

Easy determination of the addition of soybean proteins to heat-processed meat products prepared with turkey meat or pork–turkey meat blends that could also contain milk proteins

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Abstract

The addition of non-meat proteins to processed meat products is limited by regulations. Therefore, this work has investigated the determination of added soybean proteins in commercial heat-processed meat products prepared with turkey meat or pork–turkey meat blends that could also contain milk proteins. The method consisted of extracting proteins from the meat products in a Tris-HCl buffer (pH 8) and analysing the extract by high-performance liquid chromatography with a linear gradient water–acetonitrile containing 0.05% (v/v) TFA. This method enabled the detection and quantitation of up to 0.08 and 0.28% (w/w), respectively, of soybean proteins (related to 6 g initial product) in these products. Satisfactory precision and recovery data were established. Accuracy was evaluated by a comparison of soybean protein contents determined by the proposed method and the existing AOAC official method based on an enzyme-linked immunosorbent assay (ELISA) from which no statistically significant differences were observed.

Keywords: Soybean proteins, meat products, perfusion chromatography, turkey meat, milk proteins, adulteration

Introduction

The addition of foreign proteins such as milk and soybean proteins to meat products is a common practice. In fact, heat-processed meat products are meat emulsions prepared from a coarse and viscous dispersion of fat and proteins in water, which after heating is transformed into a gel filled with fat particles. In this process, meat proteins play an important role as emulsifiers to prevent the coalescence of fat. Nevertheless, not all meats have the same composition, and when meats with low protein content (e.g. offals) are used to prepare meat products, non-meat proteins are added to prevent the coalescence of fat during heating (Pearson and Gillett 1996; Yusof and Babji 1996).

Dietary health concerns and consumer demands for reduced-fat products have prompted meat companies to develop low-fat meat products (Chin et al. 1999). Fat provides succulence, texture and flavour to meat products that are altered when fat is

removed (Pietrasik and Duda 2000). In these cases, the addition of non-meat proteins (e.g. milk and/or soybean proteins) to meat systems enables a reduction of the fat content yet keeping the physical properties of the meat products unaltered (Keeton 1994; Shand 1997).

Nevertheless, the addition of soybean and milk proteins to meat products is regulated since they pose potential risk for allergic individuals. Moreover, the European Commission has amended The European Food Labelling Directive 2000/13/EC to ensure all consumers are informed of the complete contents of foodstuffs (Poms et al. 2004).

In order to ensure that a meat product is within the legal limitations and prevent potential frauds, different analytical methods for the determination of soybean proteins in meat products have been developed. Most of these methods are based on electrophoretic, immunochemical or chromatographic techniques. Nevertheless, none of these

methods can completely satisfy the required needs. The main problem appearing when using electrophoretic methods is the presence of interferences due to other components of the meat product. Moreover, although under a qualitative scope these electrophoretic methods could be useful, an absolute measurement from soybean bands is very difficult, especially at low concentrations. Furthermore, although reliable quantitation of soybean proteins has been achieved in fresh meats, the technique failed when it was applied to heated meat products (Belloque et al. 2002).

Regarding immunochemical methods, an inhibition mode of ELISA (enzyme-linked immunosorbent assay) has been adopted by AOAC International for the determination of soybean proteins in raw and heat-processed meat products (since 1988). This method is commonly used in food laboratories and is based on a very tedious and time-consuming assay. In this method, soybean proteins from a defatted 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 colour intensity is measured at 405 nm. In addition to its tediousness, cost, and complexity, the method has been considered as semiquantitative (AOAC Official Method 998.10, 1996, 1997, 1998).

High-performance liquid chromatography (HPLC) has also been applied for soybean and meat proteins separation being reversed-phase and anion-exchange the chromatographic modes most used for this purpose. Nevertheless, none of these methods enabled the clear separation of soybean proteins and the quantitation assessment was not possible. Furthermore, these methods were used with samples synthetically prepared by adding soybean proteins to raw meats and they have never been applied to commercial meat products or heated meats (Ashoor and Stiles 1987; Parris and Gillespie 1988; Belloque et al. 2002).

Our research team has developed an analytical method based on perfusion chromatography enabling the reliable determination of soybean proteins in heat-processed products made exclusively with pork meat. Since heat-processed meat products commercially available may contain not only pork meat but also another kinds of meats and even mixtures of them, our group have extended its interest to meat products prepared with turkey meat or pork–turkey meat blends. Moreover,

heat-processed meat products could contain milk proteins in addition to soybean proteins. The determination of soybean proteins in presence of milk and meat proteins have also been approached in this work.

Materials and methods

Chemicals and samples

HPLC-grade acetonitrile (ACN) (Merck, Darmstadt, Germany), trifluoroacetic acid (TFA) (Sigma, St Louis, MO, USA), and water obtained from a Milli-Q system (Millipore, Bedford, MA, USA) were used in the preparation of mobile phases. Tris(hydroxymethyl)aminomethane (Tris), 2-mercaptoethanol from Merck, and sodium hydrogen carbonate and sodium hydrogen phosphate from Panreac (Barcelona, Spain) were employed for the optimization of the sample preparation. Acetone (Merck) was employed for fat extraction. Eighteen heat-processed meat products containing turkey or pork–turkey meats and purchased in local markets in Madrid, Spain, or supplied by Campofrío Alimentación S. A. (Burgos, Spain) were used. The composition and information appearing in the label of every product are indicated in Table I. Moreover, a model meat product containing turkey meat without soybean proteins (model meat product 1), a model meat product containing pork–turkey meat blends without soybean proteins (model meat product 2), both submitted to heat treatments, and a model meat product with the same composition as the model meat product 1 but including soybean proteins and not submitted to any heat-processing (model meat product 3) were also supplied by Campofrío Alimentación S. A. These model meat products have a general composition identical to meat products A–H. All meat products were defatted as follows: 10 g meat were ground with an automatic miller, homogenized with 25 ml acetone in an Ultraturrax mixer (3 min), submitted to agitation for 15 min, and centrifuged (3362g, 30 min, 25°C). The supernatant was removed and the pellet extracted again with another 25 ml acetone following the same procedure. Finally, the pellet was dried overnight at 60°C to remove the remaining acetone. Meat solutions with concentration ranging from 90 to 250 mg ml⁻¹ (related to initial product) were used. These solutions were prepared by weighing approximately 1 g of the defatted and dried meat product (this amount corresponded to approximately 6 g initial product), solubilizing in 50 mM Tris-HCl buffer (pH 8) with ultrasonic agitation for 10 min at 50°C, and centrifuging at 3362g for 10 min to inject the supernatant in the chromatographic system. The soybean protein content in meat samples was also determined

Table I. Composition of meat products used.

Meat product	Composition
A–H and model meat product 3	Turkey meat, water, salt, soybean protein isolate, sugars (dextrose), stabilizers, antioxidants, preservatives
I	Turkey meat (60%), water, vegetable oil, starch potato, salt, soybean proteins, powdered milk, stabilizers (E-450, E-407), aroma, preservative (E-250), antioxidant (E-301)
J	Turkey meat, water, sunflower oil, milk proteins, soybean proteins, salt, dextrose, stabilizers (E-450, E-407, E-420), antioxidants (E-325, E-316), sugar, aroma, preservative (E-250)
K	Turkey meat (60%), water, vegetable oil, starch, salt, vegetable proteins, milk proteins, lactose, sugar, natural spices, stabilizers (E-450, E-407), antioxidant (E-316), preservatives (E-250, E-252)
L	Turkey breast (55%), water, starch, salt, powdered milk, vegetable proteins, sugar, dextrose, natural spices, stabilizers (E-450 and carragenate), preservatives (E-250, E-252), antioxidant
M	Turkey thighs (55%), water, starch, vegetable proteins, powdered milk, salt, sugar, dextrose, natural spices
N	Turkey meat (49%), water, starch, vegetable oil, powdered milk, salt, natural spices, vegetable proteins, stabilizers (E-450), sugar, flavour powder (E-621), antioxidant (E-316), natural spices (E-120), preservatives (E-250, E-252)
O	Pork meat (48.6%), turkey meat (5%), pork fat, water, salt, vegetable proteins, milk proteins, sugar, natural spices, stabilizers (E-450, E-407), preservatives (E-250, E-252), antioxidant (E-316), natural colouring (E-120)
P	Pork meat (60%), turkey meat (5%), water, salt, vegetable proteins, milk proteins, lactose, dextrose, sugar, natural spices, stabilizers (E-450, E-407), preservatives (E-250, E-252), antioxidant (E-316)
Q	Pork meat (60%), turkey meat (5%), water, salt, vegetable proteins, milk proteins, sugar, natural spices, stabilizers (E-450 and carragen), preservatives (E-250, E-252), antioxidant
R	Pork meat (58%), turkey meat (7%), water, salt, vegetable proteins, milk proteins, sugar, natural spices, stabilizers (E-450 and carragen), preservatives (E-250, E-252), antioxidant, natural colouring

by the ELISA procedure described in the AOAC method 988.10 (1996, 1997, 1998). The soybean protein isolate (SPI) Supro 500E (Anvisa, Madrid, Spain), used as standard of soybean proteins, presented a protein content of 86.7% (determined by Kjeldahl analysis, four replicates). Sodium caseinate, α -lactalbumin, and β -lactoglobulin from bovine milk were obtained from Sigma. The dry matter content of the SPI was determined by drying at 130°C to constant weight. Standard solutions were prepared by weighing and dissolving the standard in 50 mM Tris-HCl buffer (pH 8), sonicating for 5 min, and centrifuging at 3362g for 10 min.

High-performance liquid chromatography

A Hewlett-Packard 1100 Series liquid chromatograph (Hewlett-Packard, Pittsburgh, PA, USA) equipped with a diode array detector was used. The injected volume was 20 μ l and the detection was performed at 280 nm. The separation was accomplished with a POROS R2/H column (50 $\times$ 4.6 mm i.d.) from Perceptive Biosystems (Framingham, MA, USA) packed with 10- μ m diameter polystyrene divinylbenzene beads. The reversed-phase high-performance liquid chromatography (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⁻¹ and temperature was 50°C. Mobile phases were: A, 0.05% TFA (v/v) in Milli-Q water; and B, 0.05% TFA (v/v) in ACN. The organic modifier was filtered through 0.45- μ m nylon filters before use.

Calibration

Calibration was performed by the external standard and by the standard additions methods. Calibration by the external standard method was carried out by injecting (seven) SPI solutions over the range 0.3–6.5 mg ml⁻¹. Calibration by the standard additions was carried out by injecting extracts of the meat products spiked with known and increasing amounts of SPI (five standard solutions in the range 0–6.0 mg ml⁻¹). Integration was carried out by setting the baseline from valley to valley and the average of the area corresponding to three consecutive injections was calculated.

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). The linearity in this relationship was obtained by least-squares regression analysis. The linear model was validated by means of the analysis of residuals and variance when three replicates of every standard were injected by triplicate.

Results and discussion

Detection of soybean proteins in heat-processed turkey meat

At the starting point for the determination of soybean proteins in heat-processed turkey meat products, the experimental conditions previously optimized for

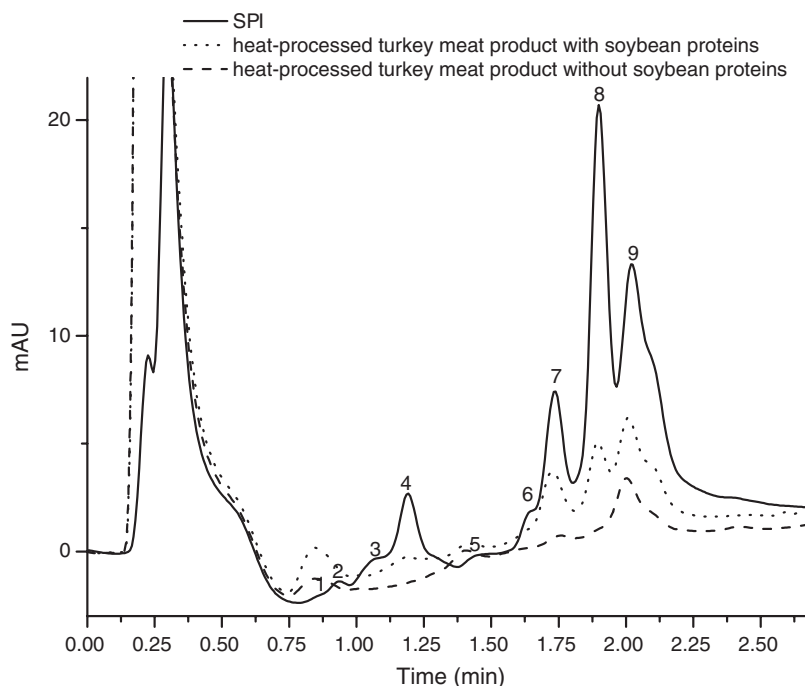


Figure 1. Chromatograms corresponding to heat-processed turkey meats with (meat product B) and without (model meat product 1) soybean proteins, and to the SPI. Chromatographic conditions: temperature, 50°C; flow-rate, 3 ml min⁻¹; gradient: 5–25% B in 0.8 min, 25–42% B in 0.8 min, 42–50% B in 0.6 min; 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. Samples were defatted with acetone and the proteins were extracted in 30 mM Tris-HCl buffer (pH 8) with 0.5% (v/v) 2-mercaptoethanol for 10 min.

the determination of soybean proteins in heat-processed pork meat products were used (Castro et al. 2005). These conditions consisted of a gradient from 5 to 25% B in 0.8 min, from 25 to 42% B in 0.8 min, and from 42 to 50% B in 0.6 min, followed by a reversed gradient from 50 to 5% B in 0.5 min to equilibrate the column to initial conditions. Samples were defatted with acetone and the proteins extracted in a 30 mM Tris-HCl buffer (pH 8) containing 0.5% (v/v) 2-mercaptoethanol with ultrasonic agitation (at 50°C for 10 min) and centrifuged (3362g for 10 min). Figure 1 shows the chromatograms obtained under these conditions for heat-processed turkey meat products with (meat product B) and without (model meat product 1) soybean proteins and for the SPI itself. Soybean proteins in the SPI were separated in nine peaks (1–9). The chromatogram corresponding to the heat-processed turkey meat product containing soybean proteins presented two peaks at retention times close to peaks 7 and 8 of SPI that did not appear in the heat-processed meat product without soybean proteins. Nevertheless, the comparison of ultraviolet light spectra and derivatives of these peaks in the SPI and the meat product containing soybean proteins yielded different signals. These differences were attributed to interferences from meat components. Although the modification of the gradient improved the separation of target peaks (peaks 7 and 8), this was not enough to eliminate these

interferences. For that purpose, the solubilization media was modified. Water, 50 mM phosphate buffer (pH 7), 50 mM Tris-HCl buffer at pH 8 and 9, and 50 mM bicarbonate buffer at pH 10 and 11 were used and the chromatograms obtained with every one for a heat-processed turkey meat and the SPI are showed in Figures 2(a) and 2(b), respectively. In comparison with Figure 1, SPI separates in less peaks being now peaks from 6 to 9 eluted in two peaks and being the peak at 1.70 min (signalled in Figure 2 with an arrow) that enabling the detection of soybean proteins in meat products. The highest response of this peak and better resolution between this peak and neighbouring peaks were observed at pH 8 with Tris-HCl buffer. Chromatograms obtained when injecting into the chromatographic system extracts from SPI and heat-processed turkey meats with and without soybean proteins using the optimized extraction media are shown in Figure 3. The peak at 1.70 min appearing in the heat-processed meat product with soybean proteins presented an ultraviolet light spectra and first and second derivatives identical to the peak at the same time in the SPI.

Detection of soybean proteins in heat-processed products containing pork and turkey meat

The optimized conditions for the detection of soybean proteins in heat-processed turkey meat

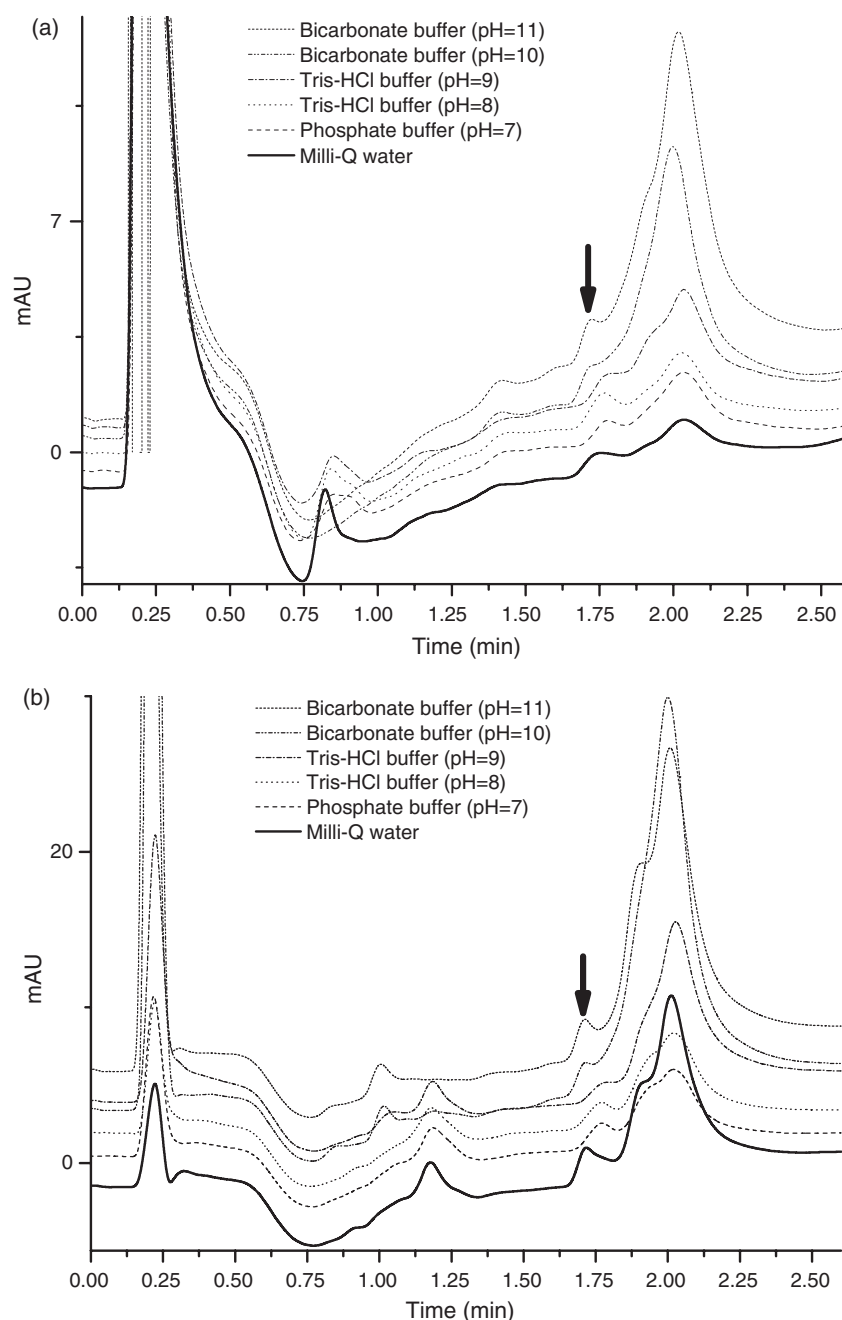


Figure 2. Chromatograms corresponding to a heat-processed turkey meat containing soybean proteins (meat product B) (a) and to the SPI (b) when using buffers at different pHs for protein solubilization. Experimental conditions as in Figure 1. The peak at approximately 1.70 min is indicated by a arrow.

products were applied to the detection of these vegetable proteins in more complex meat systems consisted of heat-processed products containing mixtures of pork and turkey meats. Figure 4 shows the chromatograms corresponding to extracts from heat-processed products with and without (model meat product 2) soybean proteins prepared with both kinds of meats. As observed, the peak appearing at 1.70 min in this meat product with soybean proteins was again that enabling the detection of the presence of soybean proteins. Thus, the method enabled the

clear detection of the presence of soybean proteins not only in turkey meat products but also in products containing pork and turkey meats.

Detection of soybean proteins in presence of milk proteins in heat-processed meat products

As stated in the Introduction, milk proteins are sometimes added to meat products in addition to soybean proteins. In order to prove the optimized method enabled the detection of soybean proteins

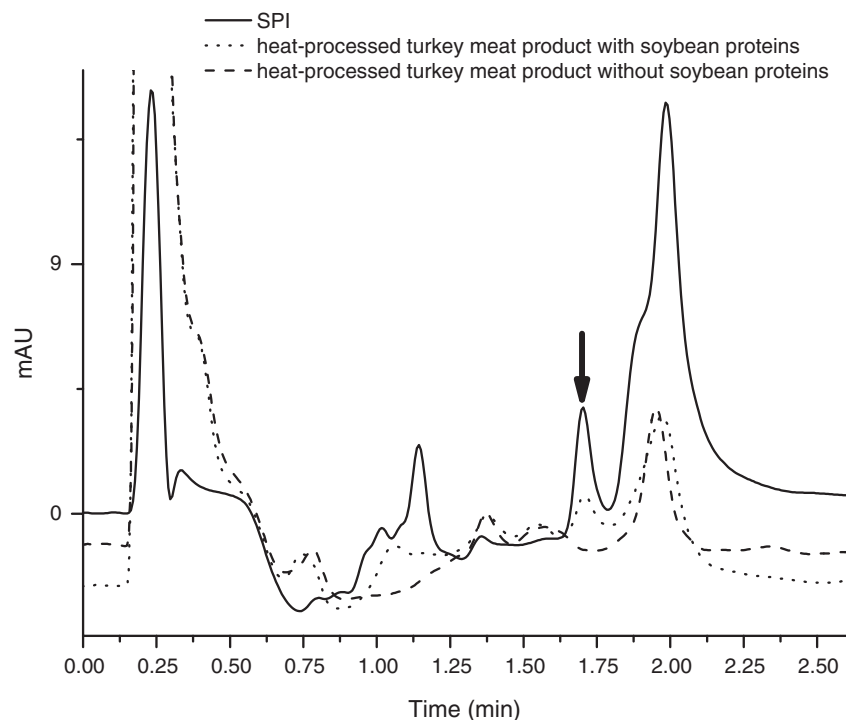


Figure 3. Chromatograms corresponding to heat-processed turkey meats with (meat product H) and without soybean proteins (model meat product 1), and to the SPI. Chromatographic conditions as in Figure 1. Samples were defatted with acetone and the proteins were extracted in 50 mM Tris-HCl buffer (pH 8) for 10 min.

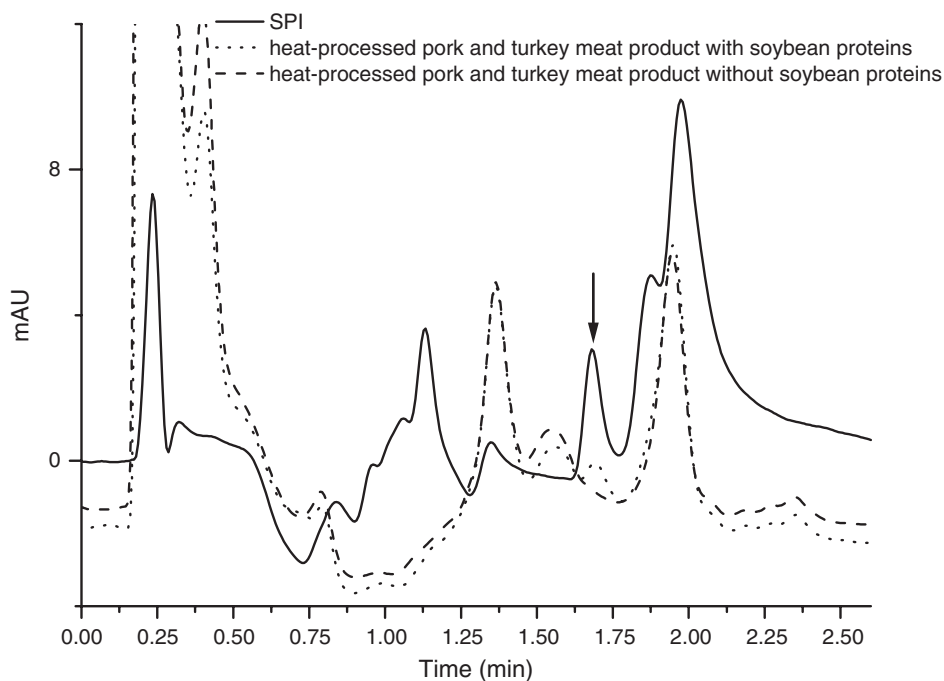


Figure 4. Chromatograms corresponding to extracts obtained from heat-processed products with and without soybean proteins containing mixtures of pork and turkey meats (model meat product 2) and to the SPI. Experimental conditions as in Figure 3.

in meat products containing milk proteins, it was used for the analysis of a commercial heat-processed meat product containing both soybean and milk proteins. Figure 5(a) shows such separation and

the chromatogram obtained for a solution of bovine caseins (which are the majority proteins in cow milks). Bovine caseins were separated into three peaks (A–C) appearing at the end of the

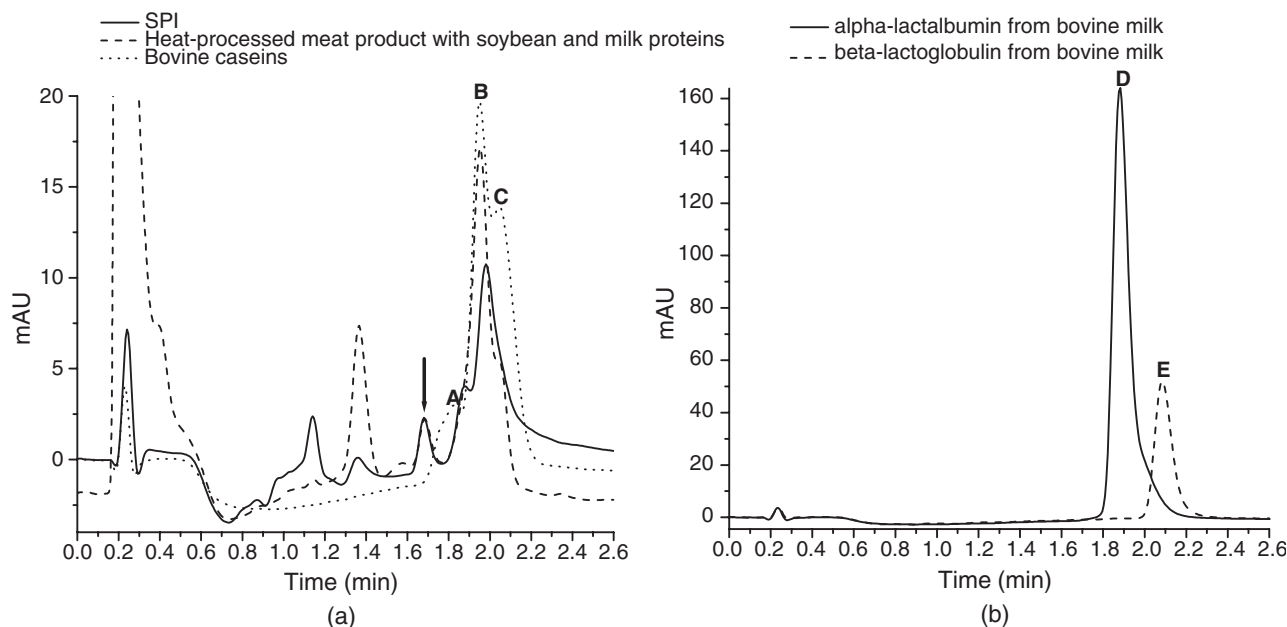


Figure 5. Chromatograms corresponding to a heat-processed meat product containing soybean and milk proteins (meat product Q), to the SPI, and to 1.12 mg ml^{-1} bovine caseins (corresponding to a casein content of 0.5% (w/w) related to 6 g initial product) (a). Chromatograms corresponding to 1.34 mg ml^{-1} α -lactalbumin (corresponding to an α -lactalbumin content of 0.5% (w/w) related to 6 g initial product) and 1.00 mg ml^{-1} β -lactoglobulin (corresponding to a β -lactoglobulin content of 0.5% (w/w) related to 6 g initial product) (b). Experimental conditions as in Figure 3.

chromatogram. Peak at 1.70 min of soybean proteins (indicated with a arrow) did not overlap with the peak appearing at the end of the chromatogram in the meat product, therefore, these proteins seemed not to interfere in detection of soybean proteins. Nevertheless, bovine milk proteins do not only consist of caseins but also of whey proteins (mainly α -lactalbumin and β -lactoglobulin) which, although they are present in a lower proportion, could interfere in the detection of soybean proteins. The chromatograms corresponding to these proteins are shown in Figure 5(b) and presented only one peak (peaks D and E) which appeared at the same retention times than peaks B and C from bovine caseins. Therefore, the method seemed to be useful for the detection of soybean proteins in these meat samples containing milk proteins.

Method validation

In order to assure the proposed method was useful for routine analysis, a standardized validation procedure for quantitative methods for food chemistry laboratories was applied (Feinberg and Ragu  n  s 1999). For this purpose, the SPI Supro 500E was used as standard of soybean proteins and the linearity of the calibration plot, the limits of detection and quantitation, existence of matrix interferences, robustness, precision, and accuracy were evaluated. Tables II and III group the results obtained in the determination of all of these parameters.

The linearity between the signal and the concentration of soybean proteins up to 10.46 mg ml^{-1} was good ($r^2 > 0.99$). Furthermore, the analysis of the residuals and variance of the linear model enabled its validation (p (%) = 5.69) in the linear concentration range. The detection limit, calculated as the concentration corresponding to a signal equal to the intercept plus three times the standard error of the calibration plot, was 0.20 mg ml^{-1} . This concentration corresponded to an addition of 0.08% (w/w) of soybean proteins in a meat product (related to 6 g initial product). The quantitation limit was 0.71 mg ml^{-1} (calculated as the concentration corresponding to a signal equal to the intercept plus ten times the standard error of the calibration plot) which means that the method enabled the quantitation of an addition of 0.28% (w/w) of soybean proteins in a meat product (related to 6 g initial product).

The slopes corresponding to the external standard and the standard additions calibration methods obtained for nine representative heat-processed meat products chosen on a set of 18 were compared for the determination of the presence of matrix interferences (Table III). For that purpose, t - and F -tests were used and t -values and significance levels (p) observed have been included in Table III. Since significance levels were lower than 5% for most heat-processed meat products, the standard additions method was selected for the determination of soybean proteins in these meat products containing

Table II. Characteristics of the proposed perfusion RP-HPLC method for the analysis of soybean proteins in heat-processed turkey or pork–turkey meat products.

Linear concentration range	Up to 10.46 mg ml ⁻¹	
Detection limit	0.20 mg ml ⁻¹ (0.08%, w/w) ¹	
Quantitation limit	0.71 mg ml ⁻¹ (0.28%, w/w) ¹	
Repeatability (RSD, %) (<i>n</i> = 10) ²	Sample ³	
Retention time	0.11	
Peak area	3.62	
Intermediate precision (RSD, %)	Sample ⁴	Standard ⁵
Retention time	0.38	0.27
Peak area	11.3	4.03
Concentration	10.56	—
Internal reproducibility (RSD, %) (<i>n</i> = 3) ⁶	Sample	
Retention time	0.08	
Peak area	0.38	
Robustness ⁷	Conventional parameters	Modified parameters
Column lot	0.72	0.76
Recovery (%) ⁸		
Processed meat spiked with 2.40% soybean proteins	92.8 ± 8.2 (<i>n</i> = 4)	

¹Limits of detection and quantitation expressed as w/w units were determined related 6 g initial product.²Number of injections.³Solution of 230 mg ml⁻¹ of a heat-processed meat product containing pork and turkey meats and milk and soybean proteins.⁴Injection of a solution of 229 mg ml⁻¹ of a heat-processed meat product prepared with pork–turkey meat blends in 3 days in 1 month.⁵Injection of a SPI solution of 3.5 mg ml⁻¹ in 8 days in 3 months.⁶Analysis of three individual samples of 90.6 mg ml⁻¹ of a heat-processed meat product prepared with pork–turkey meat blends in the same day.⁷Protein concentration (mg/100 mg sample) expressed as is basis determined when different lots of column were used.⁸Average recovery of soybean proteins when 2.4% of soybean proteins (from SPI) were added to a heat-processed turkey meat product not containing these proteins.Table III. Existence of matrix interferences using calibration by the external standard and standard additions methods in different heat-processed meat products by perfusion RP-HPLC¹.

Meat product sample	Equation of the straight line obtained by the external standard calibration method ²	Equation of the straight line obtained by the standard additions method ²	<i>t</i> ³	<i>p</i> (%) ⁴	Existence of a proportional bias for $\alpha = 5\%$
A ^{5,6}	$y = -0.559(0.202) + 5.768(0.057)x$	$y = 1.867(0.218) + 3.045(0.177)x$	7.1	0.00	Yes
C ^{5,6}	$y = -0.329(0.189) + 5.330(0.053)x$	$y = 9.058(0.203) + 3.329(0.081)x$	10.6	0.00	Yes
K ^{5,7}	$y = -0.147(0.017) + 5.557(0.045)x$	$y = 5.280(0.136) + 4.009(0.084)x$	6.9	0.00	Yes
L ^{5,7}	$y = -0.147(0.017) + 5.557(0.045)x$	$y = 1.037(0.111) + 4.076(0.066)x$	6.4	0.00	Yes
M ^{5,7}	$y = 0.156(0.219) + 5.667(0.058)x$	$y = 8.645(0.437) + 5.284(0.184)x$	2.0	8.11	No
O ^{7,8}	$y = -0.329(0.189) + 5.330(0.053)x$	$y = 5.024(0.107) + 4.386(0.083)x$	9.6	0.01	Yes
P ^{7,8}	$y = -0.329(0.189) + 5.330(0.053)x$	$y = 8.932(0.112) + 4.395(0.044)x$	13.5	0.00	Yes
Q ^{7,8}	$y = -0.051(0.140) + 5.672(0.039)x$	$y = 8.867(0.092) + 4.771(0.036)x$	16.9	0.00	Yes
R ^{7,8}	$y = -0.051(0.140) + 5.672(0.039)x$	$y = 13.42(0.246) + 4.509(0.097)x$	7.03	0.00	Yes

¹Experimental conditions as in Figure 3.²Errors in the slope and intercept of the regression lines are given in parentheses.³Calculated *t*-value.⁴Significance level (*p*) associated.⁵Heat-processed meat products containing turkey meat.⁶Heat-processed meat products containing soybean proteins.⁷Heat-processed meat products containing soybean and milk proteins.⁸Heat-processed meat products containing pork and turkey meats.

turkey meat and pork–turkey meat blends. In order to minimize these matrix interferences, the amount of initial sample used in these experiments for the preparation of standard solutions in standard additions calibration was reduced up to 0.5 g. Despite this, results observed did not significantly improve.

Precision of the method was determined by the evaluation of repeatability and intermediate

precision. The repeatability in peak area and retention time (expressed as relative standard deviation (RSD, %)) for ten consecutive injections of an extract obtained from a heat-processed meat product with soybean proteins was better than 0.20% in retention time and than 4.00% in peak area. In order to evaluate the intermediate precision one meat solution was injected in 3 days during 1 month and

Table IV. Soybean protein content determined in turkey or pork–turkey meat products by the official ELISA method and the proposed HPLC method¹.

Meat product	Protein concentration (mg/100mg sample) ²	
	ELISA ³	Perfusion HPLC ⁴
A ^{5,6}	0.98	0.62(0.08) ⁷
C ^{5,6}	1.04	0.87
D ^{5,6}	1.18	1.09
F ^{5,6}	1.12	1.11 ⁸
G ^{5,6}	1.67	1.18
Model meat product 3 ^{5,6,9}	0.63	0.75
K ^{5,10}	0.61	0.68 ⁸
L ^{5,10}	0.34	<LDQ*
M ^{5,10}	0.86	0.69 ⁸
Q ^{10,11}	0.70	0.82 ⁸
R ^{10,11}	1.22	1.31

¹All meat products were heat-processed with the exception of model meat product 3.

²Results expressed as is basis.

³Determined following the official AOAC method 998.10.

⁴Determined by the proposed method.

⁵Meat product containing turkey meat.

⁶Meat products containing soybean proteins.

⁷Mean of three individual determinations; standard deviation given in parentheses.

⁸Mean of two individual determinations.

⁹Raw meat product.

¹⁰Meat products containing soybean and milk proteins.

¹¹Meat product containing turkey and pork meat.

*Quantitation limit (LDQ).

one standard solution was injected in 8 days during 3 months. RSD values observed were better than 0.40% in retention time and better than 12.0% in peak area. The variability in the soybean protein content determined three times in one month was 10.56%. Inter-day reproducibility of the slopes of the straight lines obtained by the standard additions calibrations was 1.86%. Internal reproducibility was determined by injecting three samples of a meat product individually extracted and the RSD obtained was better than 0.40% in peak area and retention time.

The determination of the soybean protein content was performed with an identical column from another lot in order to evaluate the robustness of the method. A *t*-test was applied for comparing the soybean protein contents obtained with both columns observing no significant differences.

Recovery was determined by spiking a commercial heat-processed turkey meat product with 2.40% of soybean proteins (from SPI) observing an average media of 92.8%.

The accuracy of the method was determined by comparison of the soybean protein content obtained by the proposed method and by the official ELISA method (AOAC Official Method 998.10, 1996, 1997, 1998). For this purpose, ten commercial heat-processed meat products and one raw meat product (model meat product 3) were analysed by

both methods and results obtained are shown in Table IV. No statistical differences between the contents determined by both methods were detected when applying a paired *t*-test ($p > 5\%$). Moreover, the slope and intercept of the equation corresponding to the best straight line obtained when plotting the contents determined by the ELISA method versus the obtained by the proposed method did not significantly differ from 1 and 0, respectively (*t*-test). The comparison of the soybean protein contents determined by the proposed method in the raw meat product and in the heat-processed meat products suggested the method was not affected by the processing conditions used in the manufacture of these products containing turkey or pork–turkey meats.

Application to edible samples

The developed method was applied to the determination of the soybean protein content in heat-processed meat products prepared with turkey or pork–turkey meats that could also contain milk proteins. For that purpose, 18 heat-processed meat products commercially available were used and the SPI Supro 500E was employed as standard of soybean proteins. Table V shows the results obtained observing these contents were always lower than 1.31%. Furthermore, Table V also shows the soybean protein contents obtained by the external standard calibration method. These contents were always lower than the obtained by the standard additions method observing that the differences between them ranged from 9 to 28% with the exception of products C and E. Despite this and although the accurate determination of the soybean protein content is obtained only with the standard additions method, the external calibration one could be useful for the rapid estimation of this content or screening in these meat samples. Therefore, only for those samples whose protein contents determined by the external standard method were higher than a certain value (e.g. 1%), the standard additions method could be used to confirm whether these meat products satisfy establish regulations.

Conclusions

The detection of soybean proteins in the presence of pork and turkey proteins and milk proteins has been possible, for the first time, in this work. The analytical method was successfully applied to the determination of additions of soybean proteins in commercial heat-processed meat products containing turkey meat or pork–turkey meat blends and that could also contain milk proteins. The method consisted of the extraction of soybean proteins from

Table V. Soybean protein content determined in different commercial heat-processed turkey or pork–turkey meat products using standard additions calibration and external standard calibration by the perfusion HPLC method.

Processed meat product	Protein concentration (mg/100 mg sample) ¹	
	Standard additions calibration	External standard calibration
A ^{2,3}	0.62(0.08) ⁴	0.47(0.05) ⁴
B ^{2,3}	0.74 ⁵	0.60 ⁵
C ^{2,3}	0.87	0.56
D ^{2,3}	1.09	0.84
E ^{2,3}	1.25	0.81
F ^{2,3}	1.11 ⁵	0.96 ⁵
G ^{2,3}	1.18	0.91
H ^{2,3}	1.02	0.79
I ^{2,6}	0.60 ⁵	0.49 ⁵
J ^{2,6}	<LDQ*	
K ^{2,6}	0.68 ⁵	0.50 ⁵
L ^{2,6}	<LDQ	<LDQ
M ^{2,6}	0.69 ⁵	0.50 ⁵
N ^{2,6}	<LDQ	<LDQ
O ^{6,7}	1.27(0.04) ⁴	1.16(0.01) ⁴
P ^{6,7}	0.84(0.08) ⁸	0.68(0.09) ⁸
Q ^{6,7}	0.82 ⁵	0.69 ⁵
R ^{6,7}	1.31	1.07

¹Results expressed as is basis.

²Heat-processed products containing turkey meat.

³Heat-processed meat products containing soybean proteins.

⁴Mean of three individual determinations; standard deviation given in parentheses.

⁵Mean of two individual determinations.

⁶Heat-processed meat products containing soybean and milk proteins.

⁷Heat-processed products containing turkey and pork meats.

⁸Mean of four individual determinations. Standard deviation given in parenthesis.

*Quantitation limit (LDQ).

the defatted meat product with an aqueous buffer and its injection in a reversed-phase perfusion column. The method enabled the detection and quantitation of additions of soybean proteins up to 0.08 and 0.28% (w/w), respectively, in meat products. The proposed method was proven to be precise, accurate and robust. In comparison with the ELISA method, the proposed method enabled the reliable determination of the soybean protein content in these meat products in the cheapest and simplest way. Furthermore, the analysis time needed in the proposed method is significantly lower than the needed when using the ELISA method. Moreover, the determination of soybean proteins by the proposed method was not affected by the processing conditions used in the manufacture of meat products neither by the soybean protein source added. Consequently, the method can be considered as suitable for routine analysis and an alternative to the ELISA method for the quality control of these products.

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