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Determination of soybean proteins in commercial heat-processed meat products prepared with chicken, beef or complex mixtures of meats from different species

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Abstract

The addition of foreign proteins (mainly soybean proteins and milk proteins) to heat-processed meat products is a common practice. This work approaches the determination of additions of soybean proteins in heat-processed meat products prepared with chicken meat, beef meat, and complex mixtures of meats from different species (chicken, pork, beef, and turkey) by perfusion reversed-phase high-performance liquid chromatography. The applied method was previously developed for the determination of soybean proteins in pork and turkey meat products but it has never been tested for the determination of soybean proteins in other heat-processed meat products containing other kinds of meats. This paper demonstrates the validity of this method for the detection of soybean proteins in heat-processed meat products containing different varieties of meats and even in the presence of other foreign proteins such as milk proteins. The specificity and existence of matrix interferences have been checked for these samples and accuracy has been evaluated by the comparison of the soybean protein contents determined by the proposed method and the official ELISA method.
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1. Introduction

Heat-processed meat products consist of emulsions prepared with meats of different qualities and from one or several species. During the elaboration process, meats are ground in a cutter and mixed with ice/water, spices, and other ingredients. After homogenization, the mixture is stuffed into casings, clipped at both ends, and cooked in a humid oven with 100% water vapour between 60 and 80 °C or in a water bath to 90 °C until the internal temperature reaches 72 °C (Andersson, Andersson, & Tornberg, 2000; Pearson & Gillett, 1996). Among other ingredients, heat-processed meat products can also contain foreign proteins such as soybean and milk proteins. The addition of these proteins can be justified in different ways:

- Foreign proteins can be added to improve the emulsification of fat and water, thus, preventing the coalescence of the fat during heating when the lean meat content of the product is low (Pearson & Gillett, 1996; Yusof & Babji, 1996).
- Some non-meat proteins can also be used as fat replacers owing to their ability to bind water and to form gels, thus, responding to consumers demands for healthier and low fat products (Egbert, Huffman, Chen, & Dylewski, 1991; Pietrasik & Duda, 2000; Shand, 1997). More-

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over, there are also well known benefits associated with the consumption of soybean: reduction of cholesterol levels and menopause symptoms and reduction of risk for several chronic diseases, i.e., cancer, heart disease, and osteoporosis (Riaz, 1999).

The addition of foreign proteins to meat products has resulted in regulations limiting this practice (Legislación Alimentaria de Aplicación en España, 2002). The application of established regulations implies the use of methods enabling the determination of soybean proteins in these products.

Detection of soybean proteins in meat products has been performed by different techniques such as polyacrylamide gel electrophoresis, immunochemical techniques, and chromatographic techniques (Belloque, García, Torre, & Marina, 2002). Nevertheless, none of these methods are completely satisfactory being, in most of cases, very tedious and time consuming or even not enabling the quantitative analysis of soybean proteins. The method commonly used in food laboratories for the determination of soybean proteins in heat-processed meat products is an AOAC method based on an enzyme-linked immunosorbent assay (ELISA) (AOAC Official Method; Koppelman, Lakemond, Vlooswijk, & Hefle, 2004; Yman, 2004). In this method, soybean proteins from a meat product are submitted to denaturing conditions, renatured conditions, and, finally, analysed by an inhibition mode of ELISA. In this immunoassay, soybean proteins are made to react with an appropriate antiserum in excess and the unreacted antibody is determined, after isolation, by its reaction with a second antibody conjugated with an enzyme. Capture enzyme activity is determined by adding a chromogenic substrate yielding a product whose color intensity is measured at 450 nm. In addition to its complexity and length, this method has been considered as semiquantitative (AOAC Official Method).

During the last years our research team has focussed on the development of analytical methods enabling the detection of the soybean protein content in heat-processed meat products (Castro, García, Rodríguez, & Marina, 2005; Castro, Marina, Rodríguez, & García, in press) and cured meat products (Criado, Castro, García-Ruiz, García, & Marina, 2005). Thus, it has been possible to reliably determine soybean proteins in heat-processed meat products prepared with pork and turkey meats by the use of a simple chromatographic method that constitutes a promising alternative to the ELISA method. Nevertheless, this method has never been applied to the analysis of heat-processed products prepared with other kind of meats or with complex mixtures of meats.

The aim of this work was the determination of soybean proteins in heat-processed meat products prepared with chicken, beef or complex mixtures of meats from different species (chicken, pork, beef, and turkey).

2. Materials and methods

2.1. Chemicals and samples

Acetonitrile (ACN, HPLC gradient grade) (Merck, Darmstadt, Germany), trifluoroacetic acid (TFA) (99.5 atom% D in 0.5 ml blisters (Sigma, St. Louis, MO)), and high-purity water (>18 MΩ/cm) obtained from a Milli-Q purification system (Millipore, Bedford, MA) were used in the preparation of mobile phases. Tris(hydroxymethyl)aminomethane (Tris) was employed for sample preparation. Acetone (Merck, Darmstandt, Germany) was necessary for fat extraction. The soybean protein isolate (SPI) Supro 500E (Anvisa, Madrid, Spain) (85.37% of proteins determined by Kjeldahl analysis (2 replicates)) was used for the quantitation of soybean proteins. Sodium caseinate, α-lactalbumin, and β-lactoglobulin from bovine milk were obtained from Sigma (St. Louis, MO). Seventeen commercial meat products made with chicken, beef or different meat blends were used. These products were purchased in local markets in Madrid (Spain) or supplied by Campofrío Alimentación S.A. (Burgos, Spain). The composition of these products is detailed in Table 1. Moreover, in the case of chicken products, a model meat product without soybean proteins (model meat product 1) and two model meat products with the same composition as the model meat product 1 but including soybean proteins and not submitted to any heat-processing (model meat products 2 and 3) were also supplied by Campofrío Alimentación S.A. The composition of these model products is also detailed in Table 1. The protocol for the preparation of the samples was the following: 10 g of meat were ground with an automatic miller, homogenised with 25 ml of acetone in an Ultraturrax mixer (3 min), submitted to agitation for 15 min, and centrifuged (3362 g, 30 min, 25 °C). The supernatant was removed and the pellet was extracted again with another 25 ml of acetone following the same procedure. Finally, the pellet was dried overnight at 60 °C to remove the remaining acetone. Meat solutions with concentrations ranging from 20 to 176 mg/ml (related to initial product) were used. These solutions were prepared by weighing the appropriate amount of the defatted and dried meat product (0.1–1.0 g), solubilising in 25 ml of 50 mM Tris–HCl buffer (pH 8) with ultrasonic agitation for 10 min at 50 °C, and centrifuging at 3362 g for 10 min to inject the supernatant in the chromatographic system. The soybean protein content in meat samples was also determined by the ELISA procedure described in the AOAC method 988.10 (AOAC Official Method).

2.2. High-performance liquid chromatography

Two Hewlett–Packard 1100 Series liquid chromatographs (Hewlett–Packard, Pittsburgh, PA) equipped with a diode array detector (one of them with a microcell and the other with a standard flow cell), an injection system, a degassing system, a quaternary pump, and a compartment

Table 1
Composition of meat products used

Meat products	Composition
Meat product A–H and model meat products 2 and 3	Chicken meat, water, salt, soybean protein isolate, sugars (dextrose), stabilizers, antioxidants, preservatives
Model meat product 1	Chicken meat, water, salt, sugars (dextrose), stabilizers, antioxidants, preservatives
Meat product I	Chicken meat, water, corn starch, vegetable proteins, stabilizers, antioxidants, preservatives
Meat product J	Chicken meat, water, potato starch, wheat starch, vegetable proteins, milk proteins, sugars (dextrose), natural spices, gelling powder, emulsion powder, antioxidants, flavor powder, preservatives, coloring
Meat product K	Beef meat, water, corn starch, vegetable proteins, stabilizers, antioxidants, preservative
Meat product L	Beef meat, olives, water, potato starch, wheat starch, vegetable proteins, milk proteins, sugars (dextrose), natural spices, gelling powder, emulsion powder, antioxidants, flavor powder, preservatives, coloring
Meat product M	Pork meat, chicken meat, water, starch, salt, vegetable proteins, spices, sugar, stabilizer, antioxidant, flavor powder, preservatives
Meat product N	Beef meat and turkey meat (56%), water, starch, vegetable oil, vegetable proteins, milk proteins, salt, sugars (dextrose and lactose), aromas, spices, stabilizers, flavor powder, antioxidant, preservatives, natural coloring
Meat product O	Beef (veal) meat, turkey meat and chicken meat (65%), water, starch, vegetable oil, vegetable proteins, salt, dextrose, sugar, spices, stabilizer, flavor powder, antioxidant, preservatives
Meat product P	Pork meat, chicken meat, turkey meat, fat, water, olives (11%), starch, salt, soybean proteins, sugars, (lactose), stabilizers, milk proteins, flavor powder, spices, antioxidant, coloring, preservative
Meat product Q	Pork meat, beef meat (8%), chicken meat, turkey meat, water, starch, salt, soybean proteins, sugars (dextrose), stabilizers, aroma, flavor powder, antioxidant, preservative, powdered milk

for the column were employed. The injected volume was 20 μl and the detection was performed at 280 nm. The separation was accomplished with a POROS R2/H column (50×4.6 mm i.d.) from Perseptive Biosystems (Framingham, MA) packed with 10 μm diameter polystyrene divinylbenzene beads. The RP-HPLC method consisted of a linear binary gradient in three steps: 5–25% B in 0.8 min, 25–42% B in 0.8 min, and 42–50% B in 0.6 min. The flow-rate was 3 ml/min and temperature was 50 °C. Mobile phases were: phase A, 0.05% TFA (v/v) in Milli-Q water; phase B, 0.05% TFA (v/v) in ACN. The organic modifier was filtered through 0.45 μm nylon filters before use.

2.3. Calibration

Calibration by the external standard method was carried out by injecting SPI solutions over the range 0.10–6.5 mg/ml of soybean proteins (corrected for the purity and moisture). The peak corresponding to soybean proteins (peak at 1.70 min) was integrated by setting the baseline from valley to valley and the average area of three consecutive injections was calculated. The content of soybean proteins in the meat products was determined by interpolation of the area of that peak in the calibration curve. Calibration by the standard additions method was performed by injecting meat extracts spiked with known and increasing amounts of SPI (0–6.5 mg/ml of soybean proteins). All determinations were performed, at least, by duplicate and every solution was injected three times into the chromatographic system.

2.4. Data treatment

The peak area corresponding to soybean proteins was plotted against the injected concentrations (external standard calibration) or spiked SPI concentrations (standard additions calibration).

3. Results and discussion

3.1. General

The determination of soybean proteins in heat-processed meat products prepared with pork meat, turkey meat, and pork–turkey meat blends that can also contain milk proteins has been demonstrated (Castro et al., 2005, in press). In addition to these products, heat-processed meat products prepared with chicken or beef meats are also very popular, being very interesting the determination of soybean proteins in these products.

In order to prove that the previously developed method for the determination of soybean proteins in pork and turkey meat products is valid for other kinds of heat-processed meat products, it was applied to the analysis of heat-processed meat products prepared with chicken and beef meats. The chromatogram obtained for a heat-processed chicken meat product with (meat product D) and without SPI (model meat product 1) and the SPI itself are shown in Fig. 1(a). The chromatogram obtained for the meat product with SPI showed a broad peak at approximately 1.10 min and a peak at 1.70 min (indicated with an arrow) that disappeared in the chromatogram corresponding to the meat product without soybean proteins. These peaks could be attributed to soybean proteins since peaks at similar retention times appeared in the chromatogram corresponding to the SPI. Nevertheless, only the UV spectra and first and second derivatives obtained for the peak at 1.70 min in the SPI and the meat product containing soybean proteins matched (see Fig. 2(a)).

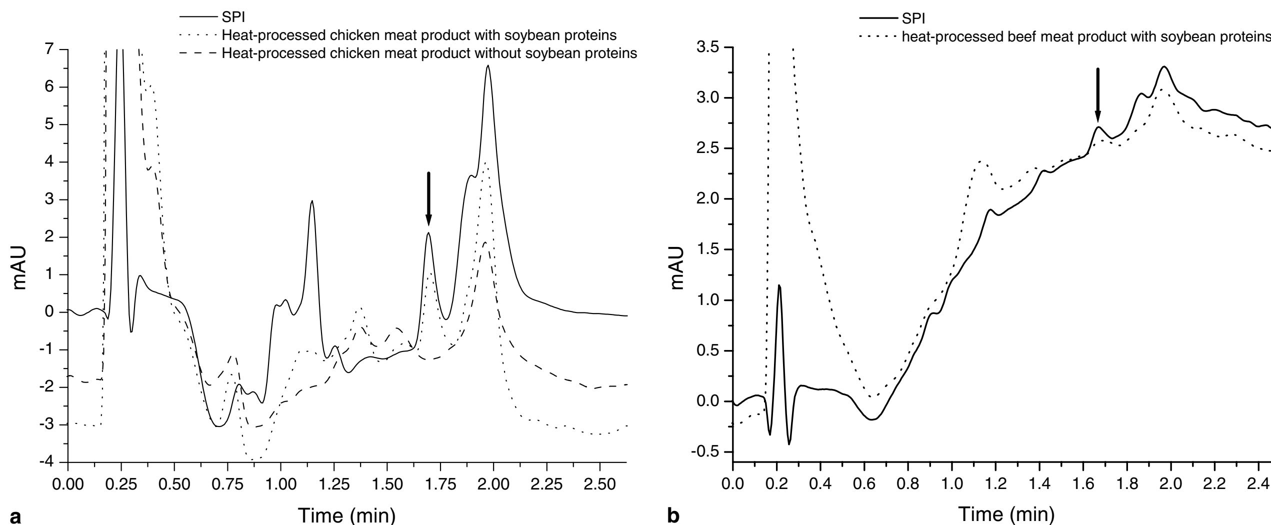

Fig. 1. Chromatograms corresponding to a heat-processed chicken meat product with (meat product D) (272 mg/ml referred to initial product) and without soybean proteins (model meat product 1) (248 mg/ml referred to initial product), and to SPI (2 mg/ml of soybean proteins) (a) and chromatograms corresponding to a heat-processed beef meat product containing soybean proteins (meat product K) (35 mg/ml referred to initial product) and to SPI (1.5 mg/ml) (b). Chromatographic conditions: temperature, 50 °C; flow-rate, 3 ml/min; gradient: 5–25% B in 0.8 min, 25–42% B in 0.8 min, 42–50% B in 0.6 min; mobile phases: A, 0.05% (v/v) TFA in water; B, 0.05% (v/v) TFA in ACN; injected volume, 20 µl; detection, 280 nm. Sample preparation: fat extraction with acetone followed by protein solubilisation in 50 mM Tris–HCl buffer (pH 8) with ultrasonic agitation for 10 min at 50 °C.

These experimental conditions were also applied to one heat-processed meat product prepared with beef meat (meat product K) (see Fig. 1(b)). A peak at approximately 1.70 min was again observed in the chromatogram of the meat product that could correspond to soybean proteins. The comparison of the UV spectra and first and second derivatives obtained for this peak in the SPI and in the meat product containing soybean proteins showed that they were identical (Fig. 2(b)).

3.2. Detection of soybean proteins in heat-processed meat products prepared with chicken or beef meats in the presence of milk proteins

The Spanish Legislation allows the addition of milk proteins to heat-processed meat products in place of or in addition to soybean proteins; therefore, the presence of both kinds of foreign proteins is very common (Legislación Alimentaria de Aplicación en España, 2002). In order to prove the validity of the proposed method for chicken or beef meat products containing milk proteins, it was applied to the analysis of one heat-processed meat product prepared with chicken and one heat-processed meat product prepared with beef that contained both soybean and milk proteins. Fig. 3(a) shows the chromatograms obtained for both products and for the SPI and Fig. 3(b) shows the chromatograms corresponding to milk proteins (bovine caseins, α-lactalbumin, and β-lactoglobulins (A + B)). All milk proteins appeared at the end of the chromatogram at retention times higher than 1.70 min. Therefore, it was possible to detect soybean proteins in these products without interference of milk proteins.

3.3. Detection of soybean proteins in heat-processed meat products prepared with complex meat blends that could also contain milk proteins

Nowadays, it is quite common to find heat-processed meat products prepared with meats from different species. The higher complexity of these products could affect the detection of soybean proteins. In order to test whether the proposed method is affected by this fact, it has been applied to the analysis of heat-processed products prepared with different meat blends. The chromatograms obtained for a product prepared with a pork/chicken blend (product M), a product prepared with a beef/turkey/chicken blend (product O) and for the SPI are shown in Fig. 4(a).

As examples of products prepared with meat blends and containing milk proteins, Fig. 4(b) shows the chromatograms corresponding to a beef/turkey product (meat product N) and to a pork/chicken/turkey product (meat product P). In all cases, the peak at 1.70 min corresponding to soybean proteins was clearly detected and separated from meat components and milk proteins (in the case of Fig. 4(b)).

3.4. Analytical characteristics of the method

The chromatographic method applied in this work has previously been validated by the determination of the linearity of the calibration plot, detection and quantitation limits, existence of matrix interferences, specificity, precision (repeatability and internal reproducibility), and accuracy using the SPI Supro 500E as standard of soybean proteins and when samples contained only pork or/and

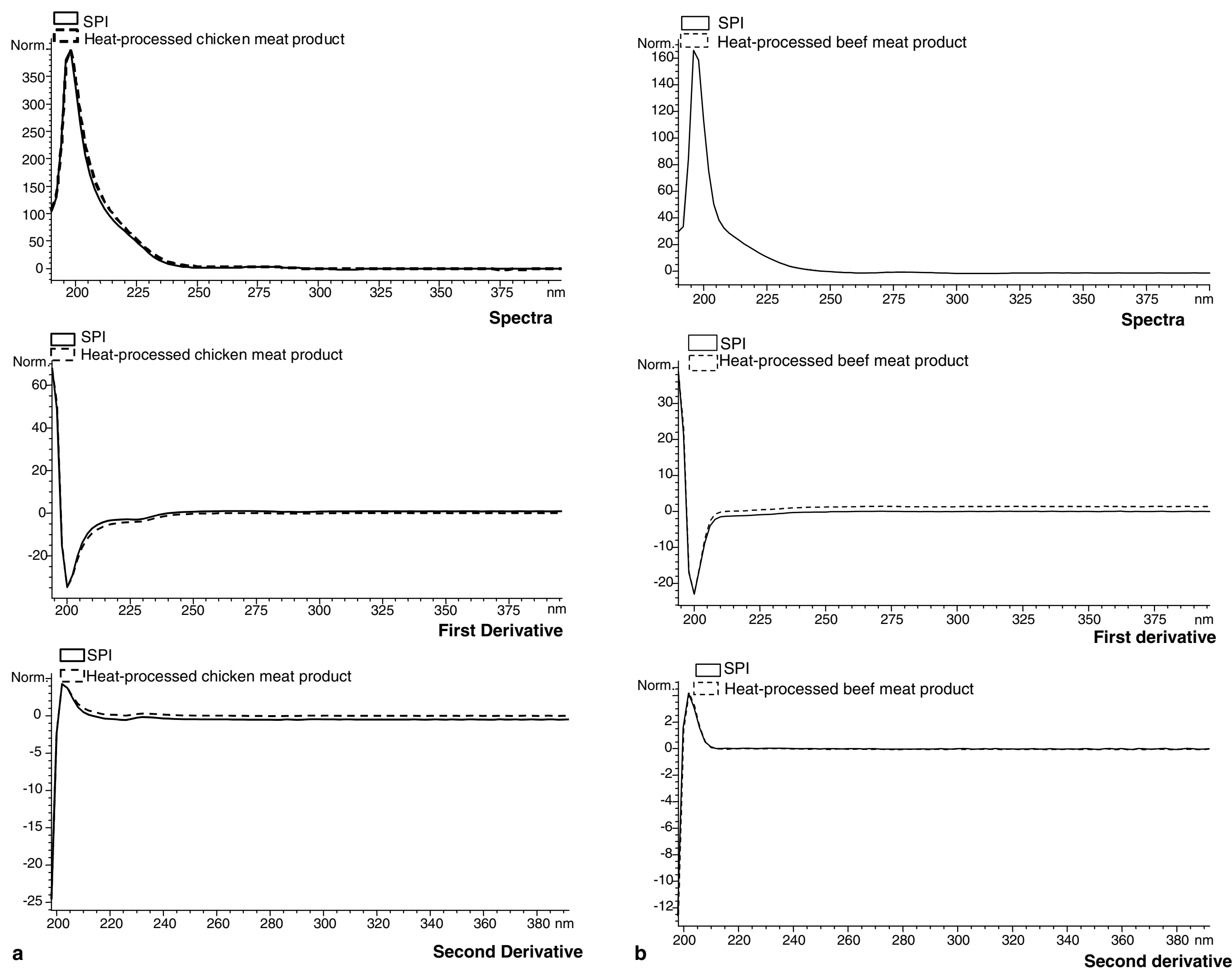

Fig. 2. UV spectra and first and second derivatives obtained for the peak at 1.7 min in a heat-processed chicken meat product with soybean proteins (meat product D), and in a heat-processed beef meat product with soybean proteins (meat product K) in comparison with the SPI. Experimental conditions as in Fig. 1.

turkey meats (Castro et al., 2005, in press). Some of these parameters (linearity and detection and quantitation limits) remain when the method was applied to other kind of meats. Nevertheless, it was necessary to check whether matrix interferences, precision, specificity, and accuracy were adequate for the application of this method to meat products prepared with other meats different to pork or turkey alone.

The presence of matrix interferences was checked by comparison of the slopes and the contents of soybean proteins obtained for the external standard and the standard additions calibration methods for five heat-processed meat products using *t*- and *F*-tests. The chosen products contained only beef or chicken meats or consisted of pork–chicken blends, beef–turkey blends or beef–turkey–chicken blends. Table 2 groups the results obtained. Since the *P*-value was greater than 5%, the absence of proportional systematic errors was confirmed and the external standard method was chosen for quantitation of soybean proteins in these meat products. In previous works, it was observed that the method resulted affected by the matrix of the product when analysing turkey products or meat blends containing high concentrations of this meat, while there were no matrix interferences when the method was applied to meat products containing only pork (Castro et al., 2005, in press).

The method specificity was verified with 12 heat-processed meat products performing one addition of SPI over every meat product covering the range from 1.50 to 6.20 mg/ml. The specificity was determined by adjusting a straight line between added and recovered concentrations of soybean proteins in these samples (see Table 3). As the slope and the intercept obtained were not statistically different from 1.0 and 0.0, respectively, the method was considered specific.

For the evaluation of the precision of the method, the repeatability and intermediate precision were determined (Table 3). Repeatability, expressed as relative standard deviation (RSD, %) in retention time and peak area and calculated by injecting 10 consecutive times a solution of

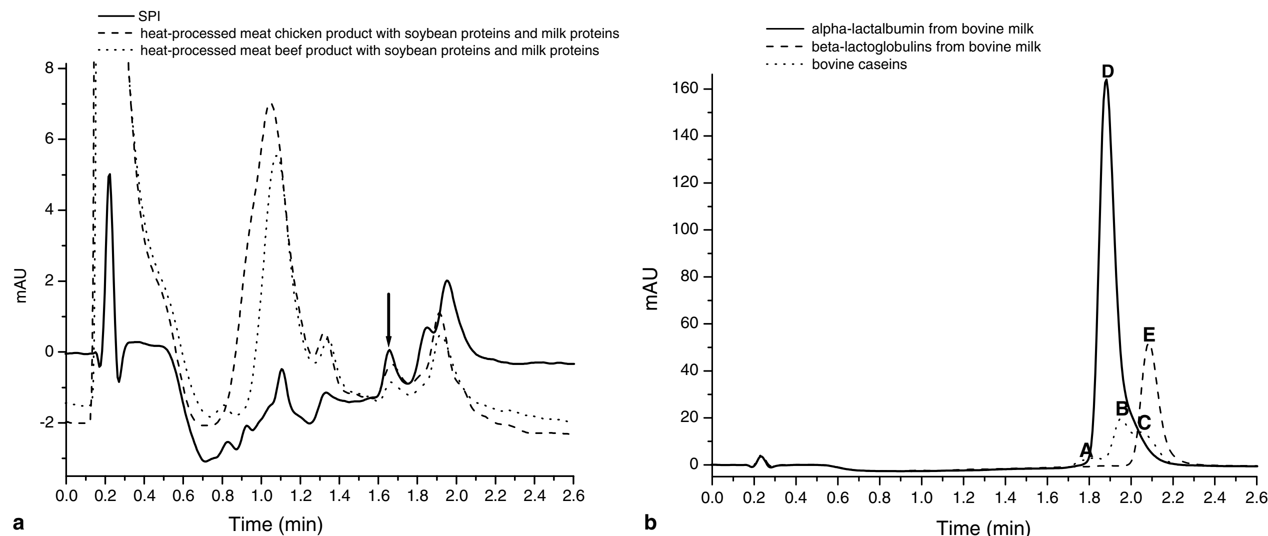

Fig. 3. Chromatograms corresponding to a heat-processed chicken meat product containing soybean and milk proteins (meat product J) (152 mg/ml referred to initial product), a heat-processed beef meat product containing soybean and milk proteins (meat product L) (31 mg/ml referred to initial product), and the SPI (1.0 mg/ml of soybean proteins) (a). Chromatograms obtained from a solution of 1.12 mg/ml of bovine caseins (corresponding to a casein content of 0.5% (w/w) related to 6 g of initial product), a solution of 1.34 mg/ml of α-lactalbumin (corresponding to a α-lactalbumin content of 0.5% (w/w) related to 6 g of initial product), and a solution of 1.00 mg/ml of β-lactoglobulin (corresponding to a β-lactoglobulin content of 0.5% (w/w) related to 6 g of initial product) (b). Experimental conditions as in Fig. 1.

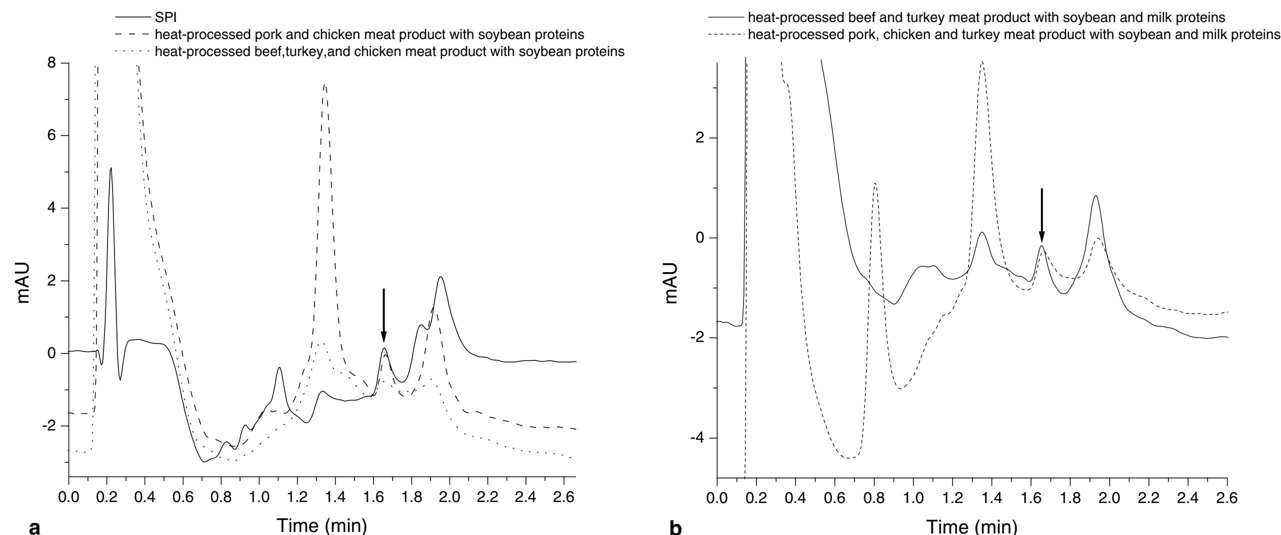

Fig. 4. Chromatograms obtained for heat-processed meat products with soybean proteins containing mixtures of pork and chicken meats (meat product M) (185 mg/ml referred to initial product) and containing mixtures of beef, turkey and chicken meats (meat product O) (176 mg/ml referred to initial product) and to SPI (1 mg/ml of soybean proteins) (a). Chromatograms obtained for heat-processed meat products with soybean and milk proteins containing mixtures of beef and turkey meats (meat product N) (192 mg/ml referred to initial product) and containing mixtures of pork, chicken, and turkey meats (meat product P) (212 mg/ml referred to initial product) (b). Experimental conditions as in Fig. 1.

32 mg/ml of a heat-processed chicken meat product (meat product J), was better than 0.9% in retention time and better than 5% in peak area. The intermediate precision in different days was determined by injecting a solution of 39 mg/ml of a heat-processed pork and chicken meat product (meat product M) in three days. The RSD values observed were better than 0.4% in retention time and close to 3.0% in peak area. The variability in the content determined in three days was close to 4.5%. The robustness of the method was determined by comparison of the results obtained by the intentional use of two different detector flow cell volumes. The soybean protein contents determined with both flow cells for a heat-processed pork and chicken meat product with soybean proteins did not differ significantly (*F*- and *t*-tests (*P* value, 9.3% > 5%)).

The recovery of the method was evaluated by spiking with different amounts of SPI the extracts obtained from five heat-processed meat products and from a raw meat

Table 2
Investigation of the existence of matrix interferences[a]

Sample	Slopes of the calibration lines[b]		Comparison of slopes (proportional bias)		Soybean protein concentration (mg/100 mg sample)[c]		Comparison of soybean protein contents	
	External standard method (ES)	Standard additions method (SA)	t-Value[d]	P-value (%)[e]	ES	SA	t-Value[d]	P-value (%)[e]
Meat product I	4.44 ± 0.09[f]	4.45 ± 0.08[f]	0.19	87.0	0.59 ± 0.17	0.37 ± 0.07	1.71	23.6
Meat product K	2.86 ± 0.16[g]	2.83 ± 0.02[g]	0.31	80.0	0.58 ± 0.03	0.57 ± 0.29	0.06	>90.0
Meat product M	4.74 ± 0.06[f]	4.61 ± 0.04[f]	2.60	13.1	0.71 ± 0.00	0.63 ± 0.08	1.49	39.9
Meat product N	4.54 ± 0.19[f]	4.43 ± 0.02[f]	0.82	49.9	0.39 ± 0.11	0.34 ± 0.19	0.23	85.0
Meat product O	2.94 ± 0.16[g]	2.67 ± 0.03[g]	2.30	16.0	0.40 ± 0.06	0.31 ± 0.01	2.02	18.7

[a] Results expressed as is basis.
[b] Average value of the slopes of two straight lines.
[c] Mean of two individual determinations.
[d] Calculated t-value.
[e] Significance level (P-value) associated.
[f] Slope obtained by using a diode-array detector with a standard flow cell.
[g] Slope obtained by using a diode-array detector with a microcell.

Table 3
Analytical characteristics of the perfusion RP-HPLC method for the analysis of soybean proteins in complex heat-processed meat products

Specificity[a]	$y = 0.969(0.015)x - 0.045(0.059)$			
Repeatability (RSD, %) (n = 10)[b]	Sample			
Retention time	0.81			
Peak area	4.80			
Intermediate precision (different days) (RSD, %)[c]				
Retention time	0.34			
Peak area	3.02			
Concentration	4.56			
Robustness (%)[d,e]	Conventional parameters[f]		Modified parameters[g]	
Detector flow cell volume	0.708(0.003)		0.761(0.024)	
Recovery (%)[h]	1.61 (mg/ml)	3.24 (mg/ml)	4.85 (mg/ml)	6.47 (mg/ml)
Model meat product 2	95.7 ± 0.1	94.7 ± 2.3	95.8 ± 0.4	–
Meat product J	95.3 ± 0.7	95.2 ± 1.5	96.5 ± 0.7	–
Meat product L	99.9 ± 2.8	97.8 ± 1.7	–	97.0 ± 1.8
Meat product N	96.7 ± 2.7	106.3 ± 0.8	99.6 ± 0.7	100.3 ± 0.4
Meat product M	100.2 ± 1.7	96.1 ± 0.9	97.2 ± 0.5	96.1 ± 0.5
Meat product O	96.7 ± 1.3	98.0 ± 1.1	95.3 ± 1.7	–
Absolute recovery (%)[i]				
Processed meat spiked with 1.95% soybean proteins	104.3 ± 0.0			
Processed meat spiked with 2.89% soybean proteins	87.2 ± 1.7			

[a] A t-test for the verification of slope and intercept were statistically equal to the unit and zero, respectively. Standard deviation of slope and intercept are given in parenthesis.
[b] Number of injections of a solution of a heat-processed chicken product with soybean and milk proteins (meat product J) (32 mg/ml referred to initial product).
[c] Analysis performed by the external standard method in different days.
[d] Injection of a solution of 39.3 mg/ml of a heat-processed pork and chicken meat product with soybean proteins (meat product M) in three days.
[e] Concentration of soybean proteins determined by the external standard method using detector flow cells of different volumes and a heat-processed pork and chicken meat product with soybean proteins (meat product M).
[f] Analysis performed using a diode array detector with a standard flow cell of 10 mm of way length and a volume of 13 μl.
[g] Analysis performed using a diode array detector with a flow microcell of 6 mm of way lenght and a volume of 1.7 μl.
[h] Recovery of soybean proteins when different amounts of SPI were added to the extract obtained from a heat-processed meat product.
[i] Recovery of soybean proteins when different amounts of SPI were initially added to heat-processed meat products.

product (model product 2). Recoveries obtained ranged from 95% to 106% not observing any difference between the recoveries obtained for the raw meat product and for the products submitted to heat processing. Moreover, the recovery (absolute recovery) was also determined by directly spiking a heat-processed meat product with two different amounts of SPI. Values of recovery of 104% and 87% were obtained.

The accuracy was also checked by comparing the soybean protein contents obtained by the proposed method and by the official ELISA for seven heat-processed meat products and for two raw meat products and the results

Table 4
Soybean protein contents determined in different commercial heat-processed meat products and in two raw meat products by the ELISA method and the proposed HPLC method[a]

Meat products	Protein concentration (mg/100 mg sample)	
	ELISA[b]	Perfusion HPLC[c]
Processed meat products with soybean proteins		
Meat product A	–	1.11
Meat product B	0.88	1.05[d]
Meat product C	–	1.89
Meat product D	1.06	0.83(0.26)[e]
Meat product E	0.98	0.87
Meat product F	–	0.61[d]
Meat product G	–	0.76
Meat product H	0.62	0.64
Meat product I	–	0.59
Meat product M	–	0.71
Meat product K	–	0.58
Meat product O	–	0.31
Meat product Q	–	<LDQ[f]
Processed meat product with soybean and milk proteins		
Meat product J	0.98	0.81
Meat product L	–	0.68[d]
Meat product N	0.44	0.38
Meat product P	1.23	0.89[d]
Raw meat products with soybean proteins		
Model meat product 2	1.12	1.22
Model meat product 3	1.24	1.16

[a] Results expressed as is basis.
[b] Determined following the official AOAC method 998.10.
[c] Determined by the proposed method. Most determinations were performed by duplicate.
[d] Only one determination.
[e] Mean of three individual determinations. Standard deviation given in parenthesis.
[f] Soybean protein content lower than the quantitation limit of the method (0.28%, w/w).

obtained are shown in Table 4. No statistically significant differences between the contents determined by both methods were detected when applying a paired *t*-test (P value $= 18.6\% > 5\%$). The slope and intercept of the equation of the straight line obtained by plotting of the soybean protein contents obtained by the proposed method against the contents obtained by the ELISA method were 0.834 (0.167) and 0.067 (0.164), respectively. These values did not significantly differ from the unit and zero, respectively, when a *t*-test was applied.

3.5. Application to edible samples

The method was applied to the determination of the soybean protein content in 17 commercial heat-processed meat products (A–Q). Since there was not a reference certified standard of soybean proteins, a soybean protein isolate was chosen as standard of soybean proteins. Three were the reasons supporting this selection: the SPI is the soybean product with the highest protein content, the SPI is commercially available, and the SPI is the soybean product most widely added in the manufacturing of heat-processed meat products. The SPI Supro 500E was chosen as standard, since it had been used in the preparation of some of the meat products studied (those prepared by Campofrío Alimentación S.A.). The soybean protein contents obtained are also shown in Table 4. The concentrations of soybean proteins determined in the meat samples containing only soybean proteins ranged from 0.40% to 1.89%. These values were within the limits authorised by the Spanish law, 3% of soybean proteins referred to the product as is basis (Legislación Alimentaria de Aplicación en España, 2002). For the meat products containing soybean and milk proteins, lower contents of soybean proteins ranging from 0.38% to 0.86% were observed. In these cases, the Spanish law admits the addition of up to a 3% of soybean + milk proteins. Thus, the lower content in soybean proteins in these samples is justified.

4. Conclusion

The validity of a previous method developed for the determination of soybean proteins in heat-processed pork and turkey meat products has been shown for the analysis of products prepared with chicken meat, beef meat, or complex mixtures of meats from different species (chicken, pork, beef, and turkey). The presence of milk proteins did not interfere in the determination of soybean proteins in these products. The proposed method did not suffer from matrix interferences and was specific and reproducible when it was applied to the analysis of these samples. Trueness of the method was checked by means of recovery studies observing values close to 100%. Furthermore, the soybean protein contents obtained for several meat-products by the proposed method and by the ELISA method were compared and no statistically significant differences were found. The main advantages of the proposed method are easy sample preparation, short analysis time, and low cost resulting in an interesting alternative to the ELISA method.

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