whey cheese prepared at 95°C firmness is close to the control but balanced by a relatively higher smoothness. Inspection of the composition data in Table 1 for the same whey cheeses, one finds nitrogen contents of 1·260% and 1·288%, moisture contents of 65·98% and 64·16%, and fat contents of 20·00% and 26·75% for the whey cheeses prepared at 90°C and 95°C, respectively; it is apparent that the protein contents of both cheeses are quite close to one another (8·04 and 8·22%, after multiplication by the classical correction factor of 6·38).

As typically found for protein coagulates (Clark & Ross-Murphy, 1987; Tunick *et al.*, 1990), the whey cheese samples tested have shown a high strain sensitivity, probably a result of the heterogeneous network formed by the proteins in the whey cheese. Typical examples of the mechanical spectra obtained for these samples are shown in Fig. 1. Qualitatively, the viscoelastic behaviour observed was similar for both the reference whey cheese and those prepared at laboratory scale using the aforementioned factorial experimental design. The storage modulus was slightly dependent upon the oscillatory frequency (Konstance & Holsinger, 1992), which can be attributed to some mobility of the elastically active protein chains that build up the gelled network. Also the difference between the two viscoelastic moduli is relatively low if compared with true gels (Clark & Ross-Murphy, 1987; Kamphuis & Jongschaap, 1985). Probably, the cross-linking density is small as a result of the relatively low protein concentration, thus preventing formation of a more elastic network, although the contribution of such other components of the whey cheese matrix as fat and calcium might also play a role in the viscoelastic behaviour of this system. Figure 2 shows the frequency dependence of the storage modulus for the whey cheeses prepared at laboratory scale as compared with the behaviour of the control whey cheese. Although qualitatively all whey cheeses tested show a similar behaviour, the control whey cheese clearly exhibits higher dynamic moduli (G' and G''), which are likely related to a lower water content: the higher water content of the experimental whey cheeses may then be attributed to the lower precipitation temperatures employed (remember that, traditionally, whey cheese is prepared at

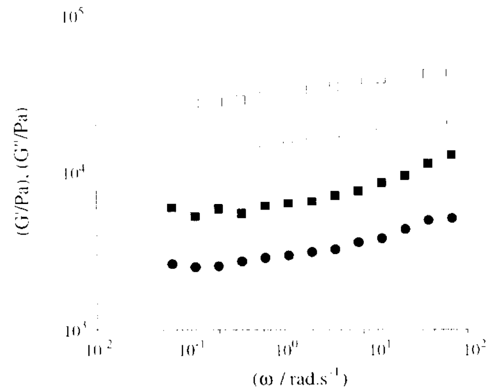

Fig. 1. Frequency dependence of G' and G'' viscoelastic moduli of control cheese (□-G',■-G'') and whey cheese prepared at 95°C for 60 min with addition of 10% ovine milk (○-G', ●-G'').

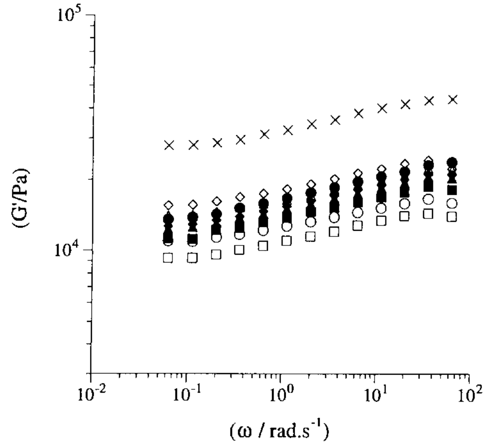

Fig. 2. Frequency dependence of G' for whey cheese prepared via heating at 95°C for 30 min with addition of 10% caprine milk (□), whey cheese prepared via heating at 90°C for 60 min with addition of 10% caprine milk (○), whey cheese prepared via heating at 95°C for 30 min with addition of 10% ovine milk (■), whey cheese prepared via heating at 95°C for 60 min with addition of 10% ovine milk (◆), whey cheese prepared via heating at 90°C for 60 min with addition of 10% ovine milk (●), whey cheese prepared via heating at 90°C for 30 min with addition of 10% ovine milk (▲), whey cheese prepared via heating at 90°C for 30 min with addition of 10% caprine milk (△), whey cheese prepared via heating at 95°C for 60 min with addition of 10% caprine milk (◇), and control whey cheese (**X**).

nearly boiling temperature). These results are in agreement with those obtained for firmness in the sensory analysis, namely that the whey cheeses manufactured possess a higher firmness than the control, and they are also confirmed by the significant negative correlation of moisture content with the storage modulus ($r = 0{\cdot}855$) and the loss modulus ($r = 0{\cdot}905$). Our results are also consistent with those reported by Pereira *et al.* (1982) who claimed that moisture content is one of the most important factors in the determination of the textural characteristics (including firmness) of a whey cheese. The observation that no important differences appear to exist between the whey cheeses prepared at different temperatures, heating times and with addition of different quantities and qualities of milk, is confirmed by the statistical analyses of the rheological data (see Table 4): low F-ratios indicate that differences between the storage modulus and the loss modulus are not significant at the 5% level of significance.

CONCLUSIONS

From the above analyses, it is apparent that choice of ideal conditions for whey cheese manufacture must result from a compromise between high yield (for which most information is obtained from the deproteinized whey) and high nitrogen content (for which most information is obtained from the whey cheese) since the (tentative) maxima for these two criteria do not coincide. If one attends preferentially to the protein (or nitrogen) enriched whey cheese (as this might drive the preference and consequently the demand by the consumer), then the processing conditions that lead to an optimum for nitrogen content of whey cheese (i.e. $T = 90°C$, $t = 48$ min and $m = 8{\cdot}2\%$ caprine milk, or $T = 95°C$, $t = 37{\cdot}5$ min and $m = 4{\cdot}4\%$ caprine milk) would be good choices for whey cheese manufacture. If one attends to the whey cheese most preferred in the sensorial analyses, then the first condition (i.e. $T = 90°C$, $t = 48$ min and $m = 8{\cdot}2\%$ caprine milk) will be the final choice.

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