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Chemometrical Analysis of Capillary Electrophoresis Casein Fractions for Predicting Ripening Times of Milk Mixture Cheese

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The effect of the ripening time on the proteolytic process in cheeses manufactured from mixtures of cow's and ewe's milk during a 167-day ripening period was monitored by capillary electrophoresis of the pH 4.6-insoluble fraction. Totals of 21 and 16 peaks were recognized and matched in the electropherograms obtained with a fused-silica capillary and a neutral capillary (hydrophilically coated), respectively. These peaks corresponded to intact bovine and ovine caseins and their hydrolysis products (e.g., α_{s1}-casein, γ-caseins). In 167-day-old cheeses, bovine α_{s0}-casein (α_{s1}-casein 9P) had been completely degraded and 6% of the residual bovine α_{s1}-casein remained intact. Breakdown of the β-casein fraction was lower than that of the α_s-casein fraction. Finally, partial least-squares regression and principal component regression were used to predict the ripening time in cheeses. The root-mean-square errors in prediction by cross-validation were <7.8 days in all cases.

KEYWORDS: Capillary electrophoresis; chemometric analysis; cow's and ewe's milk mixture cheese; ripening time

INTRODUCTION

Cheese ripening is characterized by a network of complex physical, chemical, and microbiological changes that affect the major components of the cheese matrix (*1*). Attempts to monitor this process have led to the development of objective physicochemical indices that will eventually be useful in predicting the final quality of cheese. For most hard and semihard cheese varieties proteolysis is the most commonly used index of maturation (*2*), and it is also helpful in evaluating starter culture performance (*3*) because proteolysis strongly contributes to flavor and texture development (*4*). Chemical methods (*5*, *6*) and analysis by nonspecific methods (e.g., quantification of nitrogen in different peptide and amino acid fractions) provide information about the degree of proteolysis in cheese ripening. Nevertheless, the results of these methods do not express well the complexity and specifity of proteolytic development during cheese ripening. Electrophoretic and chromatographic techniques, however, resolve peptides or groups of peptides and thereby provide so-called proteolytic profiles (*7*).

Capillary electrophoresis (CE) has shown its usefulness as an alternative to classical gel electrophoretic techniques owing to its speed of separation, high resolution, and possibility of automation (*8*). Due to these advantages, the application of CE to the assessment of proteolysis in milk and different cheese types has acquired an enormous importance (*9*−*11*). However, because mature cheese contains many different peptides, the amount of data from such analyses becomes both large and complicated. Therefore, researchers working on proteolysis during cheese ripening have need of methods for objective evaluation and data reduction and interpretation in addition to traditional visual examination of the proteolytic profiles (*7*). A combination of studies on peptide profiles using multivariate statistical analysis as a method for evaluation of the chromatograms and the identification of some peptides has been recommended as an approach to better understand the complexity of proteolysis during cheese ripening (*12*).

To date, there have been several reports on the use of multivariate statistical analysis for evaluation of the proteolytic process in cheese during ripening. Thus, Pham and Nakai (*13*) classified Cheddar cheeses according to ripening time on the basis of liquid chromatography of aqueous extracts, followed by stepwise linear discriminant analysis (LDA). A range of proteolysis parameters has also been used for characterization Spanish Manchego cheese (*14*), as well as for predicting the ripening time by means of partial least-squares regression (PLS) and other multivariate regression techniques (*15*). However, despite the extensive literature covering chemometrical analysis of chromatographic or electrophoretic profiles from studies on cheese ripening, very little research has dealt with the application of multivariate statistical analyses to CE peptide profiles (*16*), and limited data are available on PLS of CE analysis of the pH 4.6-insoluble fraction to predict the ripening time in cow's and ewe's milk mixture cheeses.

The objective of the present study was to evaluate the proteolytic process during the ripening of commercial cheeses

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manufactured from mixtures of pasteurized cow's and raw ewe's milk, on the basis of the protein patterns obtained by CE analysis of the pH 4.6-insoluble fractions. Additionally, chemometric analysis of the CE data was used to predict the ripening time of the milk mixture cheeses.

MATERIALS AND METHODS

Materials and Reagents. Citric acid, hydrochloric acid, dichloromethane, phosphoric acid, sodium hydroxide, sodium citrate, and urea were of analytical grade and obtained from Merck (Darmstadt, Germany). Dithiothreitol (DTT), hydroxypropyl methyl cellulose (HPMC), 3-morpholinopropanesulfonic acid, plasmin (EC 3.4.21.7, from bovine plasma), α_s-casein, β-casein, and κ-casein were from Sigma Chemical Co. (St. Louis, MO). All solutions were based on highly purified water (MilliQ grade).

Buffer solutions were filtered through 0.45 μm HAWP and 0.22 μm GSWP filters (Millipore Co., Billerica, MA) before use.

Cheese Samples. Cheeses, manufactured from mixtures of pasteurized cow's and raw ewe's milk, were supplied by Quesos Frías, S.A. (Burgos, Spain). Caseins were extracted from cheese ripened for 0, 16, 44, 73, 100, 133, and 167 days. Duplicate cheese samples were collected from each batch.

Extraction of Cheese. Isoelectric caseins were obtained by precipitation from 5 g of homogenized cheese in 30 mL of water by adding 2 M HCl to pH 4.6, followed by centrifugation at 3500 rpm for 15 min. To isolate the casein fraction completely from whey and eliminate the remaining fat, it was washed once with 1 M sodium acetate buffer (pH 4.6) and three times with dichloromethane/1 M sodium acetate buffer (pH 4.6) (1:1, v/v). The casein fractions obtained were lyophilized and stored at −20 °C.

Plasmin Treatment of Casein. Half-milliliter lyophilized cheese casein solutions (20 mg mL^{-1} in 0.05 M phosphate buffer, pH 7) and 0.5 mL of plasmin preparation (0.152 unit mL^{-1} in 0.05 M phosphate buffer, pH 7) were incubated at 37 °C for 240 min. The enzymatic reaction was stopped by heating to 85 °C for 10 min. The final hydrolysates were centrifuged at 2600 rpm for 20 min at 4 °C, and the pH in the supernatants was decreased to 4.6 with 0.5 M acetic acid. After equilibration for 1 h, centrifugation was repeated, and the precipitates were stored frozen until analysis.

Sample Preparations for CE. To dissociate the caseins, all samples were dissolved in a sample buffer (0.05 M phosphate buffer) containing 8 M urea and 10 mM DTT at pH 8 and left for at least 1 h at room temperature before filtration (0.22 μm Millex-GV$_{13}$, Millipore Co.) and CE analysis. The cheese caseins were dissolved at 10 mg mL^{-1}, and the precipitate obtained from cheese casein treated with plasmin was dissolved in 1 mL of the sample buffer.

CE Separations. CE was carried out with a Beckman P/ACE System 2200 (Beckman Instruments Inc., San Ramon, CA) controlled by a System Gold Software data system version 810. The separations were performed using two 50 μm i.d. capillaries, a fused-silica capillary (eCap, Beckman Instruments) of 57 cm (50 cm to the detector window) and a neutral capillary (eCAP Neutral Capillary, Beckman Instruments) of 45 cm (33 cm to the detector window) that utilizes a polyacrylamide layer covalently linked onto the inner wall to generate a hydrophilic surface. All experiments were carried out in the cationic mode (anode at the inlet and cathode at the outlet). The sample introduction was achieved by pressure injection for 5 s at 0.5 psi.

The analysis with the fused-silica capillary was performed according to the method of Ortega et al. (*17*). The run buffer was 50 mM sodium phosphate with 6 M urea (pH 3.0), containing 0.05% (w/v) HPMC. During sample analysis a constant voltage of 18.5 kV was applied, and the temperature of the separation was kept at 23 °C with circulating coolant surrounding the capillary. Between runs the capillary was purged for 5 min with run buffer.

The CE analysis carried out with the neutral capillary was performed following the method described by Albillos (*18*). The run buffer was 0.32 M citric acid/0.02 M sodium citrate with 6 M urea (pH 3.0) containing 0.05% (w/v) HPMC. Separation was performed at 21 °C and a constant voltage of 25.09 kV. The capillary was rinsed sequentially between successive electrophoretic runs, with 0.1 M HCl (2 min) and ionized water (2 min), and the rinse buffer (pH 3.0) contained 0.32 M citric acid, 0.020 M sodium citrate, 6 M urea, and 0.042 M 3-morpholinopropanesulfonic acid (5 min).

For all experiments, detection was carried out at 214 nm (data collection rate of 5 Hz). The first electropherogram in a series was always discarded.

Peaks were characterized using caseins from cow's and ewe's milk previously identified in our laboratory (*17*, *18*).

The repeatability of peak areas was assessed by replicate injections ($n = 10$) of a mixture of α_s-casein, β-casein, and κ-casein at 5 mg mL^{-1}. Relative standard deviations were lower than 6.2 and 4.9% for the fused-silica capillary and the neutral capillary, respectively.

Statistical Procedures. Partial least-squares regression (PLS), principal component regression (PCR), and principal component analysis (PCA) were applied to peak area obtained by CE for the prediction of the ripening time of the cheese. Data were autoscaled prior to model calculations. This normalization involved dividing each value of a given variable by the standard deviation of all the values for this variable over the entire sample collection period (*19*). After normalization, all variables had the same weight because they had a mean of zero and unitary variance.

The root-mean-square error of prediction (RMSEP) obtained by cross-validation was used as a measure of the ability of the two models to furnish accurate predictions and was calculated using the equation

$$\mathrm{RMSEP} = \left(\sum_{i=1}^{n}(t_i - t_{(i)})^2/n\right)^{1/2}$$

where t_i is the real ripening time for the ith sample of the standard cheese, $t_{(i)}$ is the predicted ripening time obtained with the model constructed without the ith sample, and n is the number of standard cheeses used in the calibration model ($n = 14$). This procedure is commonly called leave-one-out cross-validation, and the RMSEP value can be considered to be an approximation of the prediction error (*15*, *20*).

Calculations were performed using the Q-PARVUS 3.0 package (*21*).

RESULTS AND DISCUSSION

Proteolysis of the Casein Fraction. Electropherograms of the pH 4.6-insoluble fraction throughout a 167-day ripening period (with sampling at 0, 16, 44, 73, 100, 133, and 167 days) using a fused-silica capillary and a neutral capillary are shown in **Figures 1** and **2**, respectively. Identification of intact caseins (α_s-caseins and β-caseins) was based on the results of Albillos (*18*), and the relative migration of the components was in accordance with Molina et al. (*9*). Similar profiles were obtained with both capillaries. Nevertheless, the fused-silica capillary provides better resolution than the neutral capillary in the separation of bovine β-casein A$_2$ and ovine β_2-casein (peaks 12*s* and 12*n*, respectively) in contrast to what should be expected from the literature (*9*).

Electrophoretic profiles from 0-day-old cheeses were very similar to the pH 4.6-insoluble fraction profiles from bovine and ovine milk mixture counterparts (electropherogram analysis not shown). In general, good resolution between intact proteins and breakdown products was achieved at day 0. As can be seen, in these electropherograms bovine β-casein A$_1$ and ovine β-casein showed the same migration time, peak 12*s* (fused-silica capillary, **Figure 1**) and peak 11*n* (neutral capillary, **Figure 2**), and bovine β-casein A$_2$ overlapped ovine β_2 (peaks 13*s* and 12*n*). However, the peak belonging to κ-casein disappeared with the concomitant appearance of a new double peak corresponding to bovine and ovine para-κ-casein (peaks 1*s* and 1*n*) with migration time near that ofthe major α_{s2}-casein components (peaks 2*s*/3*s* and 2*n*/3*n*). The ovine para-κ-casein contains Val and Asp (instead of Ala) at positions 65 and 90, respectively, which are more voluminous. The different sizes of the amino

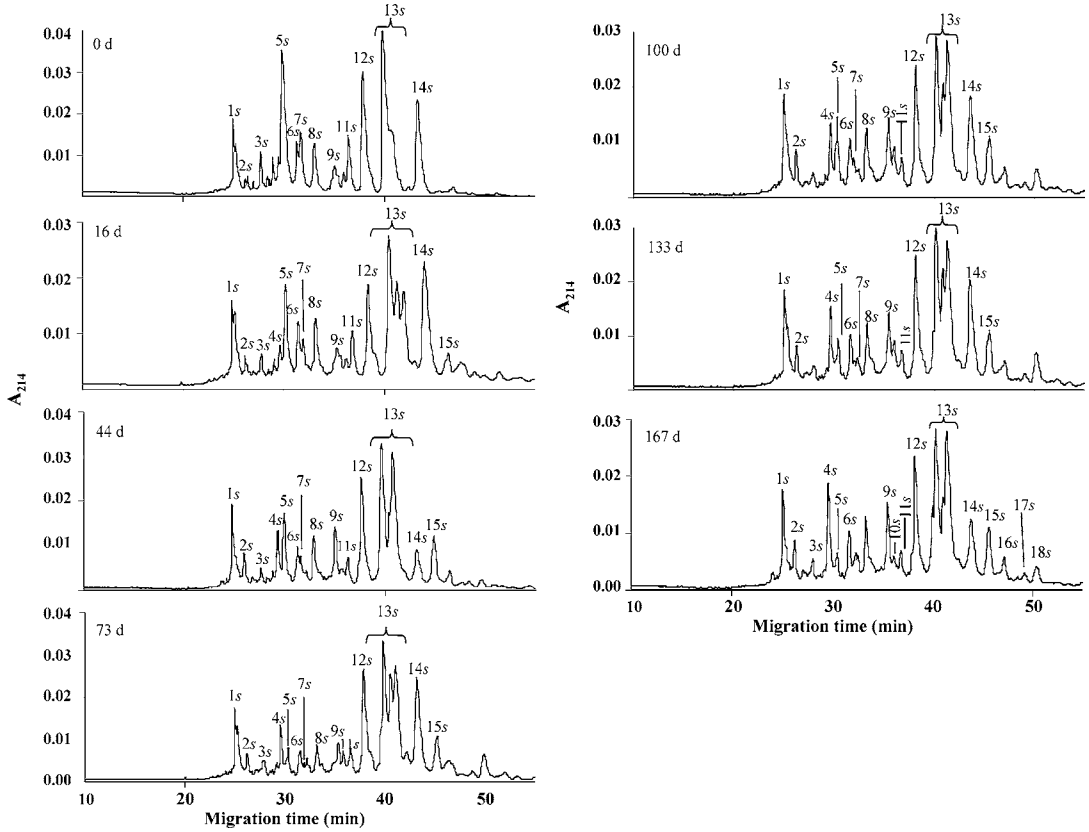

Figure 1. CE analyses of the pH 4.6 insoluble fraction of cheeses manufactured with mixtures of cow's and ewe's milk after different days of ripening. Separations were performed in a fused-silica capillary at 18.5 kV (~35 μA). Other conditions are described under Materials and Methods. Peak identification: 1*s*, bovine and ovine p-κ-CN + ovine α_{s2}-CN; 2*s*, ovine α_{s2}-CN; 3*s*, bovine α_{s2}-CN; 4*s*, γ-CN; 5*s*, bovine α_{s1}-CN; 6*s*, ovine α_{s1}-CN I; 7*s*, bovine α_{s0}-CN; 8*s*, ovine α_{s1}-CN II + γ-CN; 9*s*, ovine α_{s1}-CN III + γ-CN; 10*s*, unidentified; 11*s*, bovine β-CN B + P; 12*s*, bovine β-CN A_1 + ovine β-CN + P; 13*s*, bovine β-CN A_2 + ovine β_2-CN + P; 14*s*, ovine β_1-CN + P; 15*s*, α_{s1}-I-CN; 16*s*, unidentified; 17*s*, P; 18*s*, P. P represents peptides from the action of plasmin on caseins.

acids and the p*I* of the Asp result in a lower mobility than that of the bovine casein.

Electropherograms of the casein fraction after 16 days of ripening showed important changes compared to 0-day-old samples. The peak corresponding to α_{s1}-casein significantly decreased. In fact, the primary site of chymosin action on bovine α_{s1}-casein is the $Phe_{23}-Val_{24}$ bond with the appearance of α_{s1}-I breakdown product or α_{s1}-casein f(24/25−199) and the complementary peptide α_{s1}-casein f(1−23). This hydrolysis of α_{s1}-casein in cheeses was coincidental with the appearance of different peaks with larger migration times on the electropherograms. Thus, as confirmed by the experiments carried out using model systems (data not shown), in the electropherograms obtained using the fused-silica capillary peak 15*s* corresponded to α_{s1}-I-casein (**Figure 1**). When the coated capillary was used, this casein was integrated along with other products of casein degradation in the peak called 13*n* (**Figure 2**). α_{s1}-I-casein is further hydrolyzed by chymosin during cheese ripening, giving rise to degradation products of high migration time in electropherograms performed with a hydrophilic-coated fused silica capillary column (*22*). In fact, **Figures 1** and **2** showed peaks having migration times longer than that of α_{s1}-I-casein.

The data of these figures show that at the end of the ripening period (167 days) bovine α_{s0}-casein, which contains one phosphate group more than α_{s1}-casein (*23*), had been completely hydrolyzed and only 6% of the residual bovine α_{s1}-casein remained intact. However, higher resistance of β-caseins to enzymatic hydrolysis compared with α_s-caseins was observed. The β-casein degraded more quickly was the bovine β-casein B. Other researchers have previously reported β-casein to be more resistant to proteolysis (*24*, *25*). The overall proteolytic pattern during ripening is determined largely by the action of residual rennet enzyme(s) combined with that of enzymes produced by viable (or released by lysed) microorganisms. Although starter proteinases contribute little to the formation of large peptides (e.g., pH 4.6- or water-soluble peptides), proteinases from mesophilic streptococci are capable of hydrolyzing intact caseins in solution, especially β-casein; apparently, only a few strains are capable of hydrolyzing α_{s1}-casein, although this is hardly noticed in most cheeses because this protein is easily hydrolyzed by chymosin, the major constituent of animal rennets (*26*).

Another important change at this time of ripening was the presence of a few peaks located between α_{s1}- and α_{s2}-caseins. On the basis of their relative migration times on the electropherograms shown by different authors (*9*) and from the results obtained using model systems, they could be recognized as γ-caseins. It is known that plasmin, the principal endogenous proteinase of milk, acts mainly on β-casein, hydrolysis that leads to the formation of γ-caseins (*27*, *28*). Thus, to check whether these peaks were caused by the action of plasmin, caseins from cheeses were treated with this enzyme (**Figures 3** and **4**). As expected, there was a decrease in the β-casein peaks and a concomitant increase in peaks called 4*s*, 8*s*, and 9*s* (fused-silica capillary) and 3*n*, 4*n*, and 8*n* (neutral capillary), as a result of the appearance of the γ-casein. Furthermore, when the cheese was treated with plasmin, the peaks designated 13*s*, 14*s*, 17*s*, and 18*s* (**Figure 3**) and 13*n* and 16*n* (**Figure 4**) increased.

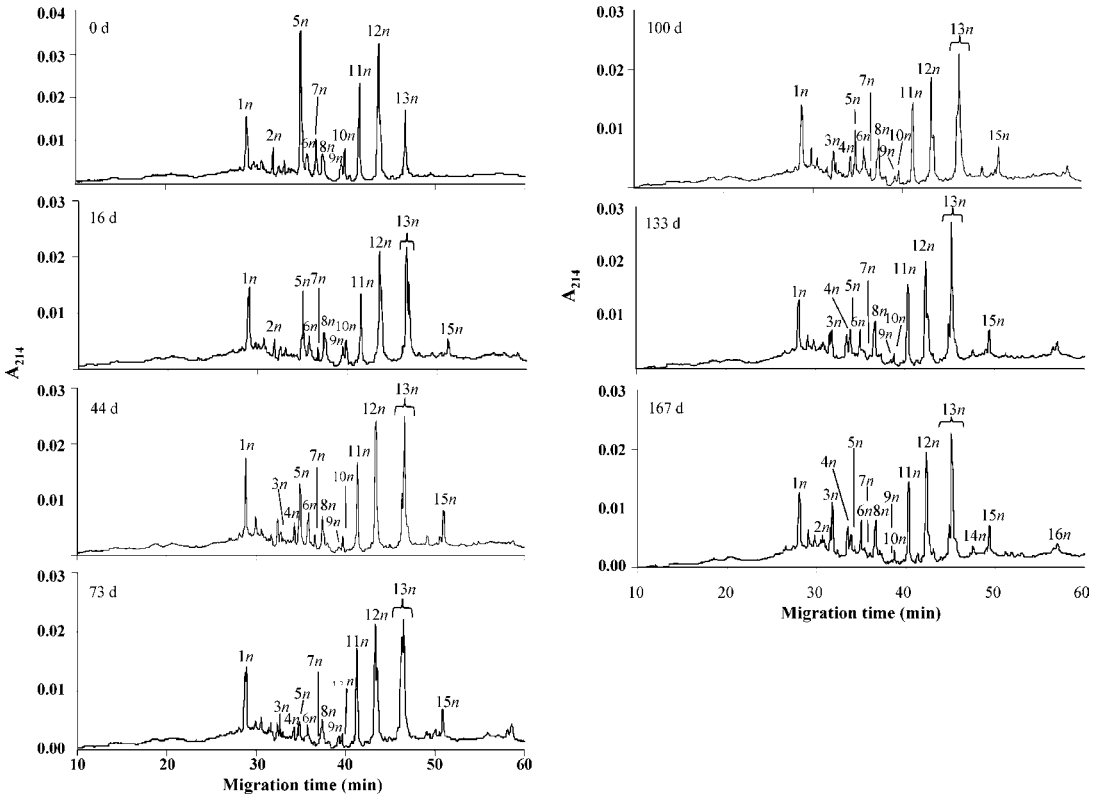

Figure 2. CE analyses of the pH 4.6 insoluble fraction of cheeses manufactured with mixtures of cow's and ewe's milk after different days of ripening. Separations were performed in a neutral capillary at 25.09 kV (~50 μA). Other conditions are described under Materials and Methods. Peak identification: 1*n*, bovine and ovine p-κ-CN + ovine α_{s2}-CN; 2*n*, bovine α_{s2}-CN; 3*n*, bovine α_{s2}-CN + γ-CN; 4*n*, γ-CN; 5*n*, bovine α_{s1}-CN; 6*n*, ovine α_{s1}-CN I; 7*n*, bovine α_{s0}-CN; 8*n*, ovine α_{s1}-CN II + γ-CN; 9*n*, ovine α_{s1}-CN III; 10*n*, bovine β-CN B; 11*n*, bovine β-CN A_1 + ovine β-CN; 12*n*, bovine β-CN A_2 + ovine β_2-CN; 13*n*, ovine β_1-CN + P + α_{s1}-I-CN; 14*n*, unidentified; 15*n*, unidentified; 16*n*, P. P represents peptides from the action of plasmin on caseins.

Table 1. Peak Visually Recognized and Matched in the Electropherograms Performed in the Fused-Silica Capillary (**Figure 1**) and Neutral Capillary (**Figure 2**)

peak[a]	casein or peptide[b]	peak[a]	casein or peptide[b]
1*s*	p-κ-$CN_{(B)}$ + p-κ-$CN_{(O)}$ + α_{s2}-$CN_{(O)}$	1*n*	p-κ-$CN_{(B)}$ + p-κ-$CN_{(O)}$ + α_{s2}-$CN_{(O)}$
2*s*	α_{s2}-$CN_{(O)}$	2*n*	α_{s2}-$CN_{(B)}$
3*s*	α_{s2}-$CN_{(B)}$	3*n*	α_{s2}-$CN_{(O)}$ + γ-CN
4*s*	γ-CN	4*n*	γ-CN
5*s*	α_{s1}-$CN_{(B)}$	5*n*	α_{s1}-$CN_{(B)}$
6*s*	α_{s1}-$CN_{(O)}$ I	6*n*	α_{s1}-$CN_{(O)}$ I
7*s*	α_{s0}-$CN_{(B)}$	7*n*	α_{s0}-$CN_{(B)}$
8*s*	α_{s1}-$CN_{(O)}$ II + γ-CN	8*n*	α_{s1}-$CN_{(O)}$ II + γ CN
9*s*	α_{s1}-$CN_{(O)}$ III + γ-CN	9*n*	α_{s1}-$CN_{(O)}$ III
10*s*	peak 10*s*	10*n*	β-$CN_{(B)}$ B
11*s*	β-$CN_{(B)}$ B + P[c]	11*n*	β-$CN_{(B)}$ A_1 + β-$CN_{(O)}$
12*s*	β-$CN_{(B)}$ A_1 + β-$CN_{(O)}$ + P[c]	12*n*	β-$CN_{(B)}$ A_2 + β_2-$CN_{(O)}$
13*s*	β-$CN_{(B)}$ A_2 + β_2-$CN_{(O)}$ + P[c]	13*n*	β_1-$CN_{(O)}$?? + P[c] + α_{s1}-I-CN
14*s*	β_1-$CN_{(O)}$ + P[c]	14*n*	peak 14*n*
15*s*	α_{s1}-I-CN	15*n*	peak 15*n*
16*s*	peak 16*s*	16*n*	P[c]
17*s*	P[c]		
18*s*	P[c]		

[a] *s*, fused-silica capillary; *n*, neutral capillary. [b] (B), bovine; (O), ovine. [c] P, peptides from the action of plasmin on caseins.

Totals of 21 and 16 peaks were visually recognized and matched in the electropherograms obtained with the fused-silica capillary and the neutral capillary, respectively. A summary of these peaks is shown in **Table 1**.

Prediction of Ripening Time by Multivariate Regression Analysis. In a preliminary analysis, PLS regression was applied to the calibration samples with ripening times from 0 to 167 days ($n = 14$), using the areas of the specified peaks (**Table 1**) as the predictor variables. A first model, with the whole data set of the standard cheeses, indicated the peaks with a low modeling power of variable [peaks 2*s*, 8*s*, 10*s*, and 14*s* (silica-fused capillary) and peaks 1*n*, 2*n*, 6*n*, 12*n*, 13*n*, and 16*n* (neutral capillary)]. **Table 2** shows the results when PLS and PCR regression were applied to the area of peaks selected (14 and 10 for the silica-fused capillary and the neutral capillary, respectively). These results include number of components selected by cross-validation (*a*), the determination coefficient (R^2), and the RMSEP. Of all of these parameters, the lowest value of RMSEP was used as a criterion to choose the most accurate method to predict the maturation time of the cheese samples (*15*).

Table 2. Partial Least-Squares Regression (PLS) and Principal Component Regression (PCR) Results for the Prediction of the Ripening Time of Cheeses Made with Cow's and Ewe's Milk Mixture, Obtained from the Capillary Electrophoresis Peak Areas

	fused-silica capillary		neutral capillary	
	PLS	PCR	PLS	PCR
a[a]	6	7	5	5
(R^2)[b]	0.9965	0.9965	0.9929	0.989
RMSEP[c]	6.9	6.3	7.8	7.5

[a] Number of components selected by cross-validation. [b] Determination coefficient. [c] Root-mean-square error of prediction (in day).

In all regression methods the percentage of the explained variance for the models was >97%, yielding good correlations

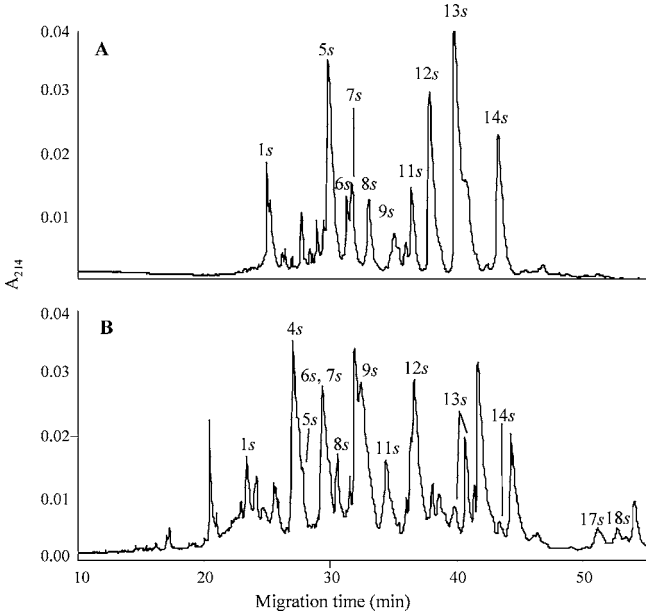

Figure 3. CE analyses of the pH 4.6 insoluble fraction of 0-day-old cheese (**A**) untreated and (**B**) treated with plasmin (7.6 units mL^{-1}, 240 min at 37 °C). Separations were performed in a fused-silica capillary at 18.5 kV (~35 μA). Other conditions are described under Materials and Methods. Peak identification: 1*s*, bovine and ovine p-κ-CN + ovine α_{s2}-CN; 4*s*, γ-CN; 5*s*, bovine α_{s1}-CN; 6*s*, ovine α_{s1}-CN I; 7*s*, bovine α_{s0}-CN; 8*s*, ovine α_{s1}-CN II + γ-CN; 9*s*, ovine α_{s1}-CN III + γ-CN; 11*s*, bovine β-CN B + P; 12*s*, bovine β-CN A_1 + ovine β-CN + P; 13*s*, bovine β-CN A_2 + ovine β_2-CN + P; 14*s*, ovine β_1-CN + P; 17*s*, P; 18*s*, P. P represents peptides from the action of plasmin on caseins.

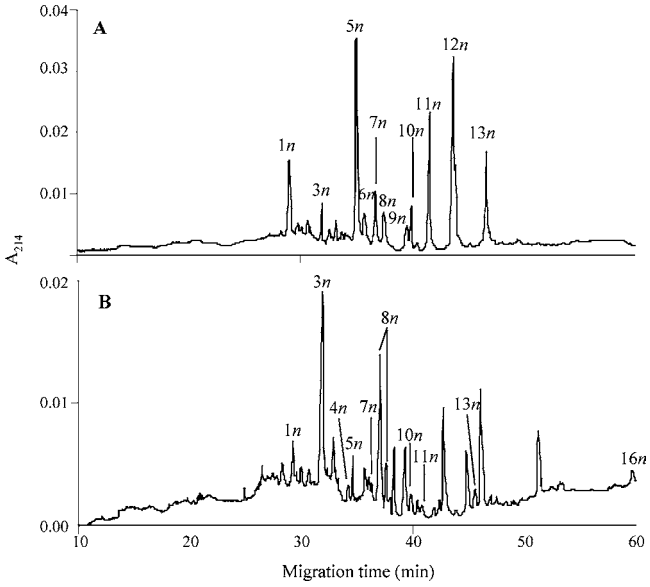

Figure 4. CE analyses of the pH 4.6 insoluble fraction of 0-day-old cheese (**A**) untreated and (**B**) treated with plasmin (7.6 units mL^{-1}, 240 min at 37 °C). Separations were performed in a neutral capillary at 25.09 kV (~50 μA). Other conditions are described under Materials and Methods. Peak identification: 1*n*, bovine and ovine p-κ-CN + ovine α_{s2}-CN; 3*n*, bovine α_{s2}-CN + γ-CN; 4*n*, γ-CN; 5*n*, bovine α_{s1}-CN; 6*n*, ovine α_{s1}-CN I; 7*n*, bovine α_{s0}-CN; 8*n*, ovine α_{s1}-CN II + γ-CN; 10*n*, bovine β-CN B; 11*n*, bovine β-CN A_1 + ovine β-CN; 12*n*, bovine β-CN A_2 + ovine β_2-CN; 13*n*, ovine β_1-CN + P + α_{s1}-I-CN; 16*n*, P. P represents peptides from the action of plasmin on caseins.

(R^2 values > 0.989) between the observed and calculated ripening times. The values of RMSEP were similar, oscillating between 6.3 days for the silica-fused capillary (PCR regression) and 7.8 days for the neutral capillary (PLS regression).

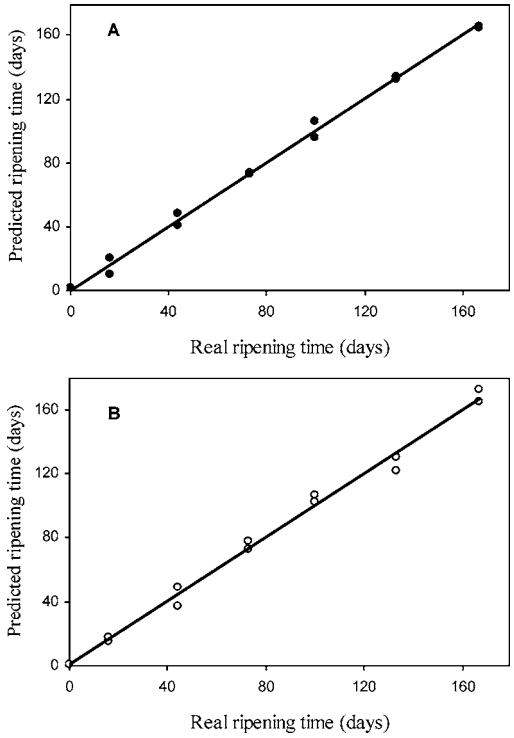

Figure 5. Correlation between the ripening times predicted by cross-validation using PLS regression and real ripening times for cheeses made with a mixture of cow's and ewe's milk analyzed by CE using a fused-silica capillary (**A**) and a neutral capillary (**B**).

The values of RMSEP mentioned above are lower than those obtained with the linear PLS model experiments. In fact, García-Ruiz et al. (*15*) obtained RMSEP of 10.3 days using PLS regression to calculate ripening time in standard Manchego cheeses on the basis of water activity, pH, and other proteolysis parameters [TN (total nitrogen), WSN (water-soluble nitrogen), WSN/TN, and N-PTA (phosphotungstic acid-soluble nitrogen)/TN].

Figure 5 depicts the relationship between the ripening times predicted by cross-validation and real ripening times for the two capillaries used. The fit for the prediction of the period between 0 and 167 days of ripening was good as shown by the values of r^2 obtained (0.9965 and 0.9929 for the fused-silica and neutral capillaries, respectively).

Additionally, PCA was applied to the area of peaks selected (except the peaks with a low modeling power of variance). When the fused-silica capillary was used to analyze the casein fraction of cheese, seven principal components (PC) were obtained, accounting for 99.7% of the total variance. PC1 explained 58.1% of the total variance, and the peak areas that correlated best with this PC were bovine α_{s1}-casein (peak 5*s*), bovine α_{s0}-casein (peak 7*s*), bovine β-casein B (peak 11*s*), and the peptide marked as peak 17*s*. In fact, these caseins showed the major modification along the ripening time. A biplot showing the projection of the samples (scores) and the variables (loading) on the plane of the first and second eigenvectors is given in **Figure 6**. It can be observed that samples appear to be separated according to their ripening time from left to right. On the other hand, a similar analysis was carried out with the peak areas obtained from the neutral capillary. PC1 explained 75.2% of the total variance, and the peaks that correlated best with this PC were bovine α_{s1}-casein, bovine α_{s0}-casein, and bovine β-casein B (peaks 5*n*, 7*n*, and 10*n*, respectively).

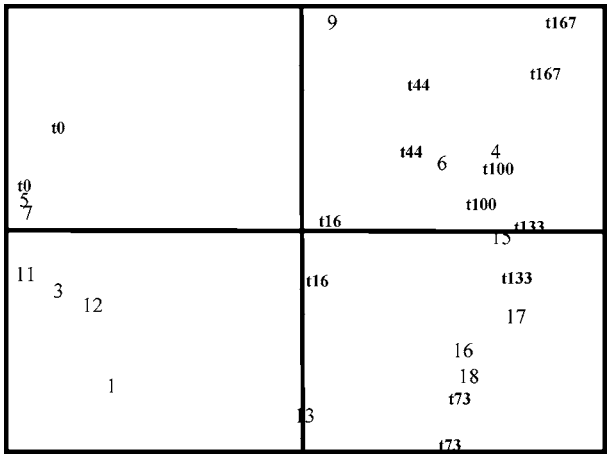

Figure 6. Biplot on the plane of the first and second eigenvectors. Training samples are represented by *ti* (where *i* is the ripening time). The numbers represent the original variables, plotted according to the respective loading.

In conclusion, chemometrical analysis of capillary electrophoresis proteolytic profiles has been shown to be a powerful method to get information on the biochemical process of proteolysis and predict the ripening time of cheeses made with mixtures of cow's and ewe's milks.

ABBREVIATIONS USED

CN, casein; CE, capillary electrophoresis; PCA, principal component analysis; PCR, principal component regression; PLS, partial least-squares regression; RMSEP, root-mean-square error of prediction.

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