



**HAL**  
open science

## Vegetable coagulant (*Cynara cardunculus*) use evidenced by capillary electrophoresis permits PDO Serpa cheese authentication

Luisa Roseiro, José Gómez-Ruiz, Mónica García-Risco, Elena Molina

### ► To cite this version:

Luisa Roseiro, José Gómez-Ruiz, Mónica García-Risco, Elena Molina. Vegetable coagulant (*Cynara cardunculus*) use evidenced by capillary electrophoresis permits PDO Serpa cheese authentication. *Le Lait*, 2003, 83 (4), pp.343-350. 10.1051/lait:2003017 . hal-00895502

**HAL Id: hal-00895502**

**<https://hal.science/hal-00895502>**

Submitted on 11 May 2020

**HAL** is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.

## Vegetable coagulant (*Cynara cardunculus*) use evidenced by capillary electrophoresis permits PDO Serpa cheese authentication<sup>1</sup>

Luisa B. ROSEIRO<sup>a</sup>, José A. GÓMEZ-RUIZ<sup>b</sup>, Mónica GARCÍA-RISCO<sup>b\*\*</sup>,  
Elena MOLINA<sup>b\*</sup>

<sup>a</sup> Unidade de Indústrias Lácteas (UIL) – DTIA – INETI, Estrada do Paço do Lumiar,  
No. 22, 1649-038 Lisboa, Portugal

<sup>b</sup> Instituto de Fermentaciones Industriales, CSIC. C/ Juan de la Cierva, 3. 28006 Madrid, Spain

Received 14 October 2002 – Accepted 6 February 2003

Published online 19 June 2003

**Abstract** – A capillary zone electrophoresis (CZE) method was applied to a Protected Designation of Origin (PDO) cheese, namely Serpa cheese, made with local pure ovine raw milk and a vegetable coagulant (an aqueous extract from dried cardoon flowers of *Cynara L.*). The electropherograms of Serpa cheese showed a peak that remained throughout maturation, and it was not detected in other cheeses made with the same technology as Serpa cheese, but with animal rennet or microbial coagulant instead of vegetable coagulant. This peak, probably arising from  $\beta$ -casein, identifies the type of coagulant employed in the cheese-making. The results obtained lead us to suggest that CZE of cheese caseins is a suitable, fast and easy-to-perform method for evaluating the authenticity of cheeses made with *Cynara L.*

**Cheese authentication / Serpa cheese / vegetable coagulant / capillary electrophoresis**

**Résumé** – La mise en évidence par électrophorèse capillaire de l'utilisation de présure végétale (*Cynara cardunculus*) dans le fromage AOP Serpa permet son authentification. L'électrophorèse capillaire de zone (ECZ) a été appliquée à l'analyse du fromage d'Appellation d'Origine Protégée (AOP) Serpa produit à partir de lait cru de brebis pur avec une présure végétale (l'extrait aqueux des fleurs du chardon desséchées de *Cynara L.*). Les profils électrophorétiques du fromage Serpa ont montré la présence d'un pic qui est conservé pendant la maturation, mais qui n'a pu être détecté sur les autres fromages élaborés avec des coagulants d'origine animale ou microbienne. Ce pic, résultant probablement de la caséine  $\beta$ , peut être représentatif de la nature du coagulant employé lors de l'élaboration du fromage. Nous pouvons conclure d'après les résultats obtenus, que la méthode ECZ des caséines du fromage est une méthode appropriée, rapide et facile à mettre en œuvre afin d'évaluer l'authenticité des fromages élaborés avec *Cynara L.*

**Authenticité du fromage / fromage Serpa / présure végétale / électrophorèse capillaire**

\* Correspondence and reprints

E-mail: e.molina@ifi.csic.es

\*\* Present address: Unidad Asociada de Ciencia y Tecnología de Alimentos UAM-CSIC, Campus de Cantoblanco, 28049 Madrid, Spain.

<sup>1</sup> Part of this work was presented as a poster at Congrilaite, held in Paris from 24th to 27th September 2002.

## 1. INTRODUCTION

Certain varieties of ovine cheeses produced in the Iberian Peninsula are traditionally made with aqueous extracts from dried cardoon flowers (*Cynara L.*) as coagulant and have a great importance from the economic point of view [18]. Some of them carry the EC label of Protected Designation of Origin (PDO), the European recognition for the Controlled Designation of Origin (CDO) of each European country under EC regulation 2081/92 [17]. This makes compulsory the use of local pure ovine raw milk and *Cynara L.* as coagulant (usually *C. cardunculus L.* or *C. humilis L.*). Serpa cheese, considered to be one of the best cheeses of Portugal, is one of these traditional Portuguese cheeses with the PDO designation. Its region of production is a demarcated area in Alentejo, the biggest and most arid province of Portugal, situated towards the south of the country. Like the other ewe's milk Portuguese cheeses, it is mainly artisanally produced, although nowadays, some are also produced by semi-industrial procedures. The main characteristics that distinguish Serpa and some other PDO Portuguese ewe's milk cheeses from other countries' ewe's milk cheeses are that they have a creamy semi-soft texture and exquisite flavour [19]. These characteristics are in part due to the type of milk and technology used, but mainly to the vegetable coagulant, which is very proteolytic. Their popularity and recent increasing demand by consumers might lead to the addition of milk from other species to extend production, and/or the substitution of vegetable coagulant by other types of coagulants, both of these procedures being considered as frauds.

In the last decade, CE has been proved to be an efficient separation technique for the analysis of food proteins in general [5, 6, 16] and milk proteins in particular [14]. In fact, CE has been applied to monitoring proteolysis in cheese [8, 12], mainly in bovine cheese, but only a few publications are concerned with the study of proteolysis

of ewe's or goat's milk cheeses by capillary electrophoresis [2, 7, 10]. Detection of milk mixtures can also be done by CE [1, 9], but detection of the coagulant origin in cheese is not yet possible. Moreover, there is not to our knowledge any characterisation of the caseins and their degradation products in cheeses made with vegetable coagulant by this methodology. Only a recent work by Roseiro and co-workers [20] refers to the study of proteolysis by CZE of cheeses produced with *Cynara L.* as coagulant.

In this study CZE was used to identify the major protein components of the casein fraction and their breakdown products of cheeses made from ewe's milk using an aqueous extract from the flowers of *Cynara L.* as coagulant. The main aim was to differentiate the use of this vegetable coagulant by comparison with cheeses made following the same manufacturing process but substituting the vegetable coagulant with animal or microbial coagulants. Model systems of ovine caseins with plasmin and different coagulants were carried out in order to study the origin of possible distinguishable peaks of the vegetable coagulant.

## 2. MATERIALS AND METHODS

### 2.1. Cheese samples

Two different dairies in the Demarcated Region for Serpa cheese in Baixo Alentejo were chosen to perform the experimental work. One is artisanal, in which about 100 L of ewe's milk from the Merino breed are transformed into cheese per day, using simple manual technology. The other is a recent dairy (about 7 years old) that produces Serpa cheese, blending the artisanal techniques with some technology on a semi-industrial scale, transforming ca. 1000 L of ewe's milk from the Lacaune breed per day.

Four batches of 9 cheeses each were made. Two batches were made in the artisanal dairy consisting of Serpa cheese

and a cheese coagulated with microbial coagulant, while the other two were made in the semi-industrial dairy, also consisting of Serpa cheese and a batch of cheese coagulated with animal rennet. All of them were manufactured on the same day in each dairy, following their own methodologies. The cheeses coagulated by animal rennet and microbial coagulant were made in each dairy from the same milk batch and according to the procedure used for Serpa cheese, but substituting *Cynara L.* with animal rennet (Bovigrand 22, 1:15000, SBI-Systems Bio-Industries, Beaune, France) and with microbial coagulant (*Rhizomucor mihei*, 1:15000, Fabre Srl, Sirtori, Italy), respectively. Laboratory trials were done using raw ewe's milk from the dairies and the animal and microbial coagulants referred to, in order to determine the amount necessary to coagulate the milk in the same time as when using *Cynara L.* aqueous extracts.

The manufacturing parameters were recorded and a curd sample (0 maturation days) was collected from each batch on the day of manufacture. During the maturation period, 3 cheeses were collected from each batch in each dairy at 30, 45 and 60 maturation days.

## 2.2. Isolation of caseins

Caseins (CN) from curd and cheese samples at different maturation times produced in both dairies were prepared. Samples of curd or cheese were homogenised with water and the caseins precipitated at pH 4.6. The precipitated caseins were then isolated by centrifugation, washed and freeze-dried prior to use.

## 2.3. Ovine caseins model systems

The specific action of each type of coagulant used in this experiment on ovine casein was evaluated by observing their effect on the whole and individual ovine caseins as model systems. The effect of plasmin (EC 3.4.21.7, from bovine plasma, SIGMA, St Louis, MO, USA) was also

evaluated. For this purpose, whole casein from raw ovine milk and  $\kappa$ -,  $\alpha_s$ - and  $\beta$ - ovine CN isolated by FPLC, obtained previously in the laboratory, were used. Fractions and whole ovine CN ( $3 \text{ mg}\cdot\text{mL}^{-1}$  phosphate buffer  $0.1 \text{ mol}\cdot\text{L}^{-1}$ , pH 6.5) were incubated with plasmin and each of the vegetable, animal and microbial coagulants used in the cheese-making experiments for 0, 10, 20, 30 and 60 min at  $37^\circ\text{C}$ . Plasmin, chymosin and microbial coagulant were incubated at enzyme:substrate ratios of  $1.67 \times 10^{-3}$ ,  $4.72 \times 10^{-3}$  and  $4.72 \times 10^{-3}$  units/mg casein, respectively. The vegetable coagulant ratio was inferred from the laboratory trials performed for the cheese-making. In all cases the reaction was stopped by dilution at 1:1.5 with CE sample buffer, pH  $8.6 \pm 0.1$  (see below).

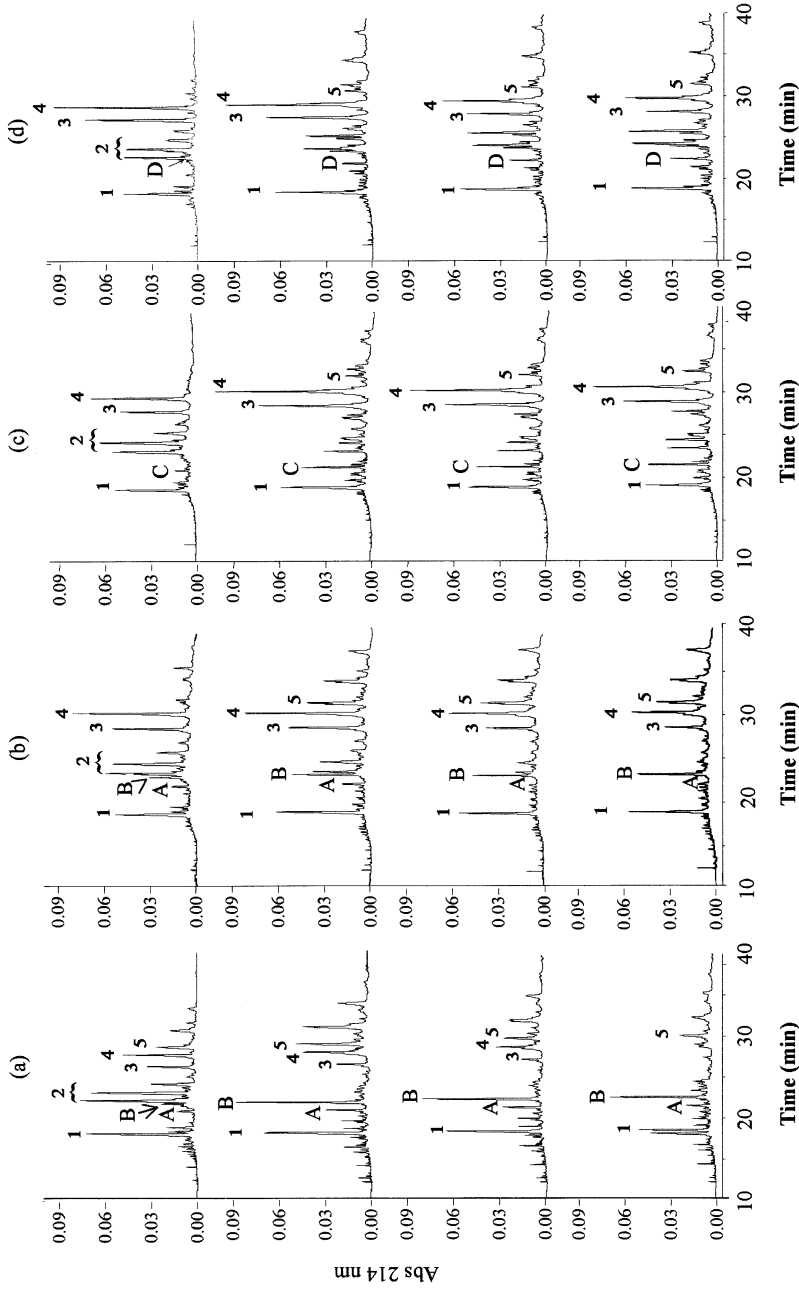
## 2.4. CZE separations

CE buffers and separation conditions were as previously described [10]. CZE was carried out in a Beckman apparatus P/ACE System 2050 controlled by a System Gold Software data system version 810 (Beckman Instruments Inc., San Ramon, CA 94583, USA). A hydrophilic-coated fused-silica capillary column (Select P1, length 0.57 m, effective length 0.50 m,  $50 \mu\text{m}$  i.d.: Supelco, Bellefonte, PA 16823, USA) was used. Prepared samples were filtered through a  $0.22 \mu\text{m}$  filter (Sterile Acrodisc, Gelman Sciences, Ann Arbor, MI 48106, USA) and injected at the anode. UV detection was at 214 nm. Each sample of the model systems was also analysed by CZE following the same conditions.

## 3. RESULTS AND DISCUSSION

### 3.1. Characterisation of Serpa cheese caseins by CZE. Comparison with cheeses made with animal or microbial coagulants

CZE electropherograms (e-grams) of caseins from samples of cheeses at 0, 30, 45 and 60 maturation days (Fig. 1) were



**Figure 1.** Electropherograms of caseins of: (a) artisanal Serpa cheese; (b) semi-industrial Serpa cheese; (c) animal rennet-coagulated cheeses; and (d) microbial-coagulated cheeses at: 0 (top); 30 (upper middle); 45 (lower middle); and 60 (bottom) days of maturation time. Peak identification: 1: para- $\kappa$ -casein; 2:  $\alpha_{S1}$ -casein; 3:  $\beta_1$ -casein; 4:  $\beta_2$ -casein; and 5:  $\alpha_{S2}$ -casein; A and B: characteristic peaks of cardoon-coagulated cheeses; C: characteristic peak of animal rennet-coagulated cheeses; and D: characteristic peak of microbial coagulant-coagulated cheeses.

obtained after a 40-min run for each sample injected. The different main caseins ( $\kappa$ -CN,  $\alpha_{s2}$ -CN,  $\alpha_{s1}$ -CN and  $\beta$ -CN) were identified on the basis of their migration times and order of migration, according to the CE data referred to in previous works [10, 15]. Different casein profiles with the different coagulant used for cheese-making can be observed, the differences being more evident during the maturation time (Fig. 1, lower middle and bottom e-grams). The higher degradation shown for cheeses coagulated with *Cynara cardunculus* L. (both artisanally and semi-industrially produced) (Fig. 1, a and b) was noteworthy compared with that of the other coagulants (animal or microbial) (Fig. 1, c and d), similar to the results reported for Los Pedroches cheese where a higher degree of proteolysis was observed when it was made with vegetable coagulant instead of animal rennet [4]. For example,  $\beta$ -casein was not detected after 60 d of maturation in cheeses coagulated with vegetable coagulant produced artisanally (Fig. 1a).

A higher extent of hydrolysis on the  $\alpha_{s1}$ -CN has been observed for Serpa cheese in comparison with animal rennet-coagulated or microbial-coagulated cheeses, as has previously been reported for other cheeses made with vegetable coagulant [4, 11].  $\alpha_{s1}$ -CN completely disappeared in Serpa cheese at 30 d in artisanal Serpa (Fig. 1a, upper middle) and at 60 d in semi-industrial Serpa (Fig. 1b, bottom). However, in animal rennet or microbial-coagulated cheeses it was not extensively degraded at 60 d of maturation (Fig. 1, c and d, bottom). Thus, the  $\alpha_{s1}$ -I-CN (peak 5;  $\alpha_{s1}$ -CN (f 24-199)) is considerably higher in Serpa cheese than in animal rennet or microbial-coagulated cheeses. Moreover, in artisanal Serpa, the  $\alpha_{s1}$ -I-CN is further hydrolysed, as a decrease in the peak 5 area was observed (data not shown) (Fig. 1, a and b) probably due to extra-susceptible bonds of  $\alpha_{s1}$ -I-CN to cardosins [13], whereas in the other cheeses (Fig. 1, c and d) the peak 5 area is maintained during maturation (data not shown).

### 3.2. Differentiation of vegetable coagulant from animal rennet and microbial coagulant in cheeses and model systems

From the e-grams of the cheese caseins, four unidentified peaks (A, B, C and D) were considered for differentiation of the three coagulants studied. Mean and standard deviation of the migration times and the corresponding corrected peak areas for these four relevant peaks are shown in Table I. Both Serpa cheeses (Fig. 1, a and b) presented peak A at mean migration times of  $22.3 \pm 0.3$  min and  $22.3 \pm 0.1$  min, respectively, which was degraded during maturation, and peak B at mean migration times of  $22.9 \pm 0.4$  and  $23.4 \pm 0.2$  min, respectively, (artisanal and semi-industrial) adjacent to  $\alpha_{s1}$ -CN, growing steeply during the ripening time and remaining prominent without further degradation, opposite to peak A. These peaks were not detected in cheeses coagulated with animal rennet and microbial coagulant, which leads us to suggest that both peaks are specific peptides from the action of the vegetable coagulant on the ovine caseins.

The study was focused on peak B, as the peptide responsible for it is not hydrolysed after its formation. Peak C was only detected on animal rennet-coagulated cheeses (Fig. 1c) and peak D was only detected on microbial-coagulated cheeses (Fig. 1d). Peaks C and D are possible markers for those coagulants; however, they were not considered in this study as good markers to differentiate the vegetable coagulant as they are not present in those cheeses coagulated by *Cynara* L.

The origin of peak B was elucidated from the model systems. It was only formed when the whole cheese casein was treated with *C. cardunculus* L. and not with plasmin, animal rennet or microbial coagulant (data not shown). Specifically, using the isolated caseins, it only appeared when  $\beta$ -casein was treated with *C. cardunculus* L. (Fig. 2). As shown in Figure 2c, after 30 min of incubation with this vegetable

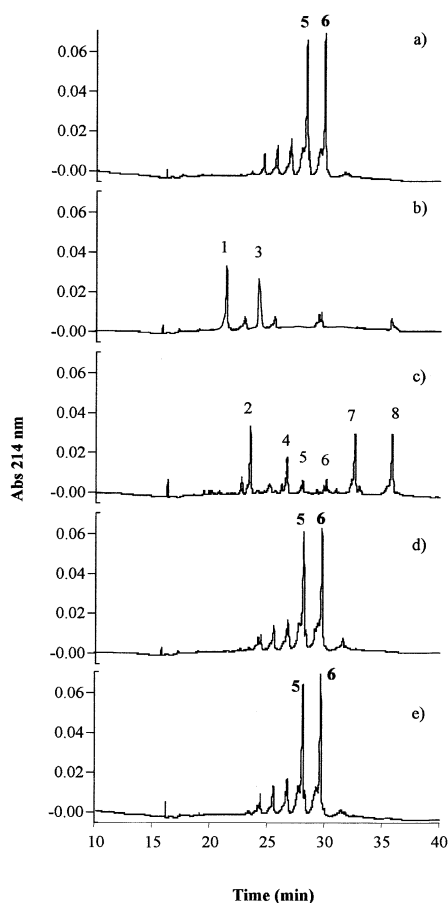
**Table I.** Migration times (MT) and corrected peak areas<sup>1</sup> (CorrA) for specific peaks from artisanal Serpa cheese, semi-industrial Serpa cheese, cheeses coagulated by animal rennet and cheeses coagulated by microbial coagulant at 0, 35, 45 and 60 d of ripening. Values are mean of three analyses (three different cheeses for each kind of them) and standard deviation is expressed in brackets.

| Type of coagulant | Cardoon (artisanal) |                |                 |                 | Cardoon (semi-industrial) |                |                 |                 | Animal          |                 | Microbial       |                |
|-------------------|---------------------|----------------|-----------------|-----------------|---------------------------|----------------|-----------------|-----------------|-----------------|-----------------|-----------------|----------------|
| Peak              | A                   |                | B               |                 | A                         |                | B               |                 | C               |                 | D               |                |
|                   | MT                  | CorrA          | MT              | CorrA           | MT                        | CorrA          | MT              | CorrA           | MT              | CorrA           | MT              | CorrA          |
| <b>0</b>          | 22.75<br>(0.35)     | 3.86<br>(0.57) | 23.45<br>(0.07) | 7.70<br>(0.28)  | 22.20<br>(0.00)           | 4.30<br>(1.35) | 23.60<br>(0.00) | 9.60<br>(0.10)  | 21.30<br>(0.10) | 2.50<br>(0.10)  | 21.03<br>(0.15) | 1.70<br>(0.10) |
| <b>30</b>         | 22.25<br>(0.07)     | 8.06<br>(0.66) | 22.85<br>(0.49) | 29.20<br>(1.84) | 22.20<br>(0.10)           | 3.73<br>(0.25) | 23.20<br>(0.00) | 14.76<br>(0.06) | 21.50<br>(0.00) | 12.67<br>(0.15) | 22.20<br>(0.00) | 3.97<br>(0.15) |
| <b>45</b>         | 22.25<br>(0.07)     | 7.50<br>(0.20) | 22.85<br>(0.49) | 26.80<br>(1.13) | 22.50<br>(0.26)           | 7.26<br>(1.21) | 23.40<br>(0.10) | 11.10<br>(0.36) | 21.53<br>(0.06) | 13.37<br>(0.25) | 22.30<br>(0.00) | 6.40<br>(0.00) |
| <b>60</b>         | 22.05<br>(0.07)     | 7.11<br>(0.54) | 22.60<br>(0.00) | 24.10<br>(0.28) | 22.20<br>(0.00)           | 7.5<br>(0.10)  | 23.26<br>(0.06) | 14.33<br>(0.95) | 21.6<br>(0.00)  | 11.86<br>(0.40) | 22.40<br>(0.00) | 8.53<br>(0.21) |
| <b>Mean</b>       | 22.33<br>(0.30)     |                | 22.94<br>(0.36) |                 | 22.28<br>(0.15)           |                | 23.37<br>(0.18) |                 | 21.48<br>(0.13) |                 | 21.98<br>(0.64) |                |

<sup>1</sup> Corrected peak area is the value obtained dividing the integrated peak area by migration time.

coagulant  $\beta$ -CN gave rise to four main degradation peaks. One of these peaks presented a similar migration time ( $22.5 \pm 0.1$ ) to the peak B found in Serpa cheeses. This peak was not seen when the  $\beta$ -CN was treated with plasmin (Fig. 2b), chymosin (Fig. 2d) or microbial coagulant (Fig. 2e). The small differences found in the peak B migration times between the model systems and cheese samples may be due to several facts, as the behaviour of caseins in model systems is not necessarily mirroring that occurring in cheese [3]. The different matrix of the cheese caseins versus the caseins in phosphate buffer of the model system could have originated the differences in the migration times, but also a small difference in the sequence of the fragment could be responsible for such differences. Two bonds in the amino acid sequence of  $\beta$ -CN have been reported to be susceptible to being cleaved by *Cynara cardunculus*: Leu<sup>127</sup>-Thr<sup>128</sup> and Leu<sup>190</sup>-Tyr<sup>191</sup> [21]. The latter is preferred, but this bond is also cleaved by chymosin, and

peak B did not appear in cheese other than Serpa cheese. Moreover, the fragment  $\beta$ -CN (f 1-190) contains various bonds susceptible to being cleaved, and peak B remained intact after 60 d of maturation. However, the bond Leu<sup>127</sup>-Thr<sup>128</sup> is also very susceptible to being degraded, but in this case only by *Cynara cardunculus* [21], producing the fragment  $\beta$ -CN (f 1-127), and no susceptible bonds have been described inside this sequence to *C. cardunculus* [22]. Although it has not been reported, from the sequence of  $\beta$ -CN, there is another Leu<sup>125</sup>-Thr<sup>126</sup> bond, which could also be cleaved, giving a peptide  $\beta$ -CN (f 1-125). It is possible that this bond could have been cleaved preferentially by *C. cardunculus* in the model system, originating a shift in the migration time in comparison with the cheese sample, as slight differences have been described for the behaviour of the same proteins depending on whether they are in model systems or in real samples [3]. In any case, the fragments  $\beta$ -CN (f 1-125) or  $\beta$ -CN (f 1-127) would



**Figure 2.** Electropherogram for ovine  $\beta$ -casein (a) untreated and incubated during 30 min with (b) plasmin; (c) *Cynara cardunculus* L.; (d) animal rennet; and (e) microbial coagulant. Peak identification: 1:  $\gamma_2$ -casein; 2: possible peak B; 3:  $\gamma_3$ -casein; 5:  $\beta_1$ -casein; 6:  $\beta_2$ -casein; and 4, 7 and 8: degradation products from the action of the vegetable coagulant on  $\beta$ -casein.

not be further hydrolysed during the ripening time, as happened with peak B in the cheese samples at different maturation times. However, further studies are needed to confirm the sequence of peak B.

This paper presents a rapid, straightforward method for the authentication of cheeses in which the use of *Cynara car-*

*dunculus* as coagulant is compulsory, that the bodies responsible for CDOs in Portugal have been demanding. These are, to our knowledge, the first results concerning the identification of the coagulant origin in cheese in relation to its authenticity. Other work [7] that also relates to the use of CZE and the type of coagulant used in ovine cheeses refers specifically to the use of two types of animal rennet (lamb, artisanal, and calf, commercial) and to their differences in terms of clotting activity. CZE of the caseins from ewe's milk cheeses made with different coagulants was shown to be appropriate for verifying the authenticity of PDO ovine cheeses coagulated with *Cynara cardunculus* L., through the presence of peak B, which seems to be specific for this coagulant and could be used as a marker. The results shown in this paper represent a valuable contribution from the CDO's point of view to the quality control of the cheeses referred to.

Further studies are required to identify the sequence of the peak B, but this first approach is promising for the authentication of vegetable coagulant used in cheese-makings and will contribute to detecting any forthcoming fraud. An additional experiment using bovine and caprine milk is also under consideration in order to broaden the applicability of the present study.

## ACKNOWLEDGEMENTS

Financial support via Project POCTI 35257/2000 and a Ph.D. fellowship to L.B. Roseiro, both from Fundação para a Ciência e Tecnologia, Ministério da Ciência e do Ensino Superior (FCT/MCES), are greatly appreciated. L.B. Roseiro is particularly grateful to Dr. M. Barbosa (Head of Dairy Section, INETI), and to Dr. M. Ramos for all the support and facilities provided by IFI-CSIC in Madrid.

## REFERENCES

- [1] Cattaneo T.M.P., Nigro F., Greppi G.F., Analysis of cow, goat and ewe milk mixtures



- by capillary zone electrophoresis (CZE): preliminary approach, *Milchwissenschaft* 51 (1996) 616–632.
- [2] Cattaneo T.M.P., Nigro F., Toppino P.M., Denti V., Characterization of ewe's milk by capillary zone electrophoresis, *J. Chromatogr. A* 721 (1996) 345–349.
- [3] Exterkate F.A., Lagerwerf F.M., Haverkamp J., van Schalkwijk S., The selectivity of chymosin action on  $\alpha_{S1}$ - and  $\beta$ -caseins in solution is modulated in cheese, *Int. Dairy J.* 7 (1997) 47–54.
- [4] Fernández-Salguero J., Sanjuán E., Influence of vegetable and animal rennet on proteolysis during ripening in ewe's milk cheese, *Food Chem.* 64 (1999) 177–183.
- [5] Frazier R.A., Ames J.M., Nursten H.E., The development and application of capillary electrophoresis methods for food analysis, *Electrophoresis* 20 (1999) 3156–3180.
- [6] Frazier R.A., Ames J.M., Nursten H.E., Capillary electrophoresis for food analysis method development, The Royal Society of Chemistry, Cambridge, UK, 2000.
- [7] Irigoyen A., Izco J.M., Ibáñez F.C., Torre P., Evaluation of the effect of rennet type on casein proteolysis in an ovine milk cheese by means of capillary electrophoresis, *J. Chromatogr. A* 881 (1996) 59–67.
- [8] Molina E., Ramos M., Cifuentes A., López-Fandiño R., Characterization of cheese proteolysis by capillary electrophoresis and reverse-phase HPLC analyses of peptides, *Z. Lebensm.-Unters. Forsch. A* 206 (1998) 259–263.
- [9] Molina E., Martín-Álvarez P.J., Ramos M., Analysis of cows', ewe's and goats' milk mixtures by capillary electrophoresis: quantification by multivariate regression analysis, *Int. Dairy J.* 9 (1999) 99–105.
- [10] Molina E., Frutos M. de, Ramos M., Capillary electrophoresis characterization of the casein fraction of cheeses made from cows', ewe's and goats' milks, *J. Dairy Res.* 67 (2000) 209–216.
- [11] Núñez M., Fernández del Pozo B., Rodríguez-Martín M., Gaya P., Medina M., Effect of vegetable and animal rennet on chemical, microbiological, rheological and sensorial characteristics of La Serena cheeses, *J. Dairy Res.* 58 (1991) 511–519.
- [12] Otte J., Ardö Y., Weimer B., Sørensen J., Capillary electrophoresis used to measure proteolysis in cheese, *Bull. Int. Dairy Fed.* 337 (1999) 10–16.
- [13] Queiroz Macedo I., Faro C.J., Pires E.M., Caseinolytic specificity of cardosin, an aspartic protease from the cardoon *Cynara cardunculus* L.: action on bovine  $\alpha_S$ - and  $\beta$ -casein and comparison with chymosin, *J. Agric. Food Chem.* 44 (1996) 42–47.
- [14] Recio I., Amigo L., López-Fandiño R., Assessment of the quality of dairy products by capillary electrophoresis of milk proteins, *J. Chromatogr. B* 697 (1997) 231–242.
- [15] Recio I., Amigo L., Ramos M., López-Fandiño R., Application of capillary electrophoresis to the study of proteolysis of caseins, *J. Dairy Res.* 64 (1997) 221–230.
- [16] Recio I., Ramos M., López-Fandiño R., Capillary electrophoresis for the analysis of food proteins of animal origin, *Electrophoresis* 22 (2001) 1489–1502.
- [17] Regulamento (CEE) No. 2081/92 do Conselho de 14 de Julho de 1992. *Journal das Comunidades Europeias* No. L 208 de 24 de Julho de 1992, 1–8.
- [18] Roseiro M.L.B., Ewe's milk cheese-making in Portugal using a vegetable rennet (a review), *Sheep Dairy News* 8 (1991) 74–75.
- [19] Roseiro M.L.B., Barbosa M., "Portuguese Artisanal Cheeses", in: *The European Commission (Ed.), Artisanal European Cheeses, Directorate-General XII Science, Research and Development, Brussels, Belgium, 1996, 79–92.*
- [20] Roseiro M.L.B., García-Risco M., Barbosa M., Ames J.M., Wilbey A., Evaluation of Serpa cheese proteolysis by nitrogen content and capillary zone electrophoresis, *Int. J. Dairy Tech.* 56 (2003) 99–104.
- [21] Sousa M.J., Malcata F.X., Identification of peptides from ovine milk cheese manufactured with animal rennet or extracts of *Cynara cardunculus* as coagulant, *J. Agric. Food Chem.* 46 (1998) 4034–4041.
- [22] Sousa M.J., Malcata F.X., Advances in the role of a plant coagulant (*Cynara cardunculus*) in vitro and during ripening of cheeses from several milk species, *Lait* 82 (2002) 151–170.