Electrophoresis 2006, *27*, 452–460

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Received May 9, 2005
Revised September 14, 2005
Accepted September 15, 2005

Research Article

Development of a capillary electrophoresis method for the determination of soybean proteins in soybean–rice gluten-free dietary products

CE has been applied for the first time to the simultaneous separation of soybean and rice proteins. Treated and untreated capillaries with different effective lengths as well as separation media at different pHs were tested. For that purpose, samples and standard solutions were prepared in 25:75 ACN–water media containing 0.3% v/v acetic acid. The use of an untreated capillary of 50 cm effective length together with an 80 mM borate buffer (pH 8.5) modified with 20% v/v ACN and UV detection at 254 nm were the conditions working the best. These conditions enabled the determination of soybean proteins in gluten-free dietary commercial products elaborated with soybean protein and/or soybean flour and rice flour using the standard additions calibration method. The method was linear up to 26 mg/mL of soybean proteins, the precision (expressed as RSD) was always better than 6%, and recoveries obtained for soybean proteins when spiking commercial products were very close to 100%.

Keywords: Bakery products / Capillary electrophoresis / Gluten-free dietary products / Rice / Soybean proteins
DOI 10.1002/elps.200500355

1 Introduction

Cereal grains are considered basic foods and have been widely used for the elaboration of human foods and for animal feeding throughout the world [1–5]. Cereals provide most caloric energy and much of the amount of proteins needed by human beings.

Cereal proteins have been traditionally classified in four fractions on the basis of their solubility [6]: (i) albumins, water soluble proteins; (ii) globulins, salt soluble proteins; (iii) prolamins, alcohol soluble proteins; and (iv) glutelins, acid or base soluble proteins. Based on their different solubility, cereal proteins have been sequentially extracted and analyzed [7–9]. Prolamins and glutelins, also called storage proteins, are the main proteins in cereals and, as previously indicated, they are not soluble in water. Moreover, cereal proteins are also characterized by having an amino acid profile limited in lysine and with a high content in sulfur amino acids.

The most employed cereals in human feeding are wheat and rice, although barley, rye, oat, and maize are also important [4, 5]. Some of these cereals (wheat, rye, and barley) are not suitable for celiac people and, therefore, alternative products containing other kinds of cereals such as rice have been developed [5].

Rice is the most important cereal in terms of people depending on it. Its content in proteins is about 7–9%, although there are some varieties with higher protein contents (up to 14%). As for the other cereals, rice proteins are deficient in lysine but have the advantage of being highly digestible [3, 10].

Soybean protein is extensively employed for the supplementation of cereals in bakery products [11]. Soybean protein amino acid profile (rich in lysine, limited in sulfur amino acids) fits nicely with grain proteins (limited in lysine, rich in sulfur amino acids) resulting in a product with a higher nutritional quality than the individual components [12]. There are three kinds of proteins in soybean: (i) proteins involved in metabolism, (ii) structural proteins,

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Abbreviation: SPI, soybean protein isolate

and (iii) storage proteins or globulins. This last group of proteins is present in soybean at a higher concentration. Unlike prolamins and glutelins (main proteins in cereals), globulins are sparingly soluble in water and soluble in salt solutions [12–14].

The addition of soybean to rice products in order to enhance its nutritional quality has provoked the need for analytical methods enabling the characterization and quality control of these products. Moreover, since some soybean proteins are potentially allergenic, this method would also be very useful for soybean-intolerant people.

Although the individual separation of soybean and rice proteins has previously been reported by chromatographic and electrophoretic techniques [1, 2, 8, 15–18], there is no analytical methodology enabling the simultaneous separation of these two groups of vegetable proteins. Only recently, our research group has proposed an analytical methodology for the separation of soybean and cereal (wheat, rice, and maize) proteins in binary mixtures by perfusion RP-HPLC. This method has also been applied to the determination of the soybean protein content in cereal-based products [19, 20]. However, there is no reference in the literature dealing with the simultaneous separation of soybean and rice proteins by CE. The use of CE for the analysis of proteins is significantly increasing due to different reasons, which makes it advantageous over other separation techniques usually employed for the analysis of proteins such as HPLC. In fact, the amount of sample needed for a CE analysis used to be very low in comparison with conventional HPLC. Moreover, the separation conditions used in CE are usually more compatible with proteins than those used in RP-HPLC in which very low pHs and high concentrations of organic modifiers are needed for the separation of proteins. These facts together with the high resolution, low analysis times, and low cost have contributed to the increasing use of this technique for protein separations.

Therefore, the aim of this work has been the development of a CE method enabling, for the first time, the simultaneous separation of soybean and rice proteins and demonstrating its applicability to the analysis of soybean–rice gluten-free dietary products.

2 Material and methods

2.1 Chemicals and samples

All reagents employed for the preparation of the separation buffers were of analytical grade. Boric acid was from Fluka (Buchs, Switzerland), sodium dihydrogen phosphate dihydrate was purchased from Merck (Darmstadt, Germany), sodium hydroxide, hydrochloric acid, and glacial acetic acid were supplied from Panreac (Barcelona, Spain), and HPLC-grade ACN was purchased from Scharlau (Barcelona, Spain). All solutions were prepared with ultrapure water from a Milli-Q system (Millipore, Bedford, MA, USA).

A soybean protein isolate (SPI) from ICN (Aurora, OH, USA) (protein content by Kjeldahl analysis, 89.1%) was used as standard of soybean proteins. Moreover, a soybean flour from El Granero (Madrid, Spain) (protein content by Kjeldahl analysis, 48.9%), a standard of rice proteins from Ferrer Alimentación (Barcelona, Spain) (purity 70%), a rice flour from El Granero, and standards of casein and egg albumin (ovoalbumin + conalbumin) from Sigma (Madrid, Spain) was also employed. The commercial gluten-free bakery products analyzed were purchased from local markets in Alcalá de Henares (Madrid, Spain). A rice biscuit (S, spiked with 2% SPI) was employed to evaluate the accuracy of the developed analytical method. The samples analyzed were three soybean–rice biscuits (M1, M2, and M3) and four soybean–rice breads (M4, M5, M6, and M7). All these products were prepared with rice flour and contained soybean proteins from one or two different sources. Products M1, M5, M6, and M7 were prepared with SPI, product M4 with soybean flour, and products M2 and M3 contained both SPI and soybean flour. Moreover, product M1 contained milk and products M2 and M4 were prepared with eggs. Moisture in SPI (6.34%) and samples was determined by drying 2 g of product at 130°C to constant weight [21].

2.2 Apparatus

All experiments were performed on an HP^{3D}CE system (Agilent Technologies, Waldbronn, Germany) equipped with an on-column diode array detector (DAD). The HP^{3D}CE ChemStation software was used for instrument control and data acquisition. Separations were performed on untreated fused-silica capillaries of 75 μm ID and 375 μm OD with a total length of 58.5 cm and an effective length of 50 cm from Composite Metal Services (Worcester, England, UK). For preliminary experiments, a treated fused-silica capillary (permanently coated with a hydrophilic polymer) from Agilent Technologies of 75 μm ID (375 μm OD) with an effective length of 50 cm (total length of 58.5 cm) as well as untreated fused-silica capillaries from Composite Metal Services of the same ID and OD with effective lengths of 50 cm (58.5 cm) and 25 cm (33.5 cm) were also employed. Capillary temperature was 25°C and UV detection was performed at 200 nm and at 254 nm with bandwidths of 10 nm and a

response time of 0.1 s. A 654 pH meter from Metrohm (Herisau, Switzerland) was used to adjust the pH of the separation buffers.

2.3 Procedure

The treated capillary was conditioned with the buffer solution (1 bar) for 15 min before first use and for 5 min between injections. Conditioning of untreated capillary consisted of flushing with 1 M NaOH (1 bar) for 15 min and with water (1 bar) for another 15 min before first use. Between sample injections, untreated capillaries were conditioned with 0.1 M NaOH (1 bar) for 2 min, Milli-Q water (1 bar) for 2 min, and, finally, with the separation buffer (1 bar) for 5 min. Injection of samples was made by injecting the sample itself by pressure (50 mbar for 4 s) followed by the injection of buffer solution (50 mbar for 4 s), and the applied voltage was 15 kV. Separation buffer solution was prepared by dissolving the appropriate amount of boric acid in Milli-Q water, adjusting the pH value at 8.5 (by addition of 1 M NaOH), and adjusting the final volume to achieve a 100 mM solution. Then, ACN was added to the buffer solution in a proportion of 20:80 v/v obtaining a 80 mM borate buffer with 20% ACN (apparent pH, 9.2). Buffer solutions were filtered through 0.45 μm pore-size nylon filter membranes from Sugelabor (Madrid, Spain) prior to use. Standard solutions were prepared by dissolving the proteins in ACN:water (25:75 v/v) containing 0.3% v/v acetic acid to achieve the desired concentration. These solutions were shaken manually for 2 min, and then centrifuged at $3362 \times g$ for 5 min at 25°C before injection in the CE system. Samples were grounded with an automatic miller and proteins were extracted with ACN:water (25:75 v/v) containing 0.3% v/v acetic acid. Resulting solutions (200 mg/mL) were centrifuged at $3362 \times g$ and injected in the CE system.

2.4 Data treatment

Corrected peak areas (A_c) and corrected migration times (t_c) were used to compensate fluctuations in electrophoretic conditions and to obtain a good reproducibility of data [22]. A_c was calculated dividing the peak area (A) by the corresponding migration time (t) and t_c was calculated dividing the migration time (t) by the migration time of the EOF (t_{EOF}), *i.e.*, $A_c = A/t$ and $t_c = t/t_{EOF}$. LODs ($3s_a/b$) and LOQs ($10s_a/b$) were determined from the standard error of the intercept (s_a) and the slope (b) of the calibration curve obtained by analysis of variance (ANOVA) [23]. Experimental data analysis and parameters were calculated using Excel Microsoft XP® and Origin 7.0. Graphs with different electropherograms were composed in Origin 7.0.

2.5 Quantitative analysis

Quantitative analysis was performed using the external standard and the standard additions calibration methods. The external calibration was carried out by injecting triplicate five standard solutions of SPI with concentrations ranging from 4.40 to 21.10 mg/mL. The standard additions calibration was performed by injecting triplicate five solutions containing 200 mg/mL of sample where 0.00, 1.70, 3.70, 5.10, and 6.80 mg/mL of SPI were added. Solutions containing known concentrations of SPI were individually prepared in 25:75 v/v ACN–water containing 0.3% v/v acetic acid to obtain the calibration curve. Peak integration was made by setting the baseline from valley to valley.

3 Results and discussion

3.1 Development of a CE method determination of soybean proteins in soybean–rice protein mixtures

In order to develop a method for the determination of soybean proteins in commercial products containing soybean and rice proteins, some preliminary experiments were performed. These experiments involved the use of treated and untreated capillaries and buffers at different pH values. In all cases, the injection of soybean and rice proteins was performed using as sample solvent a 25:75 v/v ACN–water mixture containing 0.3% v/v acetic acid. These conditions were optimized in a previous work and enabled the simultaneous solubilization of soybean proteins (water-soluble) and rice proteins (mostly insoluble in water) [19]. The separation media consisted of 80 mM phosphate buffer at different pH values (2.0, 2.5, and 7.0) and 80 mM borate buffer at pH 8.5, all of them modified with 20% v/v ACN. The addition of this organic modifier at this percentage was found to be adequate for the separation of cereal proteins by CE, particularly, for rice proteins [18, 24, 25]. All these first experiments were made using UV-absorption detection at 200 nm (peptide bond absorption) in order to avoid the missing of any peak that could be interesting for the purpose of this work.

When using treated capillaries, buffers at pH values of 2.5 and 7.0 were employed since capillary specifications did not recommend the use of pHs higher than 8.0. Figure 1 shows the electropherograms obtained when solutions of soybean flour (A) and rice proteins (B) were injected under these conditions using different capillary lengths. Those peaks from the soybean flour, which mainly appeared in the electropherogram of the SPI (not shown in this figure), are marked with an arrow. The electropherograms (a) in Figs. 1A and B correspond to the use of a

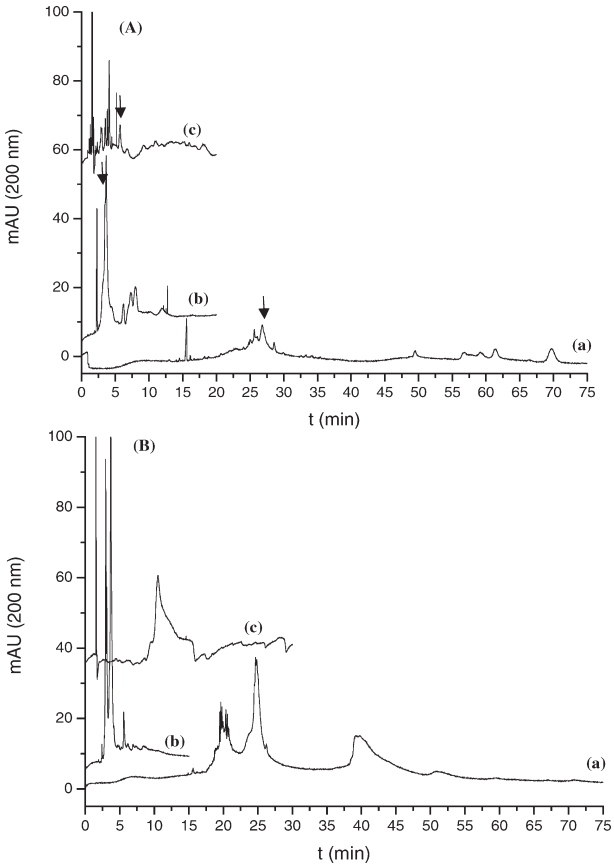

Figure 1. Electropherograms corresponding to the injection of solutions of soybean flour (40 mg/mL) (A) and rice proteins (40 mg/mL) (B) in a coated capillary with 75 μm ID (375 μm OD) and 58.5 cm total length when the separation was performed (a) in an effective length of 50 cm with 80 mM phosphate buffer at pH 2.5 containing 20% ACN at 15 kV (~75 μA), (b) in an effective length of 8.5 cm with 80 mM phosphate buffer at pH 2.5 containing 20% ACN at −15 kV (~−75 μA), and (c) in an effective length of 8.5 cm with 80 mM phosphate buffer at pH 7.0 containing 20% ACN at −15 kV (~−105 μA). Other experimental conditions: injection, 50 mbar × 3 s. UV detection at 200 ± 5 nm. Arrows indicate peaks corresponding to soybean proteins.

capillary with an effective length of 50 cm and a pH of 2.5. An analysis time close to 70 min was obtained for the soybean flour while rice proteins appeared before 45 min. In addition, a group of peaks corresponding to soybean proteins and appearing between 25 and 30 min overlapped with peaks corresponding to rice proteins. These conditions were also used for the injection of soybean and rice proteins in a capillary with an effective length of 8.5 cm. As expected, the electropherograms (b) in Figs. 1A and B showed that peaks corresponding to soybean proteins overlapped with those of rice proteins, although the analysis time was shorter than that observed previously with the longer capillary. In order to observe the effect of the pH on the separation, a separation medium at pH 7.0 while using a capillary with an effective length of 8.5 cm (electropherograms (c) in Figs. 1A and B) was used. The increase in the pH from 2.5 to 7.0 resulted in a more crowded electropherogram for soybean proteins and enabled the separation of soybean and rice proteins in an acceptable analysis time (20 min). This indicated the convenience of using neutral pH values to achieve the separation of soybean–rice protein mixtures with this treated capillary instead of acid pH values, which were the most usually employed to separate cereal proteins by CE as shown in the literature [15].

When untreated capillaries were employed, separation buffers at low or high pH were tried in order to avoid protein adsorption on the capillary wall. Thus, 80 mM phosphate buffers at pH 2.0 and 2.5 and a 80 mM borate buffer at pH 8.5, all of them modified with 20% v/v ACN, were used. Figure 2 shows the electropherograms obtained when solutions of soybean flour (A) and of rice proteins (B) were injected under these conditions using different capillary lengths. The electropherograms (a) in Figs. 2A and B correspond to the use of a capillary effective length of 50 cm and a pH of 2.5. Although in this case, soybean protein peaks did not overlap with those of rice proteins, the analysis time for this separation was very high (75 min). In comparison with the results obtained under the same conditions when using the coated capillary (Fig. 1a), the peak profile for soybean proteins was similar in both capillaries although a better separation and peak shape for peaks appearing at higher migration times was observed when using the untreated capillary. Very similar results could be observed in the case of rice proteins. The reduction of the effective capillary length while keeping the acidic conditions resulted in lower analysis times (see electropherograms (b) and (c) in Figs. 2A and B) as it was observed for the coated capillary. Moreover, these results were compared with those obtained when working at basic conditions (borate buffer at pH 8.5) (see electropherogram (d) in Figs. 2A and B). The use of these basic conditions resulted in a more crowded electropherogram for soybean proteins and, in comparison with previous electropherograms, provided the best separation of soybean and rice proteins in a shorter analysis time. Then, these conditions were chosen to investigate the possibility of determining soybean proteins in commercial foods. In addition, the use of an untreated capillary would decrease the cost of the analysis.

In order to prove the applicability of the selected method for the determination of soybean proteins in soybean–rice gluten-free dietary products, the injection of different ingredients that can also be present in these bakery

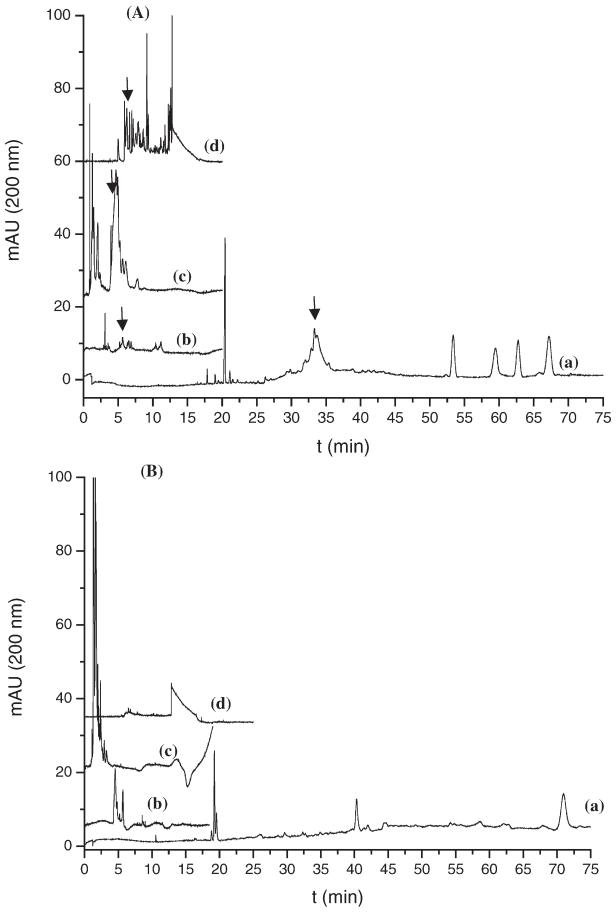

Figure 2. Electropherograms corresponding to the injection of solutions of soybean flour (40 mg/mL) (A) and rice protein (40 mg/mL) (B) in an uncoated capillary with 75 μm ID (375 μm OD) and 58.5 or 33.5 cm total length when the separation was performed (a) in an effective length of 50 cm with 80 mM phosphate buffer at pH 2.5 containing 20% ACN at 15 kV (~75 μA), (b) in an effective length of 25 cm (in this case an uncoated capillary with 50 μm ID (375 μm OD) was used) and with 80 mM phosphate buffer at pH 2.0 containing 20% ACN at 12 kV (~174 μA), (c) in an effective length of 8.5 cm (in the uncoated capillary with 50 μm ID) with 80 mM phosphate buffer at pH 2.0 containing 20% ACN at −12 kV (~−174 μA), and (d) in an effective length of 50 cm with 80 mM borate buffer at pH 8.5 containing 20% ACN at 15 kV (~15 μA). Other experimental conditions as in Fig. 1. Arrows indicate peaks corresponding to soybean proteins.

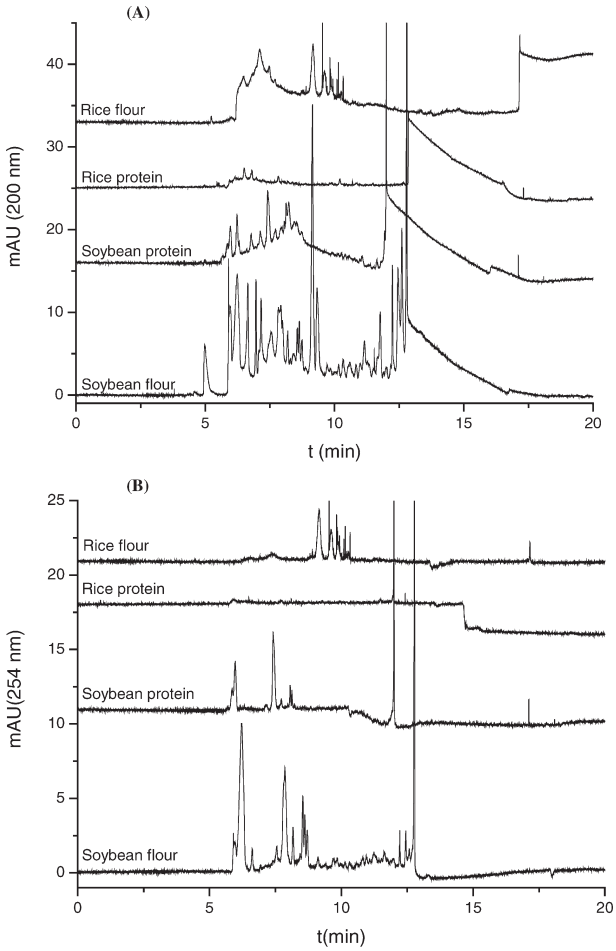

Figure 3. Electropherograms corresponding to 40 mg/mL soybean flour, 40 mg/mL soybean proteins (SPI), 40 mg/mL rice proteins, and 15 mg/mL rice flour recorded at two different wavelengths: 200 nm (A) and 254 nm (B). Separation conditions: 80 mM borate buffer at pH 8.5 containing 20% ACN, uncoated capillary with 75 μm ID (375 μm OD) and 58.5 cm total length and 50 cm effective length, and injection by pressure of 50 mbar during 4 s of sample solution followed by 50 mbar during 4 s of separation buffer. Other experimental conditions as in Fig. 1.

products was achieved under the selected conditions. In addition to soybean flour, these bakery products can contain SPI whose electropherogram appears in Fig. 3 together with the electropherograms corresponding to soybean flour, rice protein, and rice flour (main source of rice protein in bakery products). In addition, this figure shows the different selectivity observed when detection was performed at 254 nm (Fig. 3B), wavelength usually employed for the detection of soybean proteins, instead of 200 nm (Fig. 3A). In general, electropherograms obtained at 254 nm were less crowded than those recorded at 200 nm, which looked very complex. Consequently, peaks corresponding to soybean protein and soybean flour did not overlap with those corresponding to rice flour and rice protein (that did not practically show any signal) when the detection was performed at 254 nm. Regarding sensitivity, detection at 254 nm (mainly due to the absorption of the amino acid phenylalanine) was, as expected, less sensitive than at 200 nm (due to the absorption of peptide bonds).

Since some of the selected products presented other protein sources such as egg and milk, and in order to ensure that these proteins do not interfere in the detection of soybean proteins, the method was applied to the separation of standards of milk proteins (caseins) and egg proteins (egg albumins: ovoalbumin + conalbumin). No interference from peaks corresponding to caseins and egg albumins was observed when detection was performed at 254 nm, being this the wavelength selected for the detection of soybean proteins in soybean–rice gluten-free dietary products.

In order to check whether this peak could be clearly detected in commercial gluten-free commercial products containing rice flour, a control sample without soybean proteins (product S) was injected and fortified with SPI (Fig. 4A). The addition of SPI to this control product could be clearly detected by a peak at ~10 min (neighboring peaks have been indicated with lines to facilitate the identification of the soybean protein peak). These results show the applicability of the developed method to detect the presence of soybean proteins in rice dietary products. It is interesting to highlight that the migration time of the selected peak increased depending on the complexity of the mixture and on the concentration of soybean proteins. This fact may be attributed to the complexity of the sample matrix and the existence of interactions between soybean and rice proteins as reported for other cereals such as wheat. Regarding this, the 11S globulin fraction of soybean proteins can form different complexes with gluten proteins of wheat [26]. Furthermore, this also shows the extreme complexity and interaction characteristics of cereal proteins reported in the literature [1].

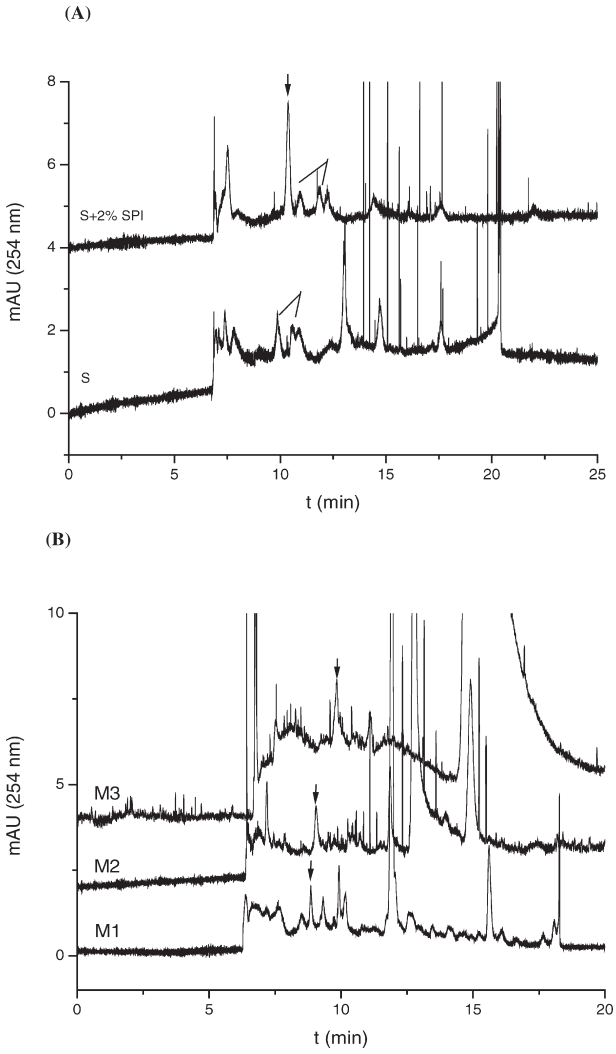

Figure 4. Electropherograms corresponding to a standard sample (commercial product S containing rice flour in its composition, 200 mg/mL) nonspiked and spiked with SPI (A) and to three commercial gluten-free dietary products containing soybean protein and/or soybean flour and rice flour (at 200 mg/mL each) (B). Arrows mark the peak corresponding to soybean proteins. Experimental conditions as in Fig. 3.

3.2 Analytical characteristics of the CE method

The possibilities of the CE method, developed in this work, to quantitate soybean proteins in commercial dietary products elaborated with soybean protein and rice flour were investigated using SPI as standard of soybean proteins. The parameters evaluated were the linearity of the calibration plot, detection and quantitation limits, existence of matrix interferences, precision, and accuracy. Table 1 groups the results obtained in the determination of all these parameters.

Good linear correlation coefficients ($r^2 > 0.99$) were observed between the area of the peak of SPI chosen to quantitate soybean proteins and the concentration of soybean proteins up to ~26 mg/mL of soybean proteins. The concentration of soybean proteins in the SPI solutions was calculated taking into account the percentage of protein of SPI (89.1%) and its moisture (6.34%). Moreover, the linear model was successfully validated in the working concentration range (from 4.40 to 21.10 mg/mL) by means of the analysis of the residuals and the variance.

The detection limit for soybean proteins was 0.36 mg/mL, which means that the method enabled the detection of a percentage of 0.18% w/w of soybean proteins in a soybean–rice commercial product (related to 2 g of initial product). The quantitation limit for soybean proteins was 1.22 mg/mL, which means that the method enabled the quantitation of 0.61% w/w of soybean proteins in a soybean–rice product (related to 2 g of initial product).

Table 1. Characteristics of the CE method for the analysis of soybean proteins in soybean–rice gluten-free dietary products

Linearity		Up to 26 mg/mL of soybean protein
Detection limit[a)]		0.36 mg/mL of soybean protein (0.18% w/w)
Quantitation limit[a)]		1.22 mg/mL of soybean protein (0.61% w/w)
Existence of matrix interferences[b)]		
–Slope by the external standard method (n = 2)		0.26 ± 0.01
–Slope by the standard additions method (n = 2)		0.30 ± 0.01
Precision		
Repeatability (n = 8)[c)]		
M1 (200 mg/mL)	A_c, RSD in %	5.65
	t_c, RSD in %	1.87
Internal reproducibility (n = 5)[d)]		
M1 (200 mg/mL)	A_c, RSD in %	2.39
	t_c, RSD in %	1.37
Intermediate precision (n = 5)[e)]		
M1 (200 mg/mL)	A_c, RSD in %	5.23
	t_c, RSD in %	0.80
Recovery[f)] (%)		
2.1 mg/mL of soybean protein added (0.85%)		94
4.3 mg/mL of soybean protein added (1.91%)		105
6.4 mg/Ml of soybean protein added (2.98%)		102
Mean value		100 ± 6

a) LODs and LOQs in percentage w/w were determined related to 2 g of sample.
b) An *F*-test for the comparison of variances and a *t*-test for the comparison of slopes were employed.
c) Number of consecutive injections of a solution of a soybean–rice biscuit (sample M1).
d) Number of individual solutions of a soybean–rice biscuit (sample M1) with the same concentration injected on the same day.
e) Number of days in which five individual solutions of a soybean–rice biscuit (sample M1) with the same concentration were injected.
f) Recovery obtained for soybean proteins when different amounts of SPI (expressed as mg/mL of soybean protein and soybean protein content in%) were added to the solution of a rice gluten-free dietary product (S, 200 mg/mL) spiked with a 2% SPI (total SPI content in the sample corresponding to 2.85, 3.91, and 4.98%).

The slopes of the calibration plots obtained by the external standard and the standard additions methods were compared in order to detect the existence of matrix interferences. The comparison of these slopes by *t*- and *F*-tests showed the existence of statistically significant differences and, therefore, the existence of matrix interferences. As a consequence, the standard additions method was used to quantitate soybean proteins in the seven commercial products analyzed.

Precision of the method was determined by the evaluation of the repeatability, internal reproducibility, and intermediate precision (see Table 1). Repeatability was calculated by injecting eight consecutive times a solution of 200 mg/mL of soybean–rice biscuits (sample M1), obtaining an RSD value of 5.65% in A_c and of 1.87% in t_c. Internal reproducibility was determined by injecting five individual solutions of 200 mg/mL of soybean–rice biscuits (sample M1) on the same day. The RSD values were 2.39% for A_c and 1.37% for t_c. Intermediate precision was evaluated by injecting five individual solutions of 200 mg/mL of soybean–rice biscuits (sample M1) on five different days. RSD values of 5.23% for A_c and 0.80% for t_c were obtained.

Accuracy of the method was determined as the recovery obtained for soybean proteins when different amounts of SPI (corresponding to 0.85, 1.91, and 2.98% related to 200 mg/mL of product) were added to solutions obtained from a commercial product elaborated with rice flour and spiked with a 2% of SPI (total content in soybean proteins corresponding to 2.85, 3.91, and 4.98%). As observed in Table 1, recoveries ranged from 94 to 105%.

Table 2. Analysis of commercial soybean–rice gluten-free dietary products by the CE method

Sample	% Soybean protein related to the product as is basis	% Soybean protein related to the dry product[a)]
M1 (soybean–rice biscuits)	1.34 ± 0.06 (n = 2)	1.55 ± 0.02 (n = 2)
M2 (soybean–rice biscuits)	2.34 ± 0.04 (n = 2)	2.52 ± 0.04 (n = 2)
M3 (soybean–rice biscuits)	6.38 ± 0.18 (n = 2)	6.96 ± 0.19 (n = 2)
M4 (soybean–rice bread)	1.46 ± 0.01 (n = 2)	1.59 ± 0.01 (n = 2)
M5 (soybean–rice bread)	1.59 ± 0.11 (n = 2)	2.52 ± 0.17 (n = 2)
M6 (soybean–rice bread)	<LOQ (~0.3)[b)]	<LOQ (~0.5)[b)]
M7 (soybean–rice bread)	<LOQ (~0.3)[b)]	<LOQ (~0.4)[b)]

a) Corrected by the moisture.
b) Below the LOQ of the CE method developed.

3.3 Quantitative analysis of soybean proteins in commercial gluten-free dietary products containing soybean and rice flour

The developed analytical method was applied to the detection of soybean proteins in seven commercial products containing soybean proteins and/or soybean flour and rice flour. Figure 4B shows the electropherograms corresponding to different soybean–rice gluten-free dietary products studied in this work. It can be observed that the peak corresponding to soybean proteins was clearly detected in all cases.

The optimized method was also applied to the determination of the soybean protein content in these products. Table 2 shows the results obtained. Soybean protein contents ranged from 1.34 to 6.38%, observing lower values of soybean proteins in breads than in biscuits. In fact, the soybean protein content was below the quantitation limit of the developed method for two soybean–rice breads. Moreover, one soybean–rice bread and one soybean–rice biscuit of the same brand, although from different lots, were also analyzed by a chromatographic method also developed by our research team [20]. The soybean protein contents determined in a different lot of the soybean–rice bread M4 and of the soybean–rice biscuit M2 were 2.19 ± 0.31 mg/100 mg (as is basis) and 1.50 ± 0.26 mg/100 mg (as is basis), respectively. The differences found between the values observed for these products by both methods could be attributed to lot-to-lot variations.

4 Concluding remarks

For the first time soybean and rice proteins have been simultaneously separated by CE. Very simple experimental conditions consisting of the use of an untreated capillary, borate buffer at basic pH, and UV-absorption detection at 254 nm enabled the detection of soybean proteins in soybean–rice mixtures in an analysis time close to 15 min. This method has been applied to the quantitation of soybean proteins in commercial gluten-free dietary products elaborated with soybean protein and/or soybean flour and rice flour using the standard additions calibration method. The method enabled the detection and quantitation of additions of 0.18% w/w and 0.61% w/w of soybean proteins, respectively, in the soybean–rice bakery products (related to 2 g of initial product). These values together with the good performance of the method enabled to consider the proposed conditions as valuable for the analysis of the soybean–rice products studied in this work.

The authors thank the Ministry of Science and Technology (Spain) for the research project BQU2002–01199 and F. J. Cabello-Murillo for technical assistance. C.G.-R. also thanks the Ministry of Science and Technology for the Ramón y Cajal program (RYC-2003–001).

5 References

[1] Bean, S. R., Bietz, J. A., Lookhart, G. L., *J. Chromatogr. A* 1998, *814*, 25–41.

[2] Bean, S. R., Lookhart, G. L., *J. Chromatogr. A* 2000, *881*, 23–36.

[3] Dendy, D. A. V., Dobraszczyk, B. J., *Cereales y Productos Derivados*. *Química y Tecnología*, Acribia, Zaragoza 2001.

[4] Cervera, P., Clapes, J., Rigolfas, R., *Alimentación y Dietoterapia*, McGraw-Hill, Madrid 2004.

[5] Belitz, H. D., Grosch, W., *Química de los Alimentos*, Acribia, Zaragoza 1997.

[6] Osborne, T. B., *The Vegetable Proteins*, Longman, London 1924.

[7] Lookhart, G. L., Bean, S., in: Kulp, K., Ponte, J. G. (Eds.), *Handbook of Cereal Science and Technology*, Marcel Dekker, New York 2000.

[8] Kruger, J. E., Bietz, J. A. (Eds.), *High Performance Liquid Chromatography of Cereal and Legume Proteins*, AACC, St. Paul, MN 1994.

[9] Rabilloud, T., *Electrophoresis* 1996, *17*, 813–829.

[10] Potter, N. N., Hotchkiss, J. H., *Ciencia de los Alimentos,* Acribia, Zaragoza 1999.

[11] Steinke, F. H., in: Waggle, D. H., Steinke, F. H., Volgarev, M. N. (Eds.), *New Protein Foods in Human Health: Nutrition, Prevention, and Therapy*, CRC Press, Boca Raton, FL 1992, pp. 59–66.

[12] García, M. C., Torre, M., Marina, M. L., Laborda, F., *Crit. Rev. Food Sci. Nut.* 1997, *37*, 361–391.

[13] Endres, J. G., *Soy Protein Products. Characteristics, Nutritional Aspects and Utilization*, AOCS Press, Champaign, IL 2001.

[14] Misra, P., Usha, M. S., Singh, S., *J. Food Sci. Technol.* 1991, *28*, 89–91.

[15] Lookhart, G. L., Bean, S. R., *Cereal Chem.* 1996, *73*, 81–87.

[16] Bean, S. R., Lookhart, G. L., *Electrophoresis* 2001, *22*, 1503–1509.

[17] Bean, S. R., Lookhart, G. L., *Electrophoresis* 2001, *22*, 4207–4215.

[18] Bean, S. R., Tilley, M., *Cereal Chem.* 2003, *80*, 505–510.

[19] Castro-Rubio, A., Castro-Rubio, F., García, M. C., Marina, M. L., *J. Sep. Sci. 28*, 996–1004.

[20] Castro-Rubio, A., Castro-Rubio, F., García, M. C., Marina, M. L., *Food Chem.* 2005, in press.

[21] *AOAC Official Methods of Analysis*, 15th Edn., Association of Official Analytical Chemists, Arlington, VA 1990.

[22] Schaeper, J. P., Sepaniak, M. J., *Electrophoresis* 2000, *21*, 1421–1429.

[23] Miller, J. N., Miller, J. C., *Statistics and Chemometrics for Analytical Chemistry*, 3th Edn., Prentice Hall, New York 2000, pp. 122–123.

[24] Bean, S. R., Lookhart, G. L., *J. Agric. Food Chem.* 2000, *48*, 344–353.

[25] Bean, S. R., Lookhart, G. L., *Cereal Chem.* 2001, *78*, 530–537.

[26] Lampart-Szczapa, E., Jankiewicz, M., *Food Chem.* 1983, *10*, 97–109.

