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Proteolysis in rennet-coagulated Spanish hard cheeses made from milk preserved by refrigeration and addition of carbon dioxide

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On-farm refrigeration of milk reduces the growth rate of mesophilic bacteria so allowing longer storage time of raw milk before processing. However, refrigeration creates a selective pressure favouring the multiplication of psychrotrophic bacteria present as normal contaminants in raw milk. Psychrotrophs are killed by most of the currently employed pasteurization and sterilization treatments of milk but they can produce extracellular heat-stable proteinases and lipases, which are capable of degrading various milk components, affecting the storage-life of heat-processed milk and the quality of dairy products (Champagne et al. 1994; Shah, 1994).

Addition of CO_2 to refrigerated raw milk inhibits the growth of psychrotrophs, allowing a further extension of the storage period of raw milk (King & Mabbit, 1982; Roberts & Torrey, 1988; Amigo et al. 1995; Ruas-Madiedo et al. 1996; Espie & Madden, 1997) and pasteurized milk (Ruas-Madiedo et al. 1998*b*; Hotchkiss et al. 1999). Biochemical quality of milk (casein, whey proteins, carbohydrates, vitamins and organic acids) is not negatively affected by carbon dioxide addition (Chang & Zhang, 1992; Olano et al. 1992; Sierra et al. 1996; Ruas-Madiedo et al. 1996, 1998*b*). Vacuum degasification before pasteurization prevents milk coagulation during heat-treatment and volume changes of the occluded gas in the pasteurizer, and renders milk acceptable for liquid consumption (Ruas-Madiedo et al. 1996). Milk preserved by refrigeration and CO_2 addition has also proved useful in cheesemaking (Uceda et al. 1994; McCarney et al. 1995; Montilla et al. 1995; Ruas-Madiedo et al. 1998*a*). We showed recently that refrigerated CO_2-treated milk can be satisfactorily used in the manufacture of Spanish hard cheeses (Ruas-Madiedo et al. 2002). However, there is limited information concerning the effect of CO_2 treatment on proteolysis in rennet-coagulated Spanish hard cheeses (Uceda et al. 1994), which is one of the most important events during ripening.

In the present work we examined the effect of previous preservation of raw milk by refrigeration and CO_2 addition on the proteolysis of two Spanish hard cheese varieties, one made from pasteurized cows' milk after 30 d of ripening and the other one made from a mixture of cows' and ewes' raw milk (90:10) after 75 d of ripening. Ripening times were chosen on the basis of legal production periods established by the EU (Real Decreto 1679/1994) and the customary consumption times of these two cheese varieties in Spain.

Materials and Methods

Milk processing and cheesemaking

Samples of raw cows' milk with initial microbial loads between $6{\cdot}1\times10^4$ and $1{\cdot}3\times10^5$ cfu/ml were collected from one farm in Asturias (northern Spain) and raw ewes' milk (total counts between $3{\cdot}2\times10^6$ and $1{\cdot}1\times10^7$ cfu/ml) was obtained from herds in Castilla-León (north-central region of Spain). Both types of milk were kept under refrigeration and transported to the pilot plant within 48 h of collection.

Two separate cheesemaking trials, each in triplicate, were carried out on a pilot scale (Table 1). For each trial, two batches were manufactured, control and experimental. To reproduce standard conditions of cheesemaking, control batches were produced from milk maintained at the pilot plant for 24 h at 4 °C before processing (fresh milk). For experimental batches, milk was preserved for 4 d by refrigeration (4 °C) and CO_2 addition (pH 6·2) (Ruas-Madiedo et al. 1996). Addition of CO_2 to milk was by an automatic system (Bada-Gancedo et al. 2000) that injected gas when pH was $>6{\cdot}25$ and stopped the supply of CO_2 when values were $<6{\cdot}15$. After the preservation period, the CO_2 from experimental milk batches was removed by heat-vacuum degasification. During the coagulation process milk was incubated at 32 °C for 2 h and then 0·2 ml calf rennet/l was

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Table 1. Differences in milk processing and cheesemaking variables between the two trials

			Milk processing		Cheese making		
Trial	Milk type	Batch	Preservation	After preservation	$CaCl_2$	Starter†	Ripening
1	Pasteurized cows'	Control	No preservation Fresh milk Refrigerated 24 h	Pasteurized (72 °C, 15 s)	0·15 g/l	YES	30 d
		Experimental	Refrigerated 4 d CO_2 added (pH 6·2)	Heat-vacuum degasified (50–52 °C, 300 mm Hg) pasteurized (72 °C, 15 s)	0·15 g/l	YES	30 d
2	Raw mixture: cows'/ewes' (90 : 10)	Control	No preservation Fresh milk Refrigerated 24 h	—	—	NO	75 d
		Experimental	Refrigerated 4 d CO_2 added (pH 6·2)	Heat-vacuum degasified (30–32 °C 350–375 mm Hg)	—	NO	75 d

† *Lactococcus lactis* spp. *lactis, Lc. lactis* spp. *cremoris, Lc. lactis* spp. *lactis* biovar *diacetylactis* and *Leuconostoc citreum* (MH18-13 series, Rhône Poulenc)

added. Once coagulation had occurred, curds were cut into 4–6 mm cubes, washed and cooked for 15 min at 37 °C. Curd was pressed for 15 min at 1·5 kg/cm^2 and ladled into moulds. Eight cheeses, approximately 2 kg in weight, were obtained per batch in each trial and they were pressed for 2–3 h at 2 kg/cm^2. Cheeses were salted for 16 h at 10 °C in brine (22 g/ml) and ripened at 12 °C and 90% relative humidity.

Cheese in trial 1 was made from pasteurized cows' milk. Conditions of heat-vacuum degasification for the experimental batch after preservation were 50–52 °C and 300 mm Hg. Additionally, both milk batches were pasteurized (72 °C for 15 s) and $CaCl_2$ (0·15 g/l) and a direct-vat starter culture (*Lactococcus lactis* ssp. *lactis, Lc. lactis* ssp. *cremoris, Lc. lactis* ssp. *lactis* biovar *diacetylactis* and *Leuconostoc citreum*; MH8-13 series, Texel, Rhône Poulenc, Epernon, France) (final concentration 12 g/l) were added prior to rennet coagulation. The ripening time was 30 d.

Cheese in trial 2 was made from a mixture of raw cows' milk and ewes' milk (90 : 10). Degasification of experimental samples after preservation was achieved at higher vacuum (350–375 mm Hg) and lower temperature (30–32 °C) than in trial 1, to avoid damage to the natural microbiota. No starter or $CaCl_2$ were added, and the ripening time was 75 d.

Samples of cheeses for analysis were taken at 3, 15 and 30 d of ripening in trial 1 and at 3, 30, 45 and 75 d of ripening in trial 2. Samples taken after 1 d of ripening were used only for the calculation of electrophoresis results as described below.

Proteolysis measured as nitrogen content in cheese fractions

Total nitrogen was determined by the Kjeldahl method (International Dairy Federation, 1993). The pH 4·6-soluble nitrogen (pH 4·6-SN) and 12% TCA-soluble nitrogen (TCA-SN) were respectively determined by measuring nitrogen soluble at pH 4·6 and nitrogen soluble in trichloroacetic acid (120 g/l). TCA-SN and pH 4·6-SN were expressed as their relative contents of the total nitrogen (% of TN).

Polyacrylamide-gel electrophoresis of casein

Casein was precipitated from cheeses with acetate buffer: samples were acidified to pH 4·6 with acetic acid (1 : 10 v/v) and then buffered by addition of an equal volume of 1 M-sodium acetate. Precipitated caseins were obtained by centrifugation (1360 ***g*** at 4 °C for 15 min), washed twice with sterile water and lyophilized. Casein solutions were prepared for electrophoresis by dissolving 0·1 g lyophilized casein in 10 ml 7 M-urea. Amounts of 23 µg of casein were electrophoresed for each sample. Urea-PAGE of caseins was carried out as described by Ramos et al. (1977) [T (acrylamide+bisacrylamide, % w/v) = 7·7%; C (bisacrylamide/acrylamide+bisacrylamide, % w/v) = 2·6%] and gels were stained with Coomassie blue R-250 (Sigma Chemical Co., St. Louis, MO, USA) as described by Winter et al. (1977). Triplicates of each cheesemaking trial were run at the same time and two independent electrophoresis runs (duplicates) performed for each sample. Hence results for a given sample were the mean of six values (three replicates, each in duplicate). Quantitative measurements were based on relative peak areas of the densitograms obtained with an Ultroscan XL densitometer (Pharmacia LKB Biotechnology, Bromma, Sweden) and Gel Scan XL 2.1 software (Pharmacia LKB Biotechnology). Residual contents of α_{s1} and β-casein were expressed as a percentage of the initial peak area of the corresponding casein fraction in one-day-old cheeses. The relation α_{s1}-I/α_{s1}-I+α_{s1}(%) was calculated (Hynes et al. 2001), where α_{s1} and α_{s1}-I represent the relative peak area for the casein and peptide, respectively.

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Analysis of water soluble nitrogen by HPLC

The water-soluble nitrogen fraction (WSF) was obtained by homogenizing 10 g of cheese with 50 ml of HPLC grade water. The mix was kept for 1 h at 40 °C and the suspension was passed through glass wool (González de Llano et al. 1995). A chromatographic system consisting of a 126M programmable solvent module (Beckman Instruments Inc., Fullerton, CA, USA), a 570 autosampler (Beckman Instruments), a 996 photodiode array detector (Waters, Milford, MA, USA) and Millennium 2010 software (Waters) was used. Before injection, samples were centrifuged at 3000 ***g*** and filtered through a 0·45-μm membrane. Separations were carried out on a 4·6 × 250 mm C18 Nucleosil 5 μm, 300 Å reversed-phase column (Macherey Nagel Düren, Düren, Germany) at a constant temperature of 40 °C. Eluent A was trifluoroacetic acid in water (0·1 : 100 v/v) and eluent B was 0·1/100 trifluoroacetic acid in acetonitrile-water (60 : 40 v/v). Separations were carried out at a flow rate of 1 ml/min with eluent A for 10 min and a linear gradient from 0 to 80% of eluent B for 80 min (González de Llano et al. 1995). The detection wavelength was 220 nm. Standard solutions of tyrosine, phenylalanine and tryptophan (Sigma-Aldrich Química S.A., Madrid, Spain) were used to determine their retention times (Lau et al. 1991). The u.v. absorption peaks from the HPLC runs were divided into portions as indicated by González de Llano et al. (1995). According to these authors, the portion with retention times <10 min was discarded because most of free amino acids, except phenylalanine and tryptophan, eluted between 0 and 10 min whereas peptides eluted between 10 and 90 min. An early, more hydrophilic portion in the chromatogram corresponded to peptides located between tyrosine and tryptophan and tryptophan-containing peptides (retention times 10–35 min). The second group of peaks with retention times between 35 and 90 min included the portion of more hydrophobic peptides and some large hydrophilic peptides (González de Llano et al. 1995). The ratio between these two portions was obtained by dividing the total area of the peaks in the hydrophobic portion by the total area of the peaks in the hydrophilic portion of the HPLC runs (Fig. 2). Results were expressed as units of chromatogram area per milligram of TS (total solids) as indicated by Picón et al. (1994). TS were determined as described by Fontecha et al. (1990).

Statistical analysis

Statistical analysis was done using the SPSS-PC+4.0 software (SPSS Inc., Chicago IL, USA). Samples of each trial were considered separately. ANOVA was carried out within each trial. Data obtained at different times of sampling (cheeses after 3, 15, 30, 45 and 75 d of ripening) were subjected to one-way ANOVA using milk-preservation as a factor with two categories: control and experimental samples. One-way ANOVA tests were also performed within each cheese batch (control and experimental) using time of ripening as factor with days as categories.

Results and Discussion

TCA-SN and pH 4·6-SN (Table 2) increased significantly ($P<0·05$; statistical analysis not shown) during ripening in cheeses from pasteurized (trial 1) and from mixed raw milk (trial 2). In cheeses of trial 1, values of TCA-SN and pH 4·6-SN were slightly lower than those reported previously for Manchego cheese (Núñez et al. 1991; Picón et al. 1994). No significant differences ($P>0·05$) were obtained between control and experimental samples, showing that refrigeration and CO_2 treatment had no effect on the extent of proteolysis in cheeses from pasteurized milk. In cheeses of trial 2, the extent of proteolysis was similar to that reported by Uceda et al. (1994) for Manchego cheese from raw ewes' milk. No significant differences ($P>0·05$) were found between control and experimental samples, although slightly lower levels of TCA-SN and pH 4·6-SN were detected after 3 d of ripening in experimental cheeses. Lower soluble nitrogen content in cheese fractions were previously recorded in several rennet and acid-coagulated cheeses manufactured from CO_2-treated milk (Uceda et al. 1994; McCarney et al. 1995; Ruas-Madiedo et al. 1998*a*) and was attributed, in Afuega'l Pitu (an acid-coagulated cheese), to the inhibition by CO_2 of microbial proteolysis during the early stages of ripening (Ruas-Madiedo et al. 1998*a*). In our case, the residual CO_2 also could have inhibited proteolysis by the natural microbiota at early stages of ripening in cheeses from mixed raw milk.

As shown in Fig. 1, in cheeses from mixed raw milk, β-casein fractions of ewes' milk (components β_1 and β_2) and cows' milk were indistinguishable with the electrophoretic conditions employed. On the other hand, α-caseins of both species were easily differentiated by their different migration patterns. However, the continuous decrease of this casein fraction during maturation and the low amounts of ewes' milk in cheeses from mixed raw milk (90 : 10 cows' to ewes' milk) makes ewes' α-caseins undetectable beyond the 30th day of ripening in this type of cheese. For these reasons, and because of the greater amounts of α_{s1}-casein with respect to α_{s2}-casein present in cows' milk, we quantified total β-casein from both species and α_{s1}-casein only from cows' milk. Table 3 shows the changes in the residual contents of casein and the relation α_{s1}-I/α_{s1}-I+α_{s1} throughout the ripening of cheeses from trials 1 and 2. The α_{s1}-I peptide is the main product obtained from the hydrolysis of α_{s1}-casein by the chymosin of rennet (Creamer & Richardson, 1974) and this relation was used as an index of α_{s1}-casein degradation (Hynes et al. 2001). In cheeses made from pasteurized milk (trial 1), the amount of α_{s1}-casein gradually decreased throughout ripening, whereas the relation α_{s1}-I/α_{s1}-I+α_{s1} increased. On the other hand, β-casein was degraded less than α_{s1}-casein during maturation, as shown previously by Lau et al. (1991) and Picón et al. (1994). No significant differences ($P>0·05$) were detected in α_{s1}-casein and β-casein levels or in the relation α_{s1}-I/α_{s1}-I+α_{s1} between control and experimental samples. Hence the previous preservation of milk and the residual CO_2 did

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Table 2. pH 4·6-SN and TCA-SN fractions of cheeses made from pasteurized cows' milk (trial 1) and from mixed (90 : 10) raw cows' and ewes' milk (trial 2) as affected by milk treatments (control *v*. experimental batches)

Values are means for $n=3$

				Soluble nitrogen fractions	
Trial	Milk type	Cheese ripening (d)	Batches	pH 4·6-SN†	TCA-SN†
1	Pasteurized cows'	3	Control	5·53	2·56
			Experimental	5·01	2·04
		15	Control	7·27	3·69
			Experimental	7·41	3·45
		30	Control	7·93	4·55
			Experimental	8·51	5·39
2	Mixed raw cows' and ewes' (90 : 10)	3	Control	5·66	3·55
			Experimental	3·90	2·62
		30	Control	10·21	6·71
			Experimental	11·49	6·64
		45	Control	14·77	8·88
			Experimental	14·48	8·71
		75	Control	18·84	12·56
			Experimental	20·05	12·74

† expressed as percentage of the total nitrogen content

Table 3. Casein fractions of cheeses made from pasteurized cows' milk (trial 1) and mixed 90:10 raw cows' to ewes' milk (trial 2) as affected by milk treatments (control *v*. experimental batches)

Values are means ± SD for $n=3$

				Casein fractions (%)		
Trial	Milk type	Ripening (d)	Batches	α_{s1}-I/α_{s1}-I+α_{s1}	α_{s1}-casein[a]	β-casein[b]
1	Pasteurized cows'	3	Control	10·97 ± 2·64	83·65 ± 13·09	94·94 ± 24·26
			Experimental	10·48 ± 3·92	69·02 ± 16·21	94·18 ± 17·22
		15	Control	35·04 ± 7·13	41·43 ± 8·92	81·26 ± 29·11
			Experimental	36·30 ± 2·01	42·75 ± 9·75	75·26 ± 24·97
		30	Control	40·81 ± 4·76	35·71 ± 19·43	67·18 ± 15·35
			Experimental	39·83 ± 2·92	35·43 ± 6·84	67·71 ± 10·56
2	Raw mixed cows' and ewes' (90 : 10)	3	Control	4·68 ± 0·51	104·51 ± 19·93	105·22 ± 10·38
			Experimental	8·67 ± 1·12***	86·36 ± 11·27	96·21 ± 18·69
		30	Control	50·11 ± 10·98	35·55 ± 7·34	86·60 ± 9·25
			Experimental	45·63 ± 1·49	39·76 ± 6·44	80·40 ± 11·54
		45	Control	60·32 ± 17·56	28·68 ± 5·69	65·03 ± 11·58
			Experimental	68·61 ± 13·27	24·39 ± 3·83	65·04 ± 8·88
		75	Control	53·99 ± 8·13	19·64 ± 6·88	49·44 ± 18·93
			Experimental	69·74 ± 4·72**	12·81 ± 4·31	42·14 ± 18·49

[a] relative to the content of α_{s1}-casein in 1-day-old cheeses
[b] relative to the content of β-casein in 1-day-old cheeses. ** $P<0·01$; *** $P<0·001$

not affect the degradation of α_{s1}-casein and β-casein during ripening of cheeses made from pasteurized milk. In cheeses from mixed raw milk (trial 2), α_{s1}-casein gradually decreased during maturation whereas the relation α_{s1}-I/α_{s1}-I+α_{s1} increased continuously until the day 45 of ripening. β-Casein was degraded less than α_{s1}-casein although its level decreased slowly throughout ripening. In general, higher values for the relation α_{s1}-I/α_{s1}-I+α_{s1} were obtained through maturation in experimental batches than in the corresponding controls (significant differences at 3 and 75 d of ripening: $P<0·01$), which indicates a greater degradation of α_{s1}-casein in experimental cheeses. Low pH values and high moisture content can favour the retention of chymosin in the curd (Holmes et al. 1977; Fox & McSweeney, 1996) or stimulate its activity (Visser, 1993) during ripening. Chemical evolution of our cheeses from mixed raw milk has

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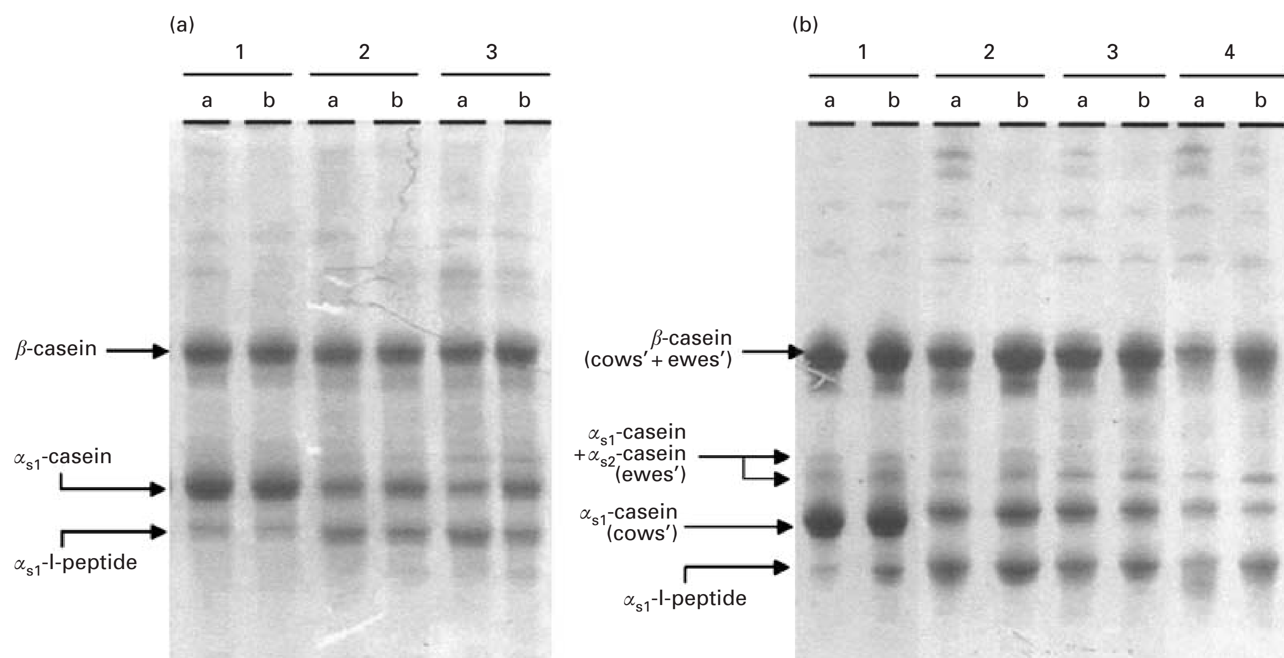

Fig. 1. Urea-PAGE of casein fractions during maturation of cheese. A) cheeses made from pasteurized cows' milk (trial 1) at 3 (lane 1), 15 (lane 2) and 30 d (lane 3). B) cheeses made from mixed cows' and ewes' milk (90 : 10) (trial 2) at 3 (lane 1), 30 (lane 2), 45 (lane 3) and 75 d (lane 4) of ripening. Slots a and b are control and experimental samples, respectively.

been published previously (Ruas-Madiedo et al. 2002): no differences ($P>0·05$) were found between batches for pH (3 d: 5·3±0·6 and 5·3±0·5; 75 d: 5·4±0·9 and 5·7±0·8, for control and experimental, respectively), TS content (3 d: 54·29±1·26 and 56·99±2·07; 75 d: 65·50±0·75 and 63·86±1·58% for control and experimental, respectively) and cheese yield (control: 12·52±0·30; experimental: 12·73±0·89%). Therefore, the greater degradation of α_{s1}-casein in our experimental samples should not be attributed to the enhancement of chymosin activity and may instead be more related to the activities of other enzymes (e.g. cathepsin D; Visser & de Groot-Mostert, 1977) in raw milk during refrigerated storage. From 45 d of ripening, a slight decrease of the relation α_{s1}-I/α_{s1}-I+α_{s1} occurred in control samples ($P>0·05$), indicating late degradation of the α_{s1}-I peptide (Fig. 1). For β-casein, no differences were found between control and experimental samples. In contrast, Uceda et al. (1994) reported that CO_2 inhibited degradation of β-casein by *Pseudomonas fluorescens* in Manchego cheese made from raw milk. In our case, the inhibitory effect of CO_2 on microbial degradation might have been counteracted by an irreversible solubilization of β-casein during refrigeration.

As cheese ages, more casein and high molecular weight peptides are broken down into smaller peptides, which may be water-soluble. The peptide content of WSF was determined by reversed-phase HPLC and detection at 220 nm. At this wavelength the total chromatographic peak area represents the light absorbed by carbonyl groups in peptide bonds. Extractability of nitrogen compounds can vary with pH, their levels being higher in WSF of cheeses of higher pH (Sousa et al. 2001). Since pH remained fairly constant in our cheeses throughout ripening and since no great differences between batches were found previously (Ruas-Madiedo et al. 2002), variations in the peptide content of WSF due to pH can be discounted. In cheeses from pasteurized milk (trial 1), peptides in the hydrophobic and hydrophilic portions of the chromatogram (Fig. 2a) increased throughout ripening, agreeing with other reports (Lau et al. 1991; Picón et al. 1994). No differences ($P>0·05$) due to milk preservation or to residual CO_2 were detected for total areas of both peptide groups; however, differences in peak profiles in zone 1 (Fig. 2b) were found between control and experimental samples throughout ripening (3–30 d). The hydrophobic to hydrophilic ratio remained fairly constant throughout ripening and no differences ($P>0·05$) were detected between control (3·13±0·68 and 3·13±1·27 at 3 and 30 d, respectively) and experimental samples (2·99±0·43 and 2·98±0·33 at 3 and 30 d, respectively). Therefore previous milk preservation and residual CO_2 caused only minor variations in peptide content of the WSF in cheeses from pasteurized milk. In cheeses from mixed raw milk (trial 2), hydrophilic peptides increased during ripening whereas those of the hydrophobic portion increased until day 30 and remained fairly constant afterwards (Fig. 2a). Considerably higher levels of peptides were found in the hydrophobic than in the hydrophilic portion. No differences ($P>0·05$) in hydrophobic areas were detected between experimental and control samples but the area of the hydrophilic portion was significantly lower ($P<0·01$) in

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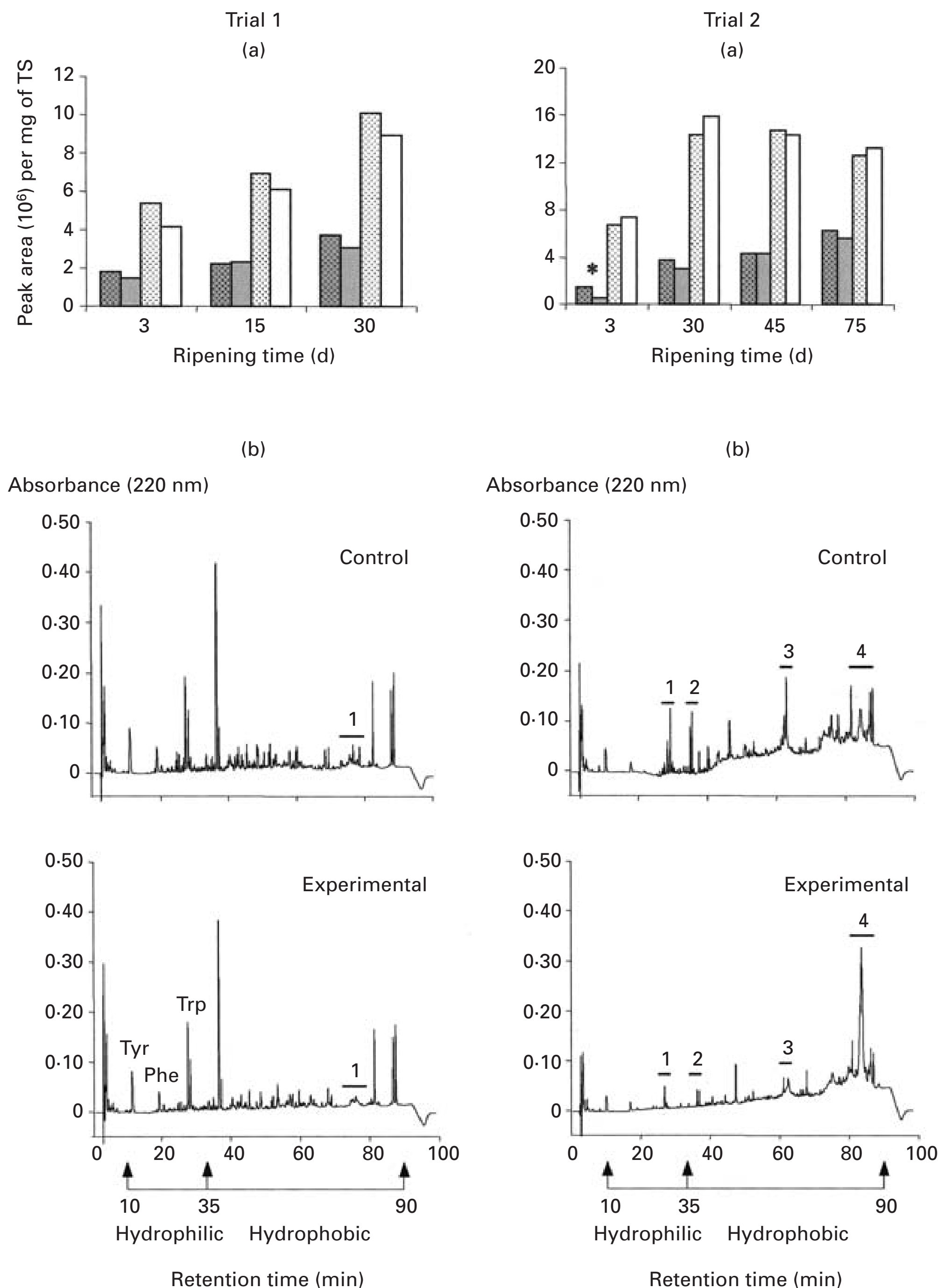

Fig. 2. Analysis of the water-soluble fraction (WSF) by reversed phase-HPLC of Spanish hard cheeses in trial 1 (pasteurized cows' milk) and trial 2 (mixed raw cows' to ewes' milk, 90:10). (a) Evolution of hydrophilic peptides (shaded bars) and hydrophobic peptides (white bars) in control (doted bars) and experimental samples (no doted bars) during ripening. (b) Chromatograms of the WSF at 3 d of ripening; numbers indicate zones displaying profile differences between batches (control and experimental) through the ripening. *$P<0{\cdot}05$.

experimental samples after 3 d of ripening. In addition, four zones (named 1, 2, 3 and 4) showing major variations in peak profiles between control and experimental samples were visually evident in chromatograms throughout ageing (3–75 d) (Fig. 2b). The lower amounts of hydrophilic peptides in experimental samples agreed with their lower levels of TCA-SN and pH 4·6-SN and might be related to the previously reported inhibitory effect of CO_2 on lactococci in the early stages of ripening in cheeses made from raw milk (Ruas-Madiedo et al. 2002). On the other hand, higher

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hydrophobic to hydrophilic ratios ($P<0{\cdot}01$) were obtained in experimental cheeses ($16{\cdot}84 \pm 3{\cdot}11$) than in the controls ($4{\cdot}48 \pm 0{\cdot}71$) after 3 d of ripening. During maturation, this ratio decreased and differences between batches lessened (control: $2{\cdot}02 \pm 0{\cdot}28$; experimental: $2{\cdot}86 \pm 0{\cdot}99$, at 75 d). Hence the previous preservation of milk and/or residual CO_2 did not cause changes in peptide levels in the hydrophobic portion but did result in lower amounts of hydrophilic peptides. In addition, some differences in peak patterns of both peptide groups were detected throughout maturation.

Finally, when the trials were compared, two facts were notable. The extent of proteolysis (TCA-SN and pH 4·6-SN) at 30 d was greater in cheeses from raw milk than in those from pasteurized milk. On the other hand, higher ratios of hydrophobic to hydrophilic peptides were found in cheeses from raw milk. These large differences might be attributable to differences in the enzymic activity of milk and its microbial composition between trial 1 (pasteurized milk, added starter) and trial 2 (raw milk, starter-free).

To summarize, in cheeses from pasteurized milk, casein breakdown was not greatly affected by residual CO_2. In cheeses made from raw milk, we detected no differences in the breakdown of β-casein attributable to the previous refrigeration and CO_2-preservation of the milk. However, the breakdown of α_{s1}-casein was enhanced in experimental samples as the cheese aged. CO_2-treatment was associated with slightly lower levels of pH 4·6-SN and TCA-SN, lower amounts of hydrophilic peptides and appeared to produce some differences in the chromatographic peptide patterns in cheeses from raw milk.

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