

Detection of 7S and 11S soy protein fractions by monoclonal antibody-based immunoassays in food

ALVORITA L. BITTENCOURT¹, IRENE FERNANDES², ISABELA R. O. PEREIRA¹, CRISTINA S. HONMOTO¹, MÁRCIA K. TANAKA¹, FLÁVIA S. P. LIMA¹, & DULCINEIA S. P. ABDALLA¹

¹Faculdade de Ciências Farmacêuticas da Universidade de São Paulo, and ²Instituto Butantan, São Paulo, Brazil

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Abstract

A panel of four monoclonal antibodies (MAbs) recognizing antigenic determinants on soy protein was produced and partially characterized. The protein fractions 7S and 11S were extracted and purified from the soy protein isolate and defatted flour of soy, respectively. To obtain hybridomas, popliteal lymph nodes of BALB/c immunized mice (10,0 µg protein/footpad) were excised and fused to SP2-0 myeloma cells. The hybridomas were screened by enzyme-linked immunosorbent assay (ELISA) using peroxidase-labelled rat anti-mouse IgG MAb and cloned under limiting dilution conditions. The specificity of these antibodies was evaluated by Western blotting. Three antibodies produced by stable clones (1H4, 2A8 and 1F9) recognized the subunits α , α' and β of the 7S soy protein fraction and one monoclonal antibody (3F2) recognized the basic subunit of the 11S fraction. ELISA assays with the MAbs anti-7S (IgG2b) and 11S (IgG1) fractions were able to detect the presence of these fractions in soy-containing commercial products as yogurt, milk and juice. The present study showed that these MAbs specifically detect the subunits of the 7S fraction and the basic subunits of the 11S fraction of soy protein and may be used in immunoassays to detect these protein fractions in soy-based products.

Keywords: Soybean, soya, food quality control, ELISA.

Introduction

Soy is a vegetable, originating in China, that belongs to the leguminous family. The seed of the soy consists mainly of proteins (48–50%), lipids (20%), water (4–10%), minerals, vitamins and phenolic compounds, among others (Garcia et al. 1997). The evidence that soy may be associated with the prevention of obesity, diabetes, hypercholesterolemia, cancer, osteoporosis, cardiovascular and renal illnesses, in addition to its low cost and high nutritional value, have all combined to a considerable increase in the consumption of this legume in occidental countries (Keeton 1991, Ishii & Yamaguchi 1992).

Correspondence: Dulcineia Saes Parra Abdalla, Universidade de São Paulo, Faculdade de Ciências Farmacêuticas, Av. Prof. Lineu Prestes, 580, Cidade Universitária – Butantã, 05508-900 São Paulo, Brasil. Tel: +55(11) 3091 3637. Fax: +55(11) 3813 2197. E-mail: dspsa@usp.br

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Although the nutritional attributes of soy are recognized and used in some foods, the industrial interest in its use is based on the functional properties of its proteins. It is known that approximately 80–90% of the total soy proteins are storage globulins and can be fractionated on the basis of their coefficients of sedimentation, determined by ultracentrifugation. Thus, the main protein fractions are named 11S globulin (glycinin; 300–350 kDa), representing 40% of the total storage proteins and 7S globulin (β conglycinin, 180–210 kDa) representing 28% of the total storage protein (Sampson & McCaskill 1985, Garcia et al. 1997). The commercial soy protein-based foods, such as yogurt, soy sauce, soy milk, soy infantile formulas, tofu, miso, natto and tempeh (Anderson & Wolf 1995) are made with the total soy, soy flour, soy protein concentrate and soy protein isolate (Lusas & Riaz 1995).

The advances in the field of immunology applied to food technology demand a constant improvement of the standardized techniques for attaining sensitive, specific, fast and less expensive procedures to be used in quality control of foods. ELISA is one of the most commonly immunoassay used to detect several proteins in biological materials. Although polyclonal antibodies may be used to detect the presence of the antigen in the sample, there is an increasing trend to the use of monoclonal antibodies, because they allow the detection of one specific epitope of the molecule in the complex food matrix. Therefore, four monoclonal antibodies directed against the 7S and 11S soy protein fractions were produced that may have applications for quality control in the food industry.

Materials and methods

Isolation of soy protein fractions

The 7S fraction was purified from the soy protein isolate and the 11S fraction from the defatted soy flour (Ceval Alimentos, São Paulo, Brazil), according to the procedures described by Burks et al. (1988) and Thanh and Shibasaki (1976), respectively, with minor modifications. The 7S fraction was extracted with 20 parts (m/v) of a solution containing 0.03 M Tris-HCl buffer (pH 6.2), 0.001 M dithioerythritol (Sigma Chem. Co., St Louis, USA), kept under agitation, at 9°C for 1 hr; afterwards, the preparation was centrifuged at $35,000 \times g$ at 4°C for 20 min. The pellet was discarded and pH of the supernatant adjusted to 4.8 with 1N HCl (Merck Co., Rio de Janeiro, Brazil) and centrifuged at $35,000 \times g$ for 20 min, at 4°C. After the elimination of the supernatant, the pellet was diluted in 10 parts of 0.03 M/L Tris-HCl buffer (pH 6.2) and pH adjusted for 7.6 with 1N NaOH (Merck Co., Rio de Janeiro, Brazil). This suspension was kept under agitation at 9°C until the pellet dissolved. The pH was adjusted again to 6.2 and the solution was centrifuged at $35,000 \times g$ for 20 min, at 4°C, and supernatant was used as the crude extract. Then, 0.4 M NaCl (Merck Co., Rio de Janeiro, Brazil) was added to this extract and the solution was chromatographed into an affinity column with Concanavalin-Sepharose 4B (Sigma-Aldrich Ltd., St. Louis, USA). The second protein peak eluted from this column was dialyzed against distilled water for 12 h and filtered on 100 kDa membranes. The retained sample in the membrane was dissolved, concentrated under vacuum and fractionated by electrophoresis on a 5–20% gradient SDS-PAGE and followed by Coomassie blue staining. The 11S fraction was extracted and purified from the defatted soy flour that was initially extracted with 20 parts of 0.03 M Tris-HCl, pH 8.0, containing 0.01 M 2-mercaptoethanol (Sigma-Aldrich Ltd., St Louis, USA). This sample was allowed to

rest for 1 hour at room temperature and afterwards was centrifuged at 10,000 rpm for 20 min, at 20°C. The first pellet was rejected and the supernatant considered as the crude extract. The pH was then adjusted for 6.4 with 2N HCl and the sample centrifuged 10,000 rpm for 20 min at 4°C. The pH of supernatant was adjusted for 4.8 and centrifuged again. The supernatant was analysed by 5–20% gradient SDS-PAGE and Coomassie blue staining. To eliminate the possible presence of the soy 2S fraction, whose MW is identical to the basic subunit of the 11S fraction, the supernatant was filtered on a 100 kDa membrane (Filtron Technol. Corp., Northborough, USA). The retained sample on the membrane was dissolved and concentrated under vacuum.

Protein concentration

The total protein concentration in the serum of mice immunized with the soy 7S and 11S protein fractions or in the supernatant of cultures of monoclonal antibodies-secreting hybridomas was evaluated according to the procedure described by Lowry et al. (1951), using bovine serum albumin (Sigma-Aldrich Ltd., St Louis, USA) as standard.

Monoclonal Antibodies (MAbs) Anti-7S and 11S soy protein fractions

Immunization. Three BALB/c mice were immunized by footpad injection with 20 µL, either the 7S or 11S purified fraction (10.0 µg protein/footpad), previously emulsified in an equal volume of complete Freund's adjuvant. The animals received two boosters with the same protein fraction doses diluted with incomplete Freund's adjuvant after two or three weeks, according to the local inflammation. Four days before the cellular fusion, an animal of the group received the last booster containing the protein fraction diluted in saline (10.0 µg protein/footpad). All procedures in this study complied with the ethical guidelines of the institution to experiments with animals.

ELISA. Polyclonal antibodies directed against the soy 7S and 11S protein fractions or the supernatant fluids of hybridomas were screened for species specific antibodies by ELISA, standardized by us. The plates (96 wells, EIA/RIA, Costar, Cambridge, USA) were coated with protein fraction (1.0 µg/well) in sodium carbonate-bicarbonate buffer, pH 9.4 and incubated overnight at 37°C. Excess protein fraction not adsorbed onto plates was removed by washing, followed by blocking with 5% fat-free milk in phosphate saline buffer (PBS), pH 7.2 for 2 h at 37°C. After washing the plates five times with PBS containing 0.5 mL/L Tween 20 (PBS-T, Fluka Chemie AG, São Paulo, Brazil), serial dilutions of the sera or supernatant fluid of hybridomas were added and incubated for 2 h at 37°C. The plates were washed again and horseradish peroxidase-conjugated polyclonal goat anti-mouse immunoglobulin (IgG) antibody (Rockland Immunochemicals for Research, Gilbertsville, PA, USA) diluted 1:1000 (v/v) in PBS-fat-free milk (1%), pH 7.2 was added. The plates were maintained at 37°C for 2 h, washed and the reaction was developed by addition of the substrate (0.53 mg of ABTS/mL in citrate-phosphate buffer, pH 4.5 and 0.25 µL of H₂O₂/mL). After 30 min of incubation at room temperature the extent of reaction was evaluated colorimetrically using the SpectraCount analyser (Packard, Canberra Company, Meriden, USA) at 405 nm.

Production of Mabs. MAbs were produced as described by Köhler and Milstein (1975), with some modifications. Popliteal lymph node cells from immunized BALB/c mice were fused with SP2-O cells (2:1) using polyethyleneglycol 50% (PEG 3000, cat. n° 3015, Sigma-Aldrich Chem. Co., St Louis, USA). Hybrids were selected in RPMI 1640 medium plus 3% HAT (hypoxanthine 10 mM, aminopterin 40 μ M and thymidine 1.6 mM) (Gibco BRL Prod., Gaithersburg, USA) containing 10% foetal calf serum (FCS) (Gibco BRL Prod., Gaithersburg, USA) at 37°C and 5% CO₂. The supernatant fluids were screened for species specific antibodies by ELISA. The clones with the highest O.D. were selected for subsequent expansion and cloned under limiting dilution conditions.

Purification of MAbs anti-7S and -11S soy protein fractions. The supernatant of hybridomas culture was delipidated with 10% dextran sulphate (20 μ L/mL ascitic liquid) and 1.0 M calcium chloride (100 μ L/mL ascitic liquid). The solution was shaken for 15 min and centrifuged at 5,000 g for 10 min. The supernatant was dialyzed in borate-buffer-saline, pH 8.5 and purified by affinity chromatography on protein-A Sepharose (HiTrap Protein-A column, Pharmacia Biotech, Uppsala, Sweden) equilibrated in TBS buffer, pH 8.5. The proteins were eluted with 0.2 M glycine/HCl buffer, pH 2.8 and NaCl 0.15 M. All peaks were collected and concentrated by vacuum. The protein content was determined according to Lowry et al. (1951).

Isotyping of MAbs anti-7S and -11S soy protein fractions. The heavy chain isotype was determined by ELISA, using monoclonal antibodies against different mice immunoglobulins classes and subclasses (Mouse monoclonal antibody isotyping reagent kit, cat. n° ISO-2 (Sigma Chem. Co., St Louis, USA) according to the manufacturer's specifications.

Immunoblotting. The ability of MAbs to recognize antigenic determinants of the soy protein immobilized on membrane of nitrocellulose was evaluated by immunoblot analysis. In this assay, samples of protein fraction, in the average concentration of 3 mg/mL (40 μ L) were subjected to the SDS-PAGE under denaturing conditions and transferred for 2 h to nitrocellulose membranes (Millipore, Bedford, USA) using the New Blot Semi-Dry system (Pharmacia Biotech, Uppsala, Sweden), calculating its amperage in accordance with the area of gel $\times$ 0.8. The efficiency of protein transfer was monitored staining of proteins with Ponceau S (Sigma, St Louis, USA). In this process, the nitrocellulose membrane was treated with 0.5 M Tris-saline buffer (TBS), pH 7.5 containing 5% fat-free milk (Molico®, Nestlé, Araçatuba, Brazil) for 2 h at room temperature in shaker (10 cycles/min). After three washings with TBS (5 min/wash), the membranes were incubated with the supernatant of culture containing the monoclonal antibodies anti-7S and -11S protein fractions for 2 h at room temperature and at constant rate (10 cycles/min) followed by similar washings. Horseradish-peroxidase-conjugate polyclonal rabbit anti-mouse immunoglobulin IgG antibody (Peroxidase Conjugated Affinity Purified anti-mouse IgG, H&L Goat, Rockland Immunochem. Res., Gilbertsville, USA) diluted 1:500 (v/v) in TBS plus 1.0 g/L fat-free milk was then added. The membranes were incubated for 2 h at room temperature, under shaking, washed and the reaction was developed with

0.05% 4-chloro-1-naphthol in 15% methanol (v/v), in presence of 0.03% H₂O₂ (v/v). (Sigma Chem. Co., St Louis, USA).

Detection of soy proteins in commercialized products

In order to standardize the ELISA technique for the purpose of detecting the soy proteins in commercialized products (see Table I), plates of 96 wells (EIA/RAI, Costar, Cambridge, USA) were used. The commercialized products (50 µL, 1 µg of protein/well) were adsorbed on the wells in carbonate-bicarbonate buffer pH 9.6 followed by incubation for 12 h at 37°C, in a wet chamber. Afterwards, the ELISA plates were emptied then blocked with 5% non-fat milk in PBS, pH 7.2 and incubated for 2 h at 37°C. The next step consisted of washing the plates with PBS + Tween 20 (0.05% v/v), the addition of 100 µL of supernatant of the culture of hybridomas producing MABs and incubation for 1 h at 37°C. After a new cycle of washing, the plates were incubated for 1 h at 37°C with 100 µL of anti IgG of mice conjugated to peroxidase (Peroxidase Conjugated Affinity Purified Anti-Mouse IgG H&L, Goat, Rockland Immunochem. Res., Gilbertsville, USA). After incubation, the plates were washed and then 100 µL of a solution containing substrate for peroxidase (ABTS, 2,2'-azino-bis(3ethylbenz-thiazoline-6-sulfonic acid) Sigma-Aldrich, St Louis, USA) was added and incubated for 30 min at room temperature. The absorbance was monitored at 405 nm.

Results

Purification of 7S and 11S fractions from soybean

The analysis by SDS-PAGE in denatured conditions (50V in the loading phase (7%) and 150V in the separation phase (20%)) confirmed the presence of purified α (MW 63.17 kDa), α' (MW 58.06 kDa) and β (MW 42.09 kDa) subunits of the 7S fraction and the acidic (MW 38.8 kDa) and basic (MW 21.04 kDa) subunits of the 11S fraction of soy (Figure 1).

Production of hybridomas

Specific MABs to the soy protein fractions were used as important tools in immunoassays with the purpose of detecting the protein fractions 7S and 11S of soy in commercialized products. In total, 960 hybridomas were generated with 47 of them showing positive reaction to the 7S fraction and 24 for the 11S fraction of soy protein. The three stable clones with the highest O.D. producing antibodies to the 7S

Table I. Soy based commercialized products

Total protein concentration (mg/mL)	Analysed commercial products
11.6	Nestogeno [®] with 5% soy
7.9	Ades [®] fruit juice
15.8	Fruit juice with soy
8.0	Mupi [®] soy based food supplement
16.0	Tonyu [®] yogurt

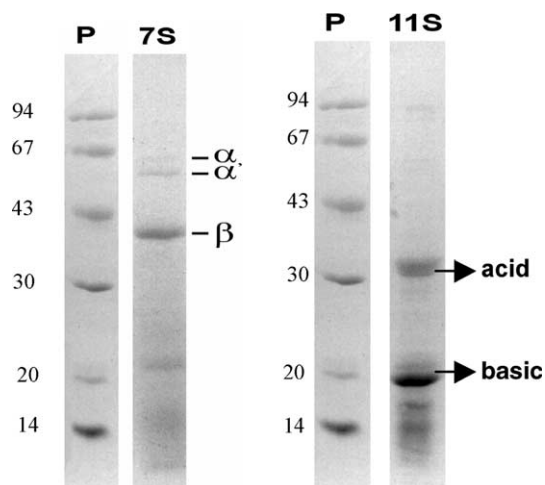


Figure 1. SDS-PAGE of the α , α' e β subunits of the 7S protein fraction and the acidic and basic subunits of the 11S protein fraction of the soy, stained with Coomassie blue. Numbers on the left indicate the migration of Mol. wt markers.

fraction were assigned as 1H4, 2A8 and 1F9, while the one directed to the 11S fraction was denominated 3F2 (Figure 2).

Reactivity of MAbs

The highest titers at which 1H4, 2A8, 3F2 and 1F9 produced an absorbance at least three times greater than negative control assays were 8000; 8000; 12,000 and 10,000, respectively (Figure 3).

Isotyping and specificity of MAbs

The heavy chain isotype, determined by ELISA using antibodies against different mice immunoglobulin classes and subclasses, showed that the MAbs anti-7S fraction

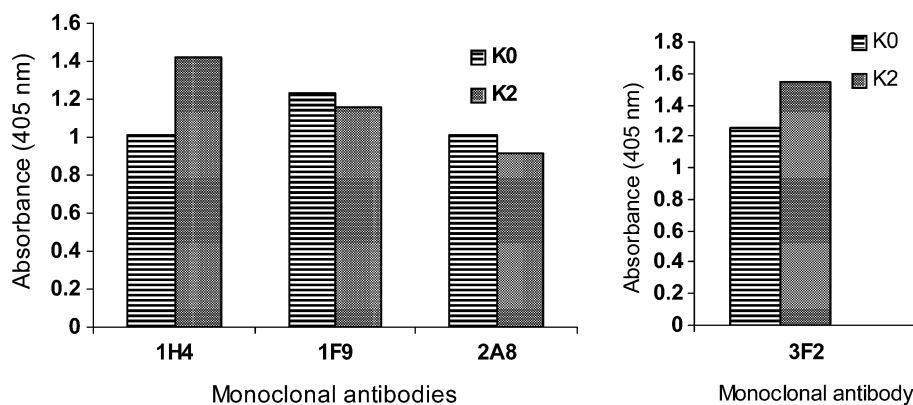


Figure 2. Reactivity of monoclonal antibodies directed against the proteic fraction 7S (1H4, 2A8 and 1F9) and 11S (3F2) of the soy. The data are expressed as the average of the analyses in duplicate, from the ELISA test. Initial KO-hybridoma and K2-hybridoma, after cloning and subcloning, respectively.

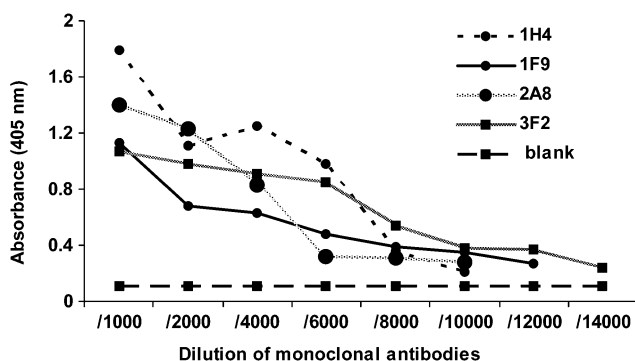


Figure 3. Evaluation of the threshold of reactivity of the monoclonal antibodies directed to the proteic fractions 7S (1H4, 2A8, 1F9) and 11S (3F2) of the soy, for ELISA. The data are expressed as the average of the analyses in duplicate.

was an IgG2b isotype, while the MAb anti-11S fraction corresponded to the IgG1 isotype (data not shown). The specificity of the MAbs, evaluated by immunoblotting, demonstrated that the MAb to the 11S fraction recognized one band of 19 kDa, corresponding to the basic subunit of the 11S soy protein fraction, while, the MAbs against the 7S recognized different antigenic determinants of the soy crude extract. The MAb 2A8 recognized the β subunit, corresponding to 52.8 KDa; the MAb 1H4 recognized the α' and β subunits, with corresponding MW of 77.57 and 52.8 KDa, while the MAb 1F9 recognized the three subunits α , α' and β , of the 7S fraction, with MW of 80.4, 77.57 and 52.8 KDa, respectively. All the MAbs described here recognized linear epitopes, since they reacted only with the protein samples reduced with 2 mercaptoethanol (Figure 4).

Detection of soy proteins in soy-based commercial products

The purpose of this protocol was to evaluate the reactivity of MAbs directed against 7S and 11S soy protein fractions and the recognition of soy proteins present in some commercialized products using the ELISA test. The results show that MAbs recognized the proteins present in different soy based-products as yogurt (Tonyo[®]), soy extract (Mupi[®]), infantile formulae with 5% soy (Nestogeno[®]) and juice (Ades[®]) (Figure 5). These same MAbs did not react with soy-free products in aliquots containing up to 5 μ g of protein per well (data not shown).

Discussion

The main components of soy are lipids (20%) and proteins (48.5%). Soy protein has one of the best essential amino acid standards, amongst proteins of vegetal origin, being sufficiently similar to the essential amino acid standard of high quality proteins of animal origin. Soy protein fractions may be detected by several methods, including polyacrylamide gel electrophoresis (Burks et al. 1988), capillary electrophoresis (Wong et al. 1994, López-Tapia et al. 1999), high performance liquid chromatography (Garcia et al. 1998), immunoblotting, ELISA (Burks et al. 1988) and immunoassays (Lipp et al. 2000, Valdivieso-Garcia et al. 2001). The advantages of using monoclonal antibodies-based immunoassays are the low cost, rapidity of the analysis, good

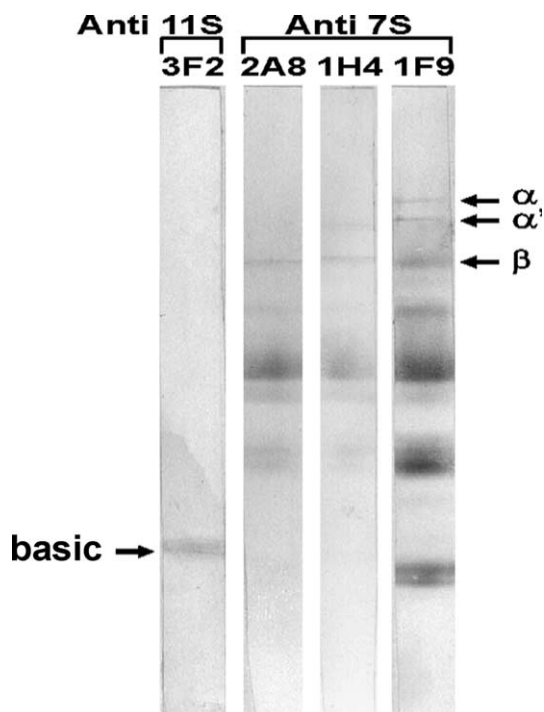


Figure 4. Specificity of MAbs against the 7S and 11S proteic fractions of the soy, evaluated by Immunoblotting.

sensitivity and specificity and the possibility of analysing a high number of samples at the same time, which is very useful for monitoring quality of food processing. Thus, the development of new methodologies is relevant for food control including the detection of soy proteins in industrialized products.

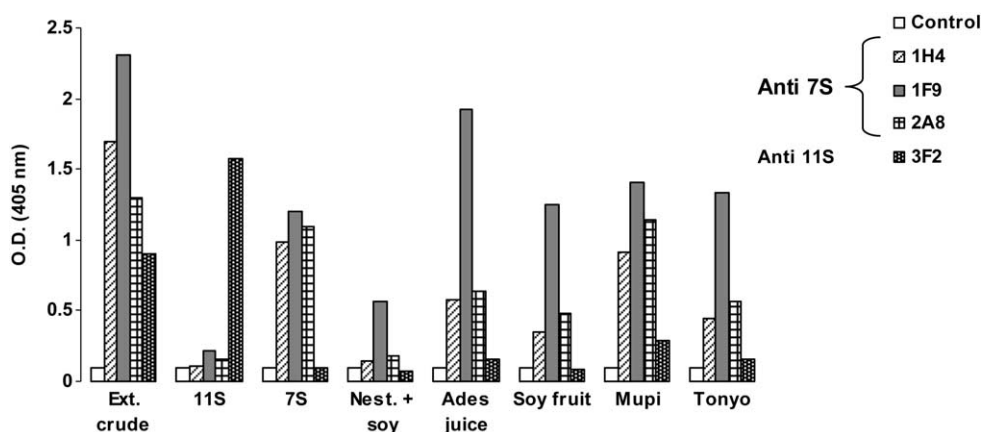


Figure 5. Identification of soy proteins present in commercialized products by MAbs 1H4, 2A8, 1F9 (anti-soy 7S fraction) and 3F2 (anti-soy 11S) in the ELISA test. The data are expressed as the mean of the analyses in duplicate.

In this work, the extraction and purification of the 7S and 11S soy protein fractions were successfully carried out as indicated by SDS-PAGE showing the molecular weights of the subunits α (63.17 kDa), α' (58.06 kDa) and β (42.09 kDa) of 7S fraction, as well as, the acid (38.8 kDa) and basic (21.04 kDa) subunits of the 11S fraction (Figure 1). Our results are in accordance with previous reports (Burks et al. 1988, Thanh & Shibasaki 1976), regarding to the subunits of 7S fraction: α (57–76 kDa), α' (57–83 kDa) and β (42–53 kDa) and the acidic and basic subunits of the 11S fraction, 34–45 kDa and 19–22 kDa, respectively (Garcia et al. 1997), assures the use of these preparations for MAb's production. After purification, 7S and 11S soy protein fractions were used as immunogens and four MAbs were obtained being three against 7S (1H4, 2A8 and 1F9) and one against 11S (3F2) soy protein fractions. The MAbs reactive to 7S subunits (1H4: β subunit; 2A8: subunits α' and β ; 1F9: subunits α , α' e β) and 11S fraction were used in immunoassays to detect these specific subunits in the commercial products (see Figure 4). Thus, ELISA assays were used to detect the 7S and 11S soy protein fractions in commercialized products (see Figure 5). The products analysed here showed that the monoclonal-based ELISA tests detected 7S and 11S proteins at concentrations up to 1 $\mu\text{g}/\text{well}$ and no positive reaction was found for products containing no soy proteins.

The official control of food safety and quality is the most important tool for the governmental protection of public health and consumers rights in foodstuffs business sector. The production of foods for an increasingly informed and selective consumer requires coordinated activities of several branches of the food chain in order to provide convenient, wholesome, tasty, safe and affordable foods (Pridmore et al. 2000). Thus, the control of quality is essential in the food industry to analyse the efficiency of the changes implanted during the processing or the maintenance of the food characteristics. In this context, immunoassays have been used to detect the content of trypsin inhibitors of processed soy products, the genetic identity of soybean seeds and the food-processing effects, among other applications (Brandon & Friedman 2002). Immunochemical techniques have enabled rapid qualitative and quantitative detection of soy proteins in complex foods. Thus, the identification of 7S and 11S soy protein fractions, will be useful in the food industry when the preparation of products excluding one of these fractions is needed. For instance, some studies have shown that 7S (Ogawa et al. 1991) and 2S (Shibasaki et al. 1980), fractions may be allergenic for animals and humans. In these conditions, food containing no allergenic fractions will be very helpful to avoid a soy allergy response in these consumers. Thus, the sensitive and specific monoclonal antibody-based immunoassays described here may be used for quality control of products containing soy or soy-derived ingredients in the food industry.

References

- Anderson RL, Wolf WJ. 1995. Compositional changes in trypsin inhibitors, phytic acid, saponins and isoflavones related to soybean processing. *J Nutrition* 125:581S–588S.
- Brandon DL, Freidman M. 2002. Immunoassays of soy proteins. *J Agricultural Food Chemistry* 50:6635–6642.
- Burks AW, Books JR, Sampson HA. 1988. Allergenicity of major component proteins of soybean determined by enzyme-linked immunosorbent assay (ELISA) and immunoblotting in children with atopic dermatitis and positive soy challenges. *J Allergy Clin Immunol* 81:1135–1142.
- Garcia MC, Torre M, Marina ML, Laborda F. 1997. Composition and characterization of soybean and related products. *Crit Rev Food Sci Nutrition* 37:361–391.

- Garcia MC, Torre M, Marina ML. 1998. A perfusion reversed-phase chromatographic method for ultrarapid determination of soybean proteins in soybean infant formulas and soybean milks: Method development and validation. *J Chromatographic Sci* 36:527–534.
- Ishii T, Yamaguchi M. 1992. Nutritive value of soy milk protein and hypocholesterolemic effect of soy milk in growing rats. *Eiyogaku Zasshi* 50:347–351.
- Keeton JT. 1991. Altering fat composition of red meat and fish products. In: Beitz DC. Food, fats and health. Council for Agricultural Science and Technology. Task force report CAST 118:42–51.
- Köhler G, Milstein C. 1975. Continuous cultures of fused cells secreting antibody of predefined specificity. *Nature* 256:495–497.
- Lipp M, Anklam E, Stave JW. 2000. Validation of an immunoassay for detection and quantitation of a genetically modified soybean in food and food fractions using reference materials: Interlaboratory study. *J AOAC Int* 83:919–927.
- López-Tapia J, García-Risco MR, Manso MA, López-Fandiño R. 1999. Detection of the presence of soya protein in milk powder by sodium dodecyl sulfate capillary electrophoresis. *J Chromatog A* 836:153–160.
- Lowry OH, Rosebrough NJ, Farr AL, Randall RJ. 1951. Protein measurement with the Folin phenol reagent. *J Biol Chem* 193:265–275.
- Lusas EW, Riaz MN. 1995. Soy protein products: Processing and use. *J Nutrition* 125:573S–580S.
- Ogawa T, Bando N, Tsuji H, Okajima H, Nishikawa K, Sasoka K. 1991. Investigation of the IgE-binding proteins in soybeans by immunoblotting with the sera of soybean-sensitive patients with atopic dermatitis. *J Nutrit Sci Vitaminol* 37:555–565.
- Pridmore RD, Crouzillat D, Walker C, Foley S, Zink R, Zwahlen MC, Brüssow H, Pétiard V, Mollet B. 2000. Genomics molecular genetics and the food industry. *J Biotechnol* 78:251–258.
- Sampson HA, McCaskill CC. 1985. Food hypersensitivity and atopic dermatitis: Evaluation of 113 patients. *J Pediatrics* 107:669–675.
- Shibasaki M, Suzuki S, Tajima S, Nemoto H, Kuroume T. 1980. Allergenicity of major component proteins of soybean. *Int Arch Allergy Applied Immunol* 61:441–448.
- Thanh VH, Shibasaki K. 1976. Major proteins of soybean seeds: A straightforward fractionation and their characterization. *J Agric Food Chemistry* 24:1117–1121.
- Valdivieso-García A, Riche E, Abubakar O, Waddell TE, Brooks BW. 2001. A double antibody sandwich enzyme-linked immunosorbent assay for the detection of *Salmonella* using biotinylated monoclonal antibodies. *J Food Protein* 64:1166–1171.
- Wong TM, Carey CM, Lin SHC. 1994. Rapid characterization of soy protein and hydrolysates by capillary electrophoresis. *J Chromatog A* 680:413–417.