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Review

Assessment of the quality of dairy products by capillary electrophoresis of milk proteins

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Abstract

This paper presents an overview of existing capillary electrophoretic methods for the study of milk proteins. The main methods of analysis of caseins, whey proteins and peptides are examined with particular attention to their application to the evaluation of the quality of dairy products. Aspects such as the study of protein polymorphism, evaluation of heat treatments, detection of adulteration and assessment of proteolysis are considered in detail. © 1997 Elsevier Science B.V.

Keywords: Dairy products; Proteins; Caseins

Contents

1. Introduction

Milk proteins are probably the best characterized of all food proteins. However, the existence of genetic and non-genetic polymorphism, as well as the application of technological treatments complicate their quantitative determination. Modifications such as heat denaturation or proteolysis, common in the manufacture of many dairy products, give rise to complex, insoluble, new compounds and smaller peptides and amino acids whose analysis is not easily performed. In addition, information on the occurrence and amount of a particular protein or derived compound is extremely useful in the assessment of processing and adulterations [1].

Analysis of milk proteins has been carried out using classical gel electrophoretic methods, isoelectric focusing and ion-exchange, hydrophobic interaction or reversed-phase HPLC among others. Each method has its own merits, but the advent of CE has resulted in the development of rapid and automated analyses with very high resolutions, which require

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very small sample and buffer volumes with a significantly reduced amount of solvent waste [2].

Over the past few years, literature reviews on the applications of CE to food analysis have been published [3–6]. This paper deals with CE separations of milk proteins with special attention to the application of these methods to the evaluation of the quality of dairy products.

2. Analysis of milk proteins and protein polymorphism

Milk proteins are readily separated into casein and whey protein fractions. In addition to the heterogeneity of these groups, genetic and non-genetic polymorphism (owing to post-translational modifications, such as phosphorylation, glycosylation and limited proteolysis) of milk proteins have been described [7]. Milk protein polymorphism has been related to production traits, composition and technological properties of milk. Therefore, rapid methods of screening and quantification of milk proteins and their variants are necessary for milk quality control and the recent developments in CE represent an important technical advance (Table 1).

The major milk proteins – α_S-casein, β-casein, α-lactalbumin (α-La) and β-lactoglobulin (β-Lg) – have been separated in an untreated fused-silica capillary, using phosphate buffer (pH 7.0), containing 4 *M* urea [8], or a highly concentrated borate buffer (pH 10.0) [9] to dissolve casein aggregates.

A better resolution was achieved by de Jong et al. [10] who obtained theoretical plate numbers in the range of 300 000 to 700 000, together with satisfactory migration time (<0.08%) and peak area (2–4%) repeatabilities. In this method, the adsorption of proteins to the capillary wall was avoided to a great extent by using a hydrophilic coated capillary and a low pH phosphate buffer (pH 2.5) containing a modified cellulose, as running electrolyte. Casein micelles were disrupted by the use of 6 *M* urea and a reducing agent in the sample buffer, and further aggregation was prevented by the inclusion of 6 *M* urea in the running buffer. This method allowed the simultaneous determination of whey proteins and caseins, including some genetic and non-genetic variants of bovine [10] and ovine [11] milk proteins, provided that the substitution involved a change in the net charge of the protein at low pH. Increasing the pH of the running buffer might allow the separation of genetic variants differing in acidic amino acid residues, such as the A and B variants of β-Lg and κ-casein and the B and C variants of α_{S1}-casein, but a slight change in pH from 2.45 to 2.5 was reported to decrease the theoretical plate number by approximately 20% [10].

This method was later modified to minimize protein absorption by optimizing the sample and running buffer [12] and was applied to the analysis of genetic variants of milk proteins from different species [13]. Fig. 1 shows the electropherograms of individual bovine, ovine and caprine milk samples containing different phenotypes of β-Lg, α_{S1}-, α_{S2}-, β- and κ-casein. The different casein fractions of ovine and caprine milks, previously isolated by anion-exchange FPLC, were identified, including ovine α_{S1}-casein D (also known as Welsh variant). Separation of the ovine β-Lg variants A, B and C was also achieved. In cows' milk, this method allowed the identification of the rare α_{S2}-casein variant D and β-casein variant C.

Regarding whey proteins, several CE methods have been used to separate, and in some cases quantify, the four major components – immunoglobulins (Ig), α-La, β-Lg and blood serum albumin (BSA) – either individually or as part of standard protein mixtures [14–20], but only the studies which included all four proteins as components of milk or whey samples will be described here (Table 1). In the next section, particular attention will be paid to the methods that have been applied to the evaluation of heat treatments.

Separation of the major whey proteins – α-La, β-Lg A and B variants, BSA and IgG – was assayed using uncoated fused-silica capillaries and high pH running buffers in combination with different organic modifiers, such as Tween 20, to modulate the electroosmotic flow and to avoid protein–wall interactions, thereby enhancing protein resolution [21,22]. However, no repeatability or quantitative data were reported and, therefore, the extent of protein adsorption on the capillary wall cannot be evaluated. The method of Paterson et al. [21] was used to phenotype β-Lg in Jersey cows and to estimate the

Table 1
Main capillary electrophoretic methods for the analysis of milk proteins

Proteins	CE mode	Quantitative analysis	Conditions	Applications	Ref.
α-La, β-Lg A and B, α_s-CN and β-CN	CZE	Peak areas	Fused-silica capillary RB: 0.5 *M* phosphate buffers (pH 6.0–9.0) with and without 4 *M* urea SB:PBS (pH 7.0) (standards). Milk injected without sample treatment	β-CN/α-La ratio to detect adulteration of fresh milk with milk powder	[8]
α-La, β-Lg, α_s-CN and β-CN	CZE	No quantitative results	Fused-silica capillary RB: 250 m*M* borate buffer (pH 10) SB: PBS (pH 7.0) (standards). Milk injected without sample treatment	Qualitative analysis of milk and egg proteins	[9]
Major casein fractions and hydrolysis products	CZE	No quantitative results	Fused-silica capillary RB: 0.1 *M* phosphate buffer, 4 *M* urea (pH 7.3) SB: 0.1 *M* phosphate buffer, 7 *M* urea, 10 m*M* dithioerythritol	Monitoring of casein hydrolysis	[52]
α-La, β-Lg A and B, BSA and major casein fractions	CZE	No quantitative results	Neutral hydrophilic coated capillary RB: 10 m*M* sodium phosphate buffer 0.05% MHEC, 6 *M* urea, (pH 2.5) and 10 m*M* sodium citrate buffer, 0.05% MHEC, 6 *M* urea (pH 2.45) SB: 5 m*M* sodium citrate, 5 m*M* dithiothreitol, 6 *M* urea (pH 8.0)	Separation of cow's, goat's and ewe's milk proteins and genetic variants. Heat-damaged caseins Qualitative analysisof cow's, goat's, ewe's and human milk. Analysis of soy proteins	[10] [45]
α-La, β-Lg A and B, and major casein fractions of ewe's milk	CZE	No quantitative results	Neutral hydrophilic coated capillary RB: 20 m*M* sodium citrate buffer 0.05% MHEC, 6 *M* urea, (pH 3.0) SB: 5 m*M* sodium citrate, 30 m*M* dithiothreitol, 9 *M* urea	Qualitative analysis of ewe's milk proteins and some genetic variants	[11]
α-La, β-Lg, BSA and major casein fractions	CZE	Area repeatability: <4% Sensitivity: 0.5 mg/100 ml α-La 6.5 mg/100 ml β-Lg Recovery: 89–107% α-La, β-Lg and BSA	Neutral hydrophilic coated capillary RB: 20 m*M* sodium citrate buffer 0.05% MHEC, 6 *M* urea, (pH 3.0) SB: 167 m*M* TRIS, 42 m*M* MOPS, 67 m*M* EDTA, 17 m*M* dithiothreitol, 10 *M* urea (pH 8.0)	Measurement of low levels of heat denatured BSA, α-La and β-Lg in the presence of a large excess of caseins Identification of casein fractions and genetic variants in cow's, ewe's and goat's milk Identification of proteolysis products from caseins	[12] [13] [53]
α-La, β-Lg A, B and C and BSA	CZE	No quantitative results	Fused-silica capillary RB: 50 m*M* MES buffer (pH 8.0) with 0.1% Tween 20. Acid whey injected directly	Qualitatitave analysis of β-Lg variants in milk from New Zealand Jersey cows Identification of β-Lg C and effect of β-Lg polymorphism on β-Lg content	[21] [23]

(*Cont.*)

Table 1. Continued

Proteins	CE mode	Quantitative analysis	Conditions	Applications	Ref.
α-La, β-Lg A and B, BSA and IgG	SDS–CGE	Standard curve for each individual whey protein	Fused-silica capillary RB: ProSort SDS–Protein Analysis Kit (Applied BioSystems) SB: 2% SDS, 5% 2-mercaptoethanol	Quantitative analysis of whey samples and whey protein concentrates. Comparison with other analytical methods	[32]
α-La, β-Lg A and B, BSA and IgG	CZE	No quantitative results	Fused-silica capillary RB: 150 m*M* sodium borate (pH 8.5) with 0.05% Tween 20 SB: 10 m*M* phosphate (pH 7.4)	Qualitative analysis of major whey proteins	[22]
α-La, β-Lg and BSA	CGE (replaceable gels)	Area repeatability: 1.9–6.5% Sensitivity: 2.7 mg/100 ml β-Lg 2.1 mg/100 ml α-La	Cross-linked polyacrylamide coated capillary RB: 40 m*M* Tris, 40 m*M* borate, 0.1% SDS, 10% (w/v) PEG 8000. Acid whey injected directly	Quantitative analysis of the major whey proteins in raw and UHT milks. Comparison with HPLC	[24]
α-La, β-Lg and BSA	CGE (bonded gels)	No quantitative results	Cross-linked polyacrylamide filled capillary RB: 20 m*M* phosphate buffer (pH 8.0) Dialysed whey injected directly	Qualitative analysis of whey proteins in real whey samples. Comparison with SDS–PEG buffer.	[29]
α-La, β-Lg A and B and BSA	CZE	Area repeatability: 2.14–5.23% Sensitivity: 0.4 mg/100 ml β-Lg B 0.4 mg/100 ml β-Lg A 0.3 mg/100 ml α-Lg B	Fused-silica capillary RB: 100 m*M* borate buffer (pH 8.2) with 30 m*M* sodium sulfate Acid whey injected directly	Evaluation of the denaturation of the major whey proteins in milks subjected to different thermal treatments	[25]
				Study of β-Lg polymorphism in several bovine breeds and its influence on the β-Lg content	[26]
				Quantification of whey proteins during the storage of UHT milks. Data compared with HPLC results. Identification of the origin of proteolysis products	[31]
α-La, β-Lg A and B, BSA and IgG	CZE	Area repeatability: 0.7–1.3% Sensitivity: 0.6 mg/100 ml α-La 0.11 mg/100 ml β-Lg. Recovery: 0.94–1.12 α-La and β-Lg	Fused-silica capillary RB: 70 m*M* phosphate buffer (pH 2.5) Whey samples diluted with RB	Quantitative analysis of real whey samples. Caseinmacropeptide was identified	[41]

BSA, blood serum albumin; CGE, capillary gel electrophoresis; CZE, capillary zone electrophoresis; EDTA, ethylenediamine tetraacetic acid disodium salt; MES, 2-(*N*-morpholino)ethanesulfonic acid; MHEC, methylhydroxyethylcellulose; MOPS, 3-*N*-(morpholino)propanesulfonic acid; PEG, polyethylene glycol; RB, running buffer; SB, sample buffer; SDS, sodium dodecyl sulfate; Tris, Tris(hydroxymethyl)aminomethane; Tween 20, (20)-sorbitan monolaurate.

effect of the β-Lg variant on the concentration of β-Lg in milk [23].

Quantitative analyses of whey proteins were performed in uncoated fused-silica capillaries using polymeric additives in the running buffer [24] and a high ionic strength and high pH separation buffer

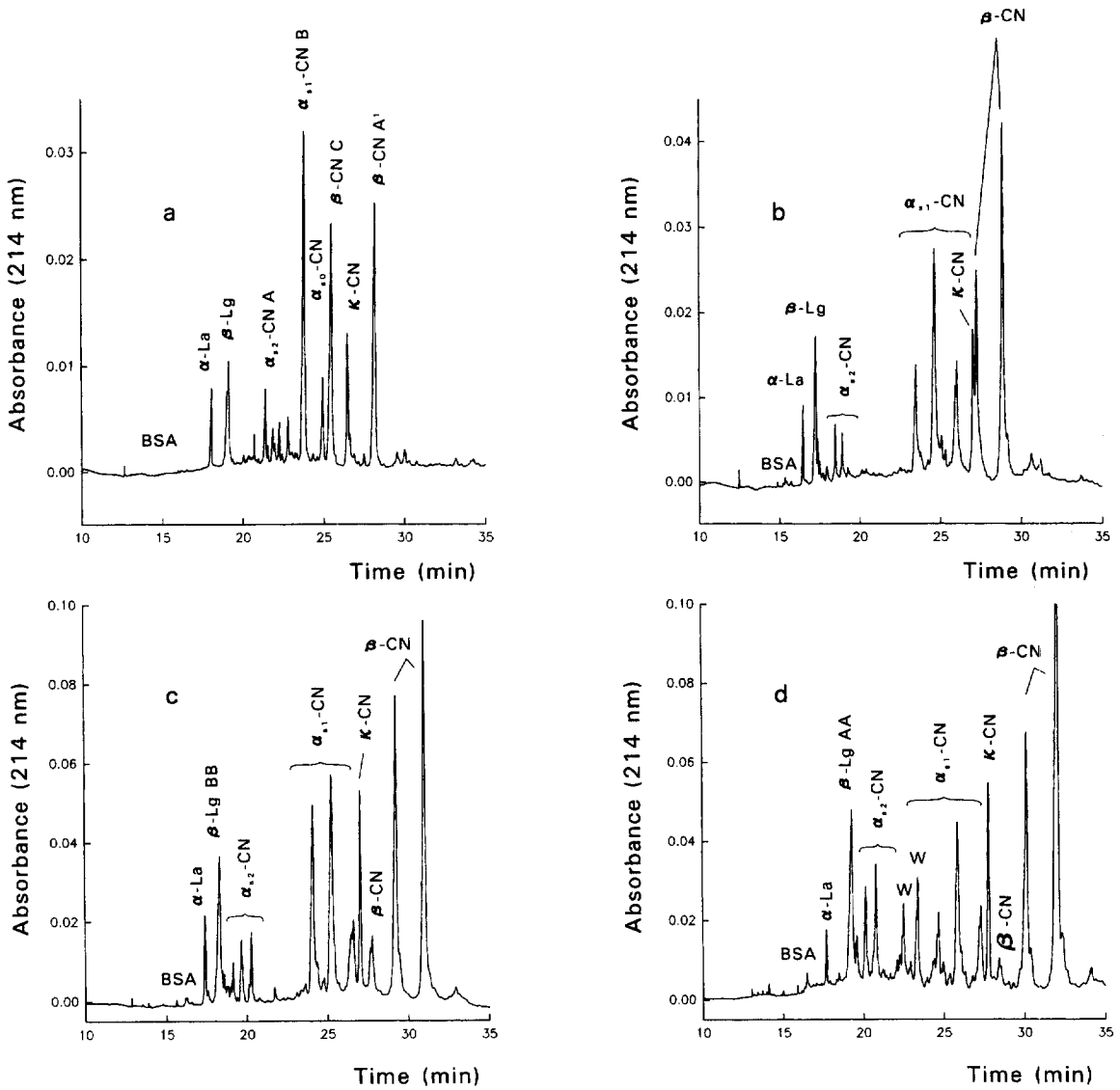

Fig. 1. Electropherograms of samples of whole cow's, ewe's and goat's milk containing different genetic variants of the milk proteins: (a) cow's milk, (b) goat's milk, (c) ewe's milk, (d) ewe's milk containing the α_{s1}-casein variant Welsh (marked as W). α-La, α-lactalbumin; β-Lg, β-lactoglobulin; CN, casein. Hydrophilic coated fused-silica capillary 57 cm×50 μm (50 cm to the detection point); temperature, 45°C; injection, 10–15 s; linear voltage gradient of 0–25 kV in 3 min, followed by a constant voltage of 25 kV; running electrolyte: 6 *M* urea, 0.32 *M* citric acid, 20 m*M* sodium citrate, 0.05% MHEC (pH 3.0). CE was carried out using a Bekman P/ACE System 2050 (Beckman Instruments, San Ramon, CA, USA). Reprinted from Ref. [13] with kind permission of Elsevier Science.

[25], which reduced adsorption of proteins to the capillary wall. Both procedures have been applied to the assessment of the heat treatment undergone by milk and will be mentioned in the next section. In addition, de Frutos et al. [26] employed one of these methods [25] for the study of bovine β-Lg polymorphism and its influence on the total whey protein content of milk.

Otte et al. [27], using a fused-silica capillary and a 70 m*M* phosphate running buffer (pH 2.5), achieved

the separation of the major whey proteins in 8 min of total analysis time. However, the use of a low pH running buffer precluded the separation of the two genetic variants of β-Lg. Different aspects of the quantitative determination were considered, including full recovery of reference β-Lg and α-La. Recovery assays are essential to guarantee that protein–capillary wall interactions are avoided [6,28].

Separation of whey proteins in non-denaturing polyacrylamide gel-filled capillaries was also attempted [29], but presented the limitations of poor migration time and injection repeatability. Low injection repeatability is due to the need to inject the sample by electromigration.

3. Evaluation of the heat treatment

In recent years, a few CE methods for the evaluation of the heat treatment of milk have been described in the literature. Denaturation of whey proteins is a good indicator of the thermal damage undergone by milk. The heat load can be estimated by the quantitative analysis of native serum proteins, which remain soluble at pH 4.6 after the heat treatment and the International Dairy Federation has proposed the content of undenatured β-Lg as a basis for the heat classification of different types of processed milks [30].

Cifuentes et al. [24] reported the quantitative analysis of the major whey proteins (β-Lg, α-La and BSA) in standard mixtures and real whey samples of raw and UHT milks. The concentration of proteins, as determined by CE, agreed with the data obtained by HPLC, except for the values of β-Lg in UHT milk, which were underestimated using CE.

Recio et al. [25] quantified the whey proteins in cow's milk subjected to different thermal processes (pasteurization, direct and indirect UHT treatments). Changes in the amounts of α-La and β-Lg were studied by different methods, during storage of direct and indirect UHT-treated milks [31]. Comparison between the data obtained by RP-HPLC and CE provided similar values at the beginning of the storage period, although the content of β-Lg, as measured by CE, was slightly higher than that determined by HPLC. However, the RP-HPLC analyses of α-La and β-Lg in stored milks revealed changes in the shape of the peaks, probably owing to the progress of the Maillard reaction and to protein aggregation during the storage period, which could result in an overestimation of the content of β-Lg in long-term-stored milks. The α-La and β-Lg contents, as determined by CE, only experienced small variations, caused by proteolysis products migrating close to the protein peaks, which hampered a more accurate quantification.

Although it has been reported that the reproducibility of the quantitative results obtained by CE for α-La and β-Lg was as good as that for HPLC [32], it is generally accepted that HPLC provides better reproducibility than CE, even when the normalized peak areas (peak area divided by migration time) relative to an internal standard are used [33,34].

Recently, a direct method to measure heat-denatured whey proteins in the casein fraction of heat-treated milks was published [12]. The electrophoretic conditions of de Jong et al. [10] were optimized in order to achieve quantitative results. Citrate buffer at pH 3.0 was used as running buffer together with a sample buffer at pH 8.6, that was able to dissociate the heat-denatured serum proteins bound to the casein micelles. Repeatabilities of 9.2%, 4.4% and 2.2% were obtained for heat-denatured BSA, α-La and β-Lg, respectively, and the recovery values were between 90–117% for the three whey proteins. This method allowed a more accurate assessment of the pasteurization processes than procedures based upon measurements of the native whey proteins. However, the unavoidable presence of small amounts of whey proteins, even in the casein fraction of raw milks, determine the detection limit.

On heating milk, lactose reacts with the side amino group of lysine in a Maillard condensation yielding lactulosyl–lysine. Hydrolysis of this product gives furosine (e-*N*-2-furoylmethyl–L-lysine), whose quantification is an indicator of the heat load, as well as a measure of blocked and thus unavailable lysine. Owing to the distinct influence of moisture and heating conditions in early Maillard reactions, the furosine content of milk can provide additional information on milk quality such as the addition of reconstituted milk powders to liquid milk [35]. Tirelli and Pellegrino [36] developed a CE method in

an untreated fused-silica capillary which allowed the detection of furosine at 280 nm with a migration time of 6.5 min. A previous step was required to vacuum dry the HCl used in the hydrolysis of ε-deoxy-fructosyl–lysine to yield quantifiable furosine. When compared with the existent ion-pair reversed-phase HPLC method, CE was found to have similar repeatability (R.S.D.<3%) but it underestimated low levels of furosine, in the range of those typical for pasteurized milk or cheese.

Corradini et al. [37] decreased the lower detection limit to 0.4 μg ml^{-1} by employing a pH 2.5 running buffer containing the additive 60 m*M* *N,N,N′,N′*-tetramethyl-1,3-butanediamine, which prevented interactions of furosine with the capillary wall and reverted the electrosmotic flow. The use of 2-amine-4-picoline as internal standard aided quantification. However, these authors used mainly pure furosine and only reported the analysis of one sample of dried milk.

Maillard reaction products have also been analysed by CE on uncoated capillaries with or without derivatization and detection at different wavelengths [38,39]. According to these authors, CE afforded a quicker and better resolution than HPLC of the complex reaction products present in model Maillard systems, which, in many cases are neither clearly polar nor distinctly hydrophobic.

4. Detection of adulterations

Determination of the fraudulent addition of rennet whey solids to dairy products is usually based upon detection and quantification of caseinomacropeptide (CMP). CMP is the hydrophilic fragment 106–169 of κ-casein (mono or diphosphorylated and glycosylated to various degrees) released by chymosin during milk clotting. CMP is a specific component of rennet whey, which should be absent from milk, however, the activity of certain bacterial proteinases, that progressively split κ-casein in milk, might give rise to degradation products similar to CMP, as for instance pseudo-CMP (fragment 107–169 of κ-casein), leading to false positive results [40]. In addition, the investigation of CMP is gaining considerable interest because of its recently found functional, nutritional and physiological properties and because it is a way of following the enzymatic hydrolysis of κ-casein that leads to milk coagulation.

Otte et al. [41] achieved the separation of non-glycosylated CMP and other 3 minor components, free from interference of whey proteins, using an uncoated capillary in combination with 40 m*M* phosphate buffer (pH 2.5).

Van Riel and Olieman [42] increased the resolving power over the above mentioned method, from 0.1×10^6 to 0.5×10^6 plate numbers/m, using a hydrophilic coated capillary in combination with 6 *M* urea and methylhydroxyethyl cellulose in citrate buffer at pH 3.0. This method could not discriminate between the two genetic variants of CMP, but allowed the separation of mono and diphosphorylated non-glycosylated forms of CMP, as well as some glycosylated forms and afforded a detection limit of 0.4% of rennet whey solids. In addition, CMP and pseudo-CMP (CMP lacking the N-terminal Met_{106} residue) could be adequately separated. This can prevent false positive results in buttermilk powder, since bacteria commonly used as starters in this cultured product can split κ-casein at position 106–107 [43].

However, it has recently been found [44] that the proteolytic activity of psychrotrophic proteinases in raw and UHT milk leads to the appearance of CMP, as well as to pseudo-CMP and an unidentified third peak, when using the CE method of van Riel and Olieman [42] (Fig. 2). Although this might give false positive results for the presence of rennet whey in the case of milks manufactured from raw materials of poor microbiological quality, low area ratios of pseudo-CMP to CMP can allow the presence of rennet whey solids to be suspected. In addition, the electropherograms provide an assessment of the degree of proteolytic degradation, pointing to bad storage conditions, or a poor bacterial quality of the milk used.

The methods that allow a simultaneous analysis of caseins and whey proteins [12] could be used for the determination of the serum protein to casein ratio and therefore, detect the addition of acid whey solids to dairy products.

It has been found that the processing practices involved in the manufacture of milk powders lead to dramatic changes in the CE patterns of caseins, including alterations in peak shapes, disappearance

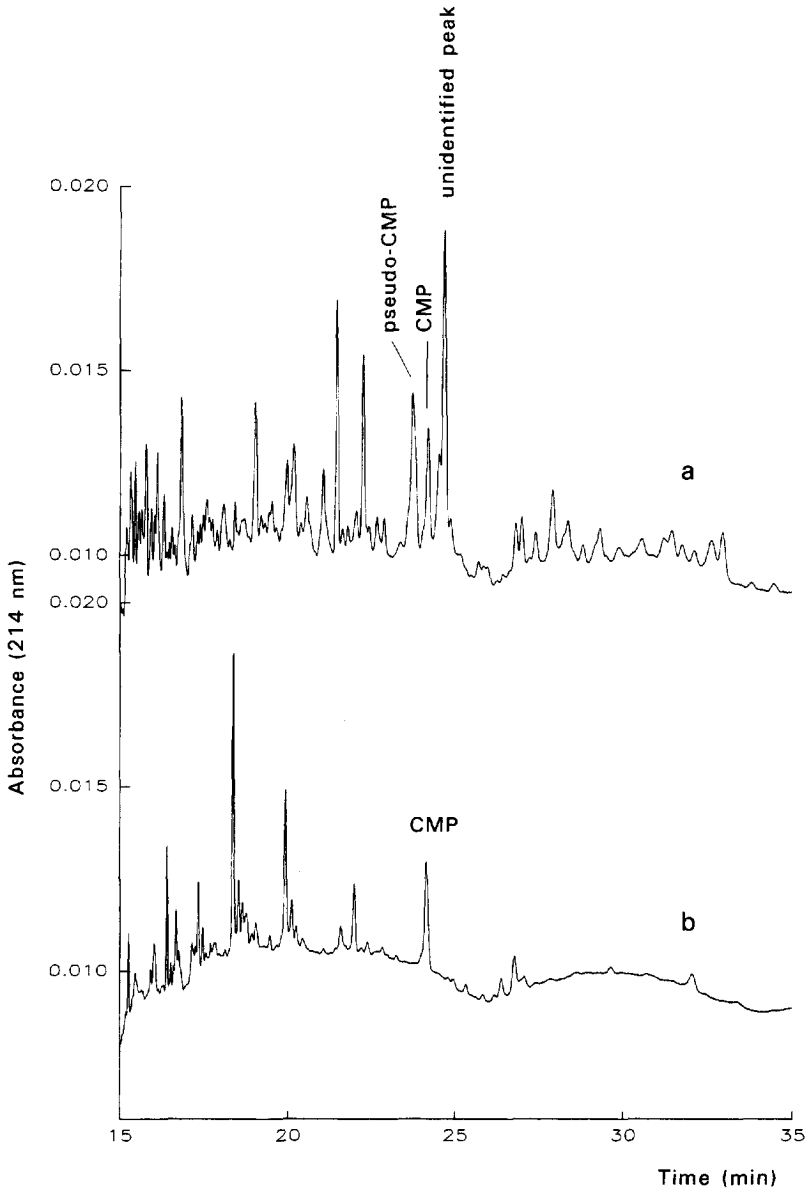

Fig. 2. Electropherograms of (a) raw milk inoculated with *Pseudomonas fluorescens* at bacterial count of $1 \cdot 10^7$ cfu ml^{-1}, after 130 h of incubation at 6°C, (b) sample of skim milk powder containing 5% (w/w) rennet whey powder. Hydrophilic coated fused-silica capillary 37 cm×50 mm (30 cm to the detection point); temperature, 45°C; injection, 60 s; linear current gradient of 0–50 μA in 10 min, followed by a constant current of 50 μA; running electrolyte: 6 *M* urea, 0.14 *M* citric acid, 10 m*M* sodium citrate, 0.05% MHEC (pH 3.0). CE equipment as in Fig. 1. Reprinted with permission from Ref. [44], copyright 1996, American Chemical Society.

of α_{S1}-casein and appearance of new peaks [10] and it has been suggested that this modifications could be used to detect the adulteration of fresh milk with milk powder [8,10].

Adulterations of dairy products with soya or milk from different species could potentially be detected on the basis that different CE patterns were obtained for soya proteins, cows', goats', ewes' and human

milks [45]. In fact, Tienstra et al. [46] reported the detection of 1% of cow's milk in ewe's milk.

5. Proteolysis in milk or cheese

In addition to other well known advantages, the application of CE to the study of proteolysis permits the simultaneous determination of changes in casein hydrolysis and the appearance of degradation products without limitations in molecular size. This technique is being increasingly used, specially for following proteolyis during cheese ripening [47–51].

Kristiansen et al. [52] used an uncoated capillary with 100 m*M* phosphate buffer containing urea at pH 7.3, to follow the hydrolysis of purified α_S- and β-casein with chymosin in buffer at pH 6.2. The lower molecular mass peptides soluble at pH 4.6 and 70% ethanol, that resulted from the hydrolysis, were also analysed in the absence of urea in the running buffer. The authors concluded that the method could be useful for monitoring proteolysis in cheese, but no real samples were analysed nor was the identification of the degradation products undertaken.

The CE method developed by Recio et al. [25], using an uncoated capillary at alkaline pH, was also applied to the study of the peptides soluble at pH 4.6, produced by heat resistant enzymes that often cause proteolysis during the storage of long life products, thus impairing their quality [31]. With this procedure it was possible to distinguish those peptides derived from the action of plasmin (milk native protease), from those produced by proteinases of psychrotrophic bacteria in UHT milk, while allowing a simultaneous determination of the whey proteins (Fig. 3). However, as previously mentioned, high levels of proteolysis products could impair the quantification of low levels of β-Lg.

The CE method of Recio and Olieman [12], using a hydrophilic-coated capillary and a low pH buffer containing urea, was used to follow the proteolytic action of plasmin and chymosin on isolated casein

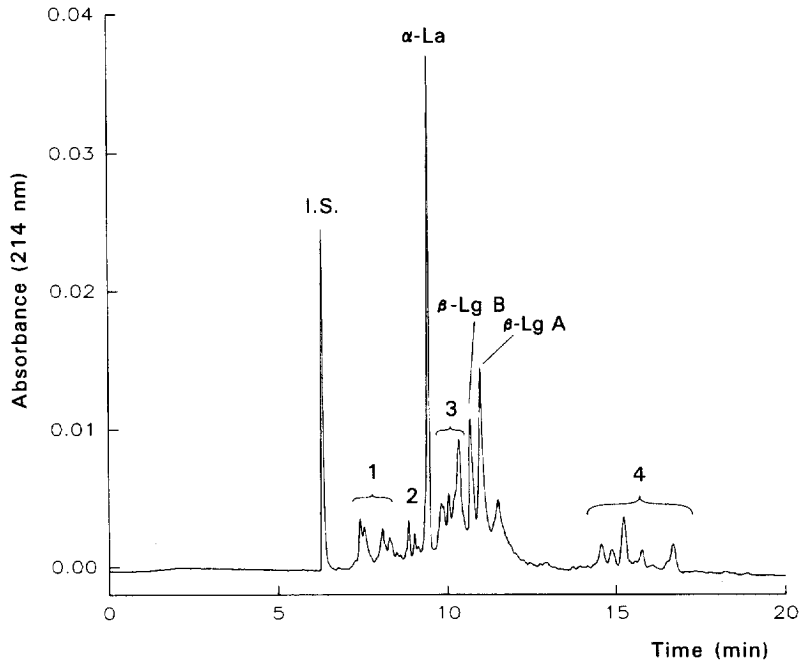

Fig. 3. Electropherogram of a sample of whole direct UHT milk kept for 30 days at 22°C after the heat treatment. Peak identities: I.S., internal standard (Lys–Trp–Lys); 1 and 4, peaks from the action of bacterial proteases on caseins; 2 and 3, peaks from the action of plasmin on caseins. Fused-silica capillary 37 cm×75 μm (30 cm to the detection point); temperature, 25°C; injection, 2 s; linear voltage gradient of 0–7 kV in 1 min, followed by a constant current of 7 μA; running electrolyte: 100 m*M* borate (pH 8.2) with 30 m*M* sodium sulfate added. CE equipment as in Fig. 1. Reprinted with permission from Ref. [31], copyright 1996, American Chemical Society.

fractions, whole casein and individual milk samples [53]. Several of the main casein breakdown products were identified, including γ_1-, γ_2- and γ_3-casein arising from different genetic variants of β-casein, as well as α_{S1}-I-casein, α_{S1}-casein f(1–23), *para*-κ-casein and CMP produced by chymosin action on α_{S1}-CN and κ-CN respectively. The identification of the degradation products arising from different enzymes made this method suitable to study proteolysis in milk and cheese, although the quantification of individual proteins has not been carried out yet.

CE has been extensively used for high sensitivity peptide mapping. Compared with RP-HPLC, CE presents the advantage of providing rapid and very efficient separations, from much smaller amounts of protein, down to the nanogram level. This is essential for the characterization and identification of many proteins of interest which are often isolated in extremely small quantities. Nevertheless, one disadvantage of CE over RP-HPLC is that the peptide peaks cannot be easily collected and used for subsequent analyses. However, since the mechanisms of separation are different, both techniques provide complementary information about the sample [54].

Separations of as little as 80 [55] and 14 fmol of β-casein tryptic digest [56] are possible by CE, with reported R.S.D. values of peak retention or migration times lower than 1% [55]. Using trypsin immobilized in capillaries it was possible to digest as little as 50 ng of protein [55]. The advantage of enzyme-modified capillaries lies in that they can be coupled directly to the separation capillary enabling on-line protein digestion and separation [56].

In general, good separations from enzymatic digests of casein have been achieved at neutral or basic pH with uncoated capillaries, with no noticeable loss of sample due to adsorption of peptides onto the surface [55,57–59]. In addition to conventional UV absorbance detection, laser-induced fluorescence detection following an arginine-selective derivatization reaction [55] and indirect detection methods, in which the displacement of an additive such as salicylate [60] or quinine [61] results in negative flourescence peaks, have also been used.

Several CE methods for the separation of amino acids have been developed. Since most mixtures of amino acids include neutral, acidic and basic components, the best results have been obtained using CE with coated capillaries or micellar electrokinetic capillary chromatography [62]. Pre- and post-column derivatization, together with different detection systems, have been attempted with satisfactory results. However, to the best of our knowledge, an application involving milk and dairy products has not been reported as yet.

6. Conclusions and future prospects

CE has proven to be a valuable technique for milk protein analysis and quality control of dairy products. For instance, the detection of adulterations with rennet whey has now become easier owing to the CE separation of CMP and pseudo-CMP, which is very difficult to achieve by the use of other analytical techniques. Similarly, this technique allows the simultaneous analysis of peptides of any size and solubility, many of which would not be retained or stained in ordinary polyacrylamide gels. However, although quantitative results have been obtained in the analysis of milk proteins, further efforts are necessary to provide reliable quantitative data and validated methods. In addition, peak identification, which is commonly performed by coinjection with standards, remains a problem in CE.

The different separation mechanism of CE with respect to HPLC makes these techniques complementary tools in a research laboratory. However, progress in capillary pretreatment procedures and permanent coatings is at the same level as advances in chromatography decades ago. Therefore, more work is required to further develop new separation modes for capillary electrophoresis, such as capillary isoelectric focusing, SDS-capillary gel electrophoresis, etc and new detection methods that could aid identification and improve the detection limits [63] (CE–mass spectrometry, laser-induced fluorescence detection, electrochemical detection, laser light scattering...) so as to achieve ranges similar to those reached by HPLC.

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