

The Allergenicity of Soybean-Based Products Is Modified by Food Technologies

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Key Words

Soybean products · Food processing · Texturized soy proteins · Allergenicity

Abstract

Background: Numerous products based on soybean are available and various food technologies are applied for their production. The allergenicity of natural soybean may be modified by these treatments. **Objectives:** To compare the allergenicity of native soybean proteins with those of soy milk and texturized protein products. To show additional allergens. **Methods:** Three commercial products and two infant formulas were studied: Soybean flour, soy milk, texturized soy proteins, two infant formulas; the first containing total proteins and the second containing a soy protein hydrolysate. Sera from 9 patients allergic to soy protein were tested by immunoblotting (IB). IB inhibition was achieved by incubating sera with protein extract from soybean flour. **Results:** The SDS-PAGE profile of soybean flour protein and soy milk showed a difference in the proportions of the various protein fractions, with a higher concentration of 37-kD protein in flour and 33-kD protein in milk. Infant formula 1 contained proteins with a molecular weight below 28 kD. The texturized extract contained high proportions of 31- to 34- and 38-kD proteins. Immunoblot-

ting revealed a lack of allergenicity in infant formula. Sera recognizing the 38- and 50-kD proteins in texturized soy protein also recognized the 37- and 49-kD proteins in soybean flour and in soy milk, suggesting a protein glycation by texturization processes. The 30- to 34-kD band in texturized proteins was devoid of any allergenicity. This study seems to indicate that the 30-kD allergen (Gly m Bd 30) disappears during the production of texturized soy protein. **Conclusion:** All technologies applied to soybean-based products induce striking variation in the protein profile and allergenicity. Texturized protein could lack the major allergen Gly m Bd 30. Further studies or texturization might generate modified technologies in order to create hypoallergenic texturized proteins.

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Introduction

Soybean is a major source of proteins in human and animal nutrition [1, 2]. Numerous products derived from soybean flour are used in food products: ingredients of minced meat, stuffing, pizza flour, soy milk and yogurts, sauces, flavor enhancers, tofu and fermented products.

Allergy to soybean varies in frequency between countries; this may be due to soybean consumption: in the USA, 14% of children allergic to milk [3] and 25% of chil-

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Table 1. Clinical features and allergologic investigations in 6 patients allergic to soybean and 3 patients allergic to legumes and cross-sensitized to soybean

No.	Sex	Age years	Symptoms	Foods	Prick tests, mm					RAST		
					cod.	SF	SM	ST	MM	Cap KU/l	lab. chall. grade	BPCFC (DB or SB) mg to soy flour
1	M	12	anaphylactic shock	ST	2.5	4	10	9	4.5	34.5	+gr 2	-965
2	F	16	acute asthma	ST	2	2.5	4	4	3	12	n.d.	-7,000
3	M	7	asthma, atopic dermatitis		2	15.5	10.5	2	1.5	11	+gr 2	+265 - asthma
4	M	45	anaphylactic shock	chinese meal	3.5	-	-	-	-	1.2	-	-965
5	F	16	anaphylactic shock	soy protein isolate	3.5	2	n.d.	1	n.d.	3.4	-	+9,000 - rhinitis, conjunctivitis activity
6	M	10	facial AE and asthma	soy milk	2	9	9	7	10.5	>100	n.d.	n.d.
7	M	8	vomiting and cough	peanut	2	3	13	10.5	12	13.8	n.d.	n.d.
8	M	3	vomiting, AE	peanut, green peas, lentils	2	2	3	2.5	5	n.d.	n.d.	n.d.
9	M	5	urticaria	green peas, lentils	3	4.5	4	2	3	4.2	n.d.	n.d.

cod. = codein; SF = soy flour; SM = Sojasun milk; ST = texturized soy protein; MM = Modilac milk; Cap = Cap system; lab. chall. = labial challenge; AE = acute edema; DB = double blind; SB = single blind; BPCFC = blind placebo-controlled food challenge; n.d. = not determined.

dren with atopic dermatitis caused by food allergies [4] present soybean allergy. In Italy, soybean allergy is less frequent: it affects only 3% of children with atopic dermatitis caused by food allergies [5–7]. There is a risk of serious shock [8] and of potentially lethal risks due to the ingestion of hidden allergens [9].

Several soybean allergens have been isolated from seeds of defatted flour: the major Gly m Bd allergen (P34) [10, 11], various allergens corresponding to the acid chain of glycinin G1 [12] or glycinin G2 [13], but also allergens specific to soybean that are not present in peanuts, such as proteins with molecular weights of 21, 46 [14] and 70 kD [15]. Other allergens have been identified in lecithins [16].

The allergenicity of food proteins has been shown to be modified by food-processing technologies, such as heating or fermentation. The allergenicity of soy proteins is destroyed in fermented soy products [17, 18]. The aim of the present study is to compare the allergenicity of soybean proteins in different products: soy flour, a soy milk (Sojasun®), two infant formula milk (total proteins and hydrolysate) and a texturized soy protein product (Sojatop®), using the sera of 9 allergic or highly sensitized patients. We found that allergenicity was preserved in soybean-derived products that underwent industrial heating and texturing processes, but we found no evidence that these processes resulted in the development of additional allergenicity.

Materials and Methods

Patient Sera

We studied sera from 9 patients. Specific IgE concentrations differed considerably from one patient to another (1.2 KU/l to more than 100 KU/l). The clinical characteristics of these patients are reported in table 1.

Preparation of Soybean Extracts

We studied various manufactured products derived from soybean: (1) soybean flour (Euronat, France); (2) soy milk (Sojasun®); (3) texturized soy protein (Sojatop®) and two infant formulas: soy milk (Modilac soja®) and soy protein hydrolysate (Prégomine®). We suspended 50 mg of flour or texturized soy protein in 5 ml of buffer pH 8 (0.1 M Tris, 0.14 M NaCl, 2 mM EDTA, 0.05% Triton, 0.05% deoxycholate and 0.1% nonidet P40) by overnight incubation with shaking at 4°C. The suspension was ultracentrifuged for 15 min at 17,000 rpm and then filtered. The soy milk was diluted 1:10 in distilled water and the infant formula milk was reconstituted (200 mg in 5 ml water). The milk (3.75 ml) was mixed with extraction buffer (7.25 ml). The samples were treated as described above. The protein content of the extracts was determined with the pyrogallol red method [19].

SDS-PAGE and Immunoblotting

Extracts were heated at 100°C for 10 min in a classic denaturing buffer (2.5% SDS, 5% mercaptoethanol, 10% glycerol). Electrophoresis was carried out in a PhastSystem (Pharmacia) with a 10–15% polyacrylamide gradient gel (Pharmacia). We loaded the equivalent of 4 µg of protein for each extract. A molecular weight standard consisting of biotinylated proteins (Covalab, Dako) was also run on the gels. After electrophoresis, the proteins were transferred to a cellulose membrane with 0.45-µm pores. The membrane was then saturated by incubation in 5% bovine serum albumin (BSA), 0.05% Tween in phosphate-buffered saline (PBS) for 1 h, followed by overnight incu-

bation at 4°C in a 1:5 dilution of the serum to be tested in 1% BSA, 0.05% Tween. The membrane was washed four times in 0.05% Tween in PBS and was then incubated for 1 h at room temperature in a 1:10,000 dilution of peroxidase-conjugated human anti-IgE antibody (Dako) in 1% BSA, 0.05% Tween in PBS. The membrane was washed four times and then immersed in a solution of luminol in the presence of H₂O₂ (Covalab, Dako) directly on a Kodak Digital Science 1D image analyzer. An image was acquired after an exposure time of 6.6 min (20 captures).

The membrane was then incubated for 15 min in the presence of peroxidase-conjugated avidin (diluted 1:2,000). Then follows the detection procedure described above, to visualize the molecular weight standards in order to determine the molecular weights of the bands observed.

A negative control immunoblot (serum replaced by 1% BSA) was carried out in strictly identical conditions to ensure that there was no nonspecific binding of the secondary antibody. Sera from two nonallergic patients were also tested as negative controls.

Dot Blot and Dot Blot Inhibition

A dot blot was performed with cat allergenic extract. The inhibition by preincubation of the allergic serum with the soy extract was carried out to detect a potential protease activity.

Immunoblot Inhibition

We incubated 500 µl of serum overnight at 4°C in the presence of 500 µl of a protein extract from soybean flour (1.5 mg/ml). As described above, an immunoblot was carried out, with this preblocked serum.

The specificity of the inhibition was controlled by incubating 500 µl of serum overnight at 4°C in the presence of 500 µl of a standardized extract of *Dermatophagoides pteronyssinus* (Allerbio Lab, Varennes-en-Argonne, France). As described above, an immunoblot was carried out with this preblocked serum.

Results

Protein Determination

The final protein concentrations after extraction were 1.5 g/l for soybean flour, 2.9 g/l for texturized soy protein and 2.3 g/l for Sojasun milk. For the infant formula, we took into consideration the concentrations indicated by the manufacturers 1.8 g/100 ml in Modilac soy milk, 2 g/100 ml in Prégomine.

SDS-PAGE

The electrophoretic profiles (fig. 1) of the soybean seed proteins and the Sojasun milk were very similar but the proportions of the various protein fractions were different. The major flour proteins were 24, 33, 37, 49 and 71 kD in size. Those in Sojasun milk were 24, 29, 33, 37, 49 and 71 kD in size. For texturized soy protein, we mainly observed proteins with a molecular weight of 50 kD or less, with a high proportion of 31-, 34- and 38-kD pro-

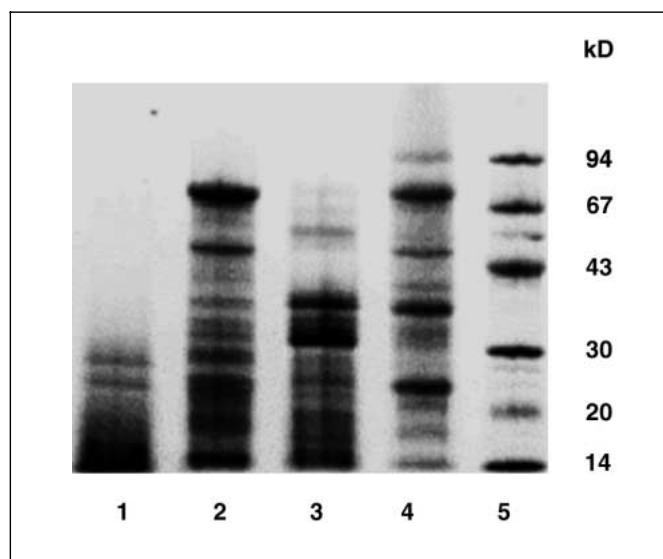


Fig. 1. SDS-PAGE Electrophoresis of the various protein extracts, stained with Coomassie brilliant blue. 1 = Infant formula milk; 2 = Sojasun soy milk; 3 = texturized soy protein; 4 = soybean flour; 5 = molecular mass standards.

teins. Infants formula milk (prepared from a soybean protein isolate) contained exclusively proteins with molecular masses less than 28 kD. No proteins were observed in the Prégomine hydrolysate.

Dot Blot of Cat Extract

No inhibition by the soy extract was observed (data not shown).

Immunoblotting

The control sera showed negative results. The 9 sera tested reacted with the soybean seed proteins and with Sojasun milk (fig. 2). Most of the allergenic proteins were present in both extracts: 24, 33, 37, 49, 71–73 kD. According to the serum tested (table 2), individual variants were identified, A 55-kD allergen was detected among soybean seed proteins in 1 case. A 59-kD allergen, not present in seeds, was detected in Sojasun milk in 3 cases. Immunoblots with texturized soy protein revealed the presence of two main allergens, 38 and 50 kD in size, which reacted with 7 sera (6 sera reacted with a 38-kD protein and 6 sera reacted with a 50-kD protein).

None of the sera reacted with the proteins in the Modilac infant formula.

Prior incubation with a soybean flour protein extract completely inhibited binding to immunoblots of Sojasun

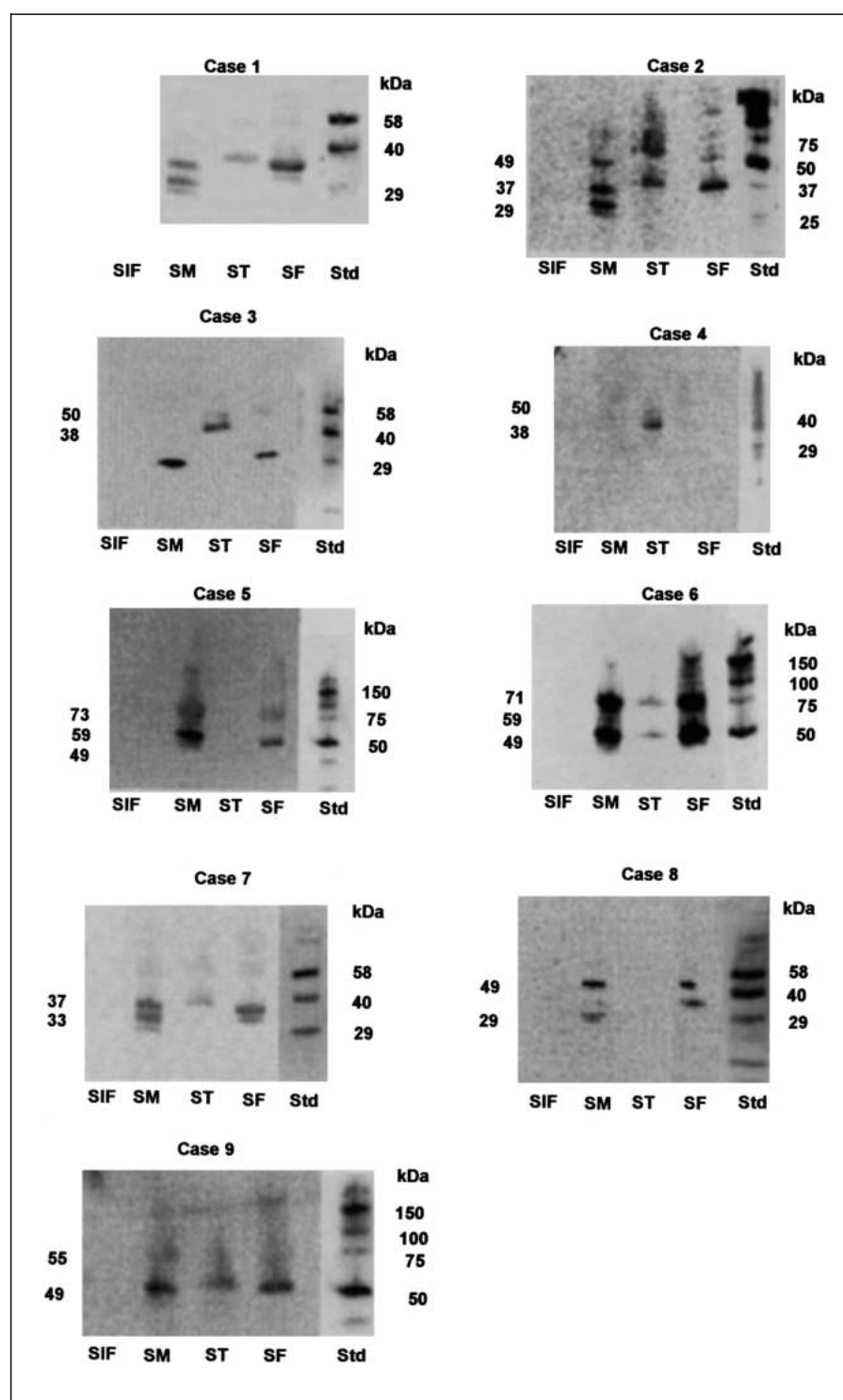


Fig. 2. Immunoblot carried out with 9 patients' sera: after protein transfer, the membrane was incubated with the serum diluted 1:5. Detection by chemiluminescence on a Kodak 1D Digital Science image analyser; the membrane was then incubated with avidin peroxidase in order to visualize the molecular weight standards. SIF = Infant formula soy milk; ST = texturized soy protein; SM = Sojasun soy milk; SF = soybean flour; Std = molecular weight standard.

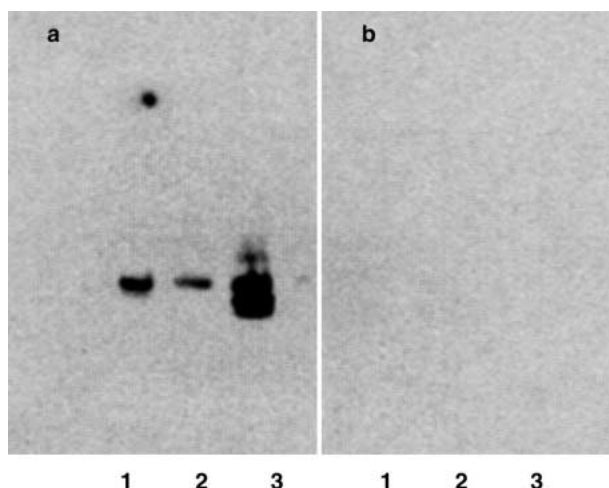


Fig. 3. Inhibition of immunoblot. The immunoblot was carried out as previously described, in the presence of serum from case 1 which was preincubated overnight with an extract of soybean seed proteins at 4°C without pre-incubation (a) or with preincubation (b) of the serum with soybean seed proteins. 1 = Soybean seeds; 2 = texturized soy protein; 3 = Sojasun.

Table 2. Immunoblotting: molecular weight (kD) of the proteins detected by specific IgE of the sera in the various protein extracts

Case No.	Soybean seeds	Sojasun	Texturized soy
1	33-37-49	24- 33-37-49	38
2	37-49-71	29-37 -(49)	38-50
3	31-(40)	33-37-41	(38)-(50)
4	–	–	38-50
5	49-71	49- 59-73	–
6	49-71	24- 49-59-73	50
7	24- 33-37 -(49)	(24)- 33-37	(18)-(27)- 38
8	33-49	29- 49	–
9	49-55-73	49-59	50

Bold and underlined = very strong response; bold = strong response; figures in parentheses = weak; – = no response.

milk and texturized soy protein (fig. 3) except for serum 6, which retained the same response profile: two bands, 49 and 71 kD in size, for soybean seeds and Sojasun milk.

Preincubation with a standardized extract of *D. pteronyssinus* did not inhibit IgE binding to immunoblots of Sojasun milk, texturized soy protein and soybean flour proteins (fig. 4).

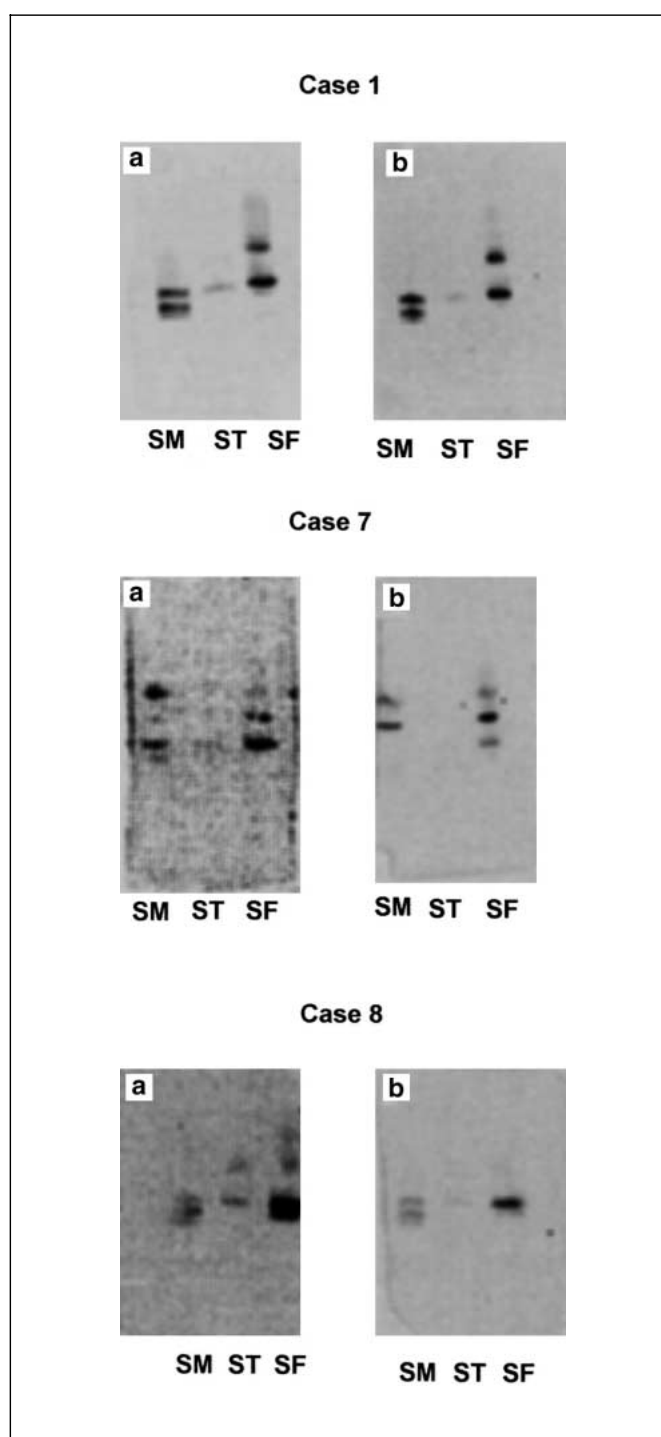


Fig. 4. Inhibition of immunoblot. The immunoblot was carried out, as previously described, in the presence of sera from cases 1, 7, 8 which were preincubated overnight at 4°C with a standardized extract of *D. pteronyssinus* without preincubation (a) or with preincubation (b) of the sera. SF = Soybean flour; ST = texturized soy protein; SM = Sojasun milk.

Table 3. Principal soybean allergens described in the literature

Allergen	Molecular weight, kD	Reference	Nature
Gly m 3	14	12	Profilin
20-kD protein	20	13	G2 glycinin (basic chain) (11S)
21-kD protein	21	13	trypsin inhibitor (2S)
Gly m Bd 28K	28	22	–
Gly m Bd 30K (major allergen)	30–34	10	thiol protease
	40	12	G1 glycinin (acid chain) (11S)
	45	13	20-kD aggregates
	68–70	12	α -subunit of β -conglycinin (7S)
	7, 12, 20, 39, 57	16	protein bands identified in soy lecithin

Discussion

Soybean seeds are rich in storage proteins (37–41%), the composition of which depends on the variety, ranging from 32 to 43% [20, 21] with a mean of 90% globulins (11S, 7S and 2S) and 10% albumins [1]. Glycinin, or 11S globulin, accounts for 31% of the globulins. High-molecular-mass oligomers are constituted by acidic α -subunits (molecular weight 34–45 kD) and basic β -subunits (molecular weight 18–20 kD), linked by disulfide bonds.

The β -conglycinin, or 7S globulin, accounting for 37% of soybean proteins is a trimeric protein, composed of three subunits. The α -conglycinin, or 2S protein fraction, accounts for 22% of total proteins. Other proteins are albumins (7–21 kD) and lectins (120-kD tetrameric glycoproteins composed of 30-kD subunits).

Eight main allergens have been described (table 3) [10, 12, 13, 16, 22].

The seed flour is a commercial product elaborated from a mixture of multiple varieties. It is produced by different processing steps including oil extraction and heating [20, 21]. The preparation of soy milk by grinding seeds in the presence of variable quantities of water, followed by ultrafiltration, has been shown to modify protein content [1]. We then compared a milk available in supermarkets and two infant formulas, Modilac and Prégomine in order to study potential differences of protein profiles. The hydrolysate is commercially available: it is produced from a soy isolate that needed an alkaline extraction. It is said to contain only small peptides of unknown molecular weight.

Moreover, we assessed the allergenicity of soy flour compared to texturized soy proteins, because of the processes involved in the production of texturized soy protein including heating, mechanical pressure and acid treatment (pH 4.5).

The proportions of the various protein fractions markedly differed between the extracts tested with a higher concentration of the 37-kD protein in seed flour and a higher concentration of the 33-kD protein in Sojasun soy milk. No protein band was observed for the soy protein hydrolysate (data not shown). No immunoblot was performed. Modilac infant formula contained only proteins with molecular weights of less than 28 kD (fig. 1).

The protein extract obtained from texturized soy protein displayed a different electrophoretic profile, with a high proportion of 31- to 34- and 38-kD proteins. The process used to produce texturized soy protein confers a fibrous structure on a globulin protein, leading to protein denaturation, with the loss of secondary, tertiary and quaternary structures. Limited proteolysis is also applied to the product [15, 23]. The processes used to treat the natural proteins thus modify both the molecular weight of the proteins extracted and their relative proportions.

Numerous IgE-binding proteins were observed between 24 and 74 kD.

The lack of similarity of the immunoblot profiles obtained from soybean flour and Sojasun suggests quantitative modifications associated with the manufacture of soy milk. In fact, SDS-PAGE showed a 55-kD protein in flour that is absent in Sojasun milk. Correlatively, the IgE-binding 55-kD protein was found in soy flour and not in milk (case 9). Similarly, the 71-kD allergen was detected in soybean flour but not in soy milk (cases 2, 5, 6).

The study of texturized soy proteins was triggered by a clinical case of anaphylactic shock following the ingestion of texturized soy protein (case 1). The immunoblot presented a single protein signal at 38 kD for the extract obtained from texturized soy protein and several bands, 33, 37 and 49 kD in size, for the flour and milk extracts. The 38-kD protein was also detected on four other immu-

noblots, in some cases associated with a 50-kD protein. On the whole, the 38- and 50-kD texturized proteins were recognized by 7 of the 9 sera.

On the other hand, sera recognizing the 38- and 50-kD proteins in texturized soy protein recognized the 37- and 49-kD proteins in soybean flour and in Sojasun. These bands could contain the same allergen: the thermal effect upon protein may induce Maillard reactions leading to Amadori products, as a result of glycation [1, 24]. This linking of carbohydrate residues might explain the difference in molecular weight of allergens in soybean flour and in texturized soy.

It is interesting to note that the 31- to 34-kD band observed in texturized soy does not bind IgE. Moreover, we observed no allergenic activity with Modilac infant formula proteins, even when studying sera which recognized 24-kD proteins in Sojasun soy milk (cases 1, 6, 7).

Total inhibition is observed in sera preincubated with seed protein extracts (8 out of 9 cases). This inhibition is specific since it was not observed when sera were preincubated with standardized extract of *D. pteronyssinus*. Otherwise, the soybean extracts did not inhibit dot blots carried out with extract of cat hairs. The lack of inhibition confirms the absence of protease activity (data not shown). It can then be concluded that the processes used in soybean treatment do not necessarily lead to the appearance of new allergens.

The 38-kD allergen present in texturized soy protein may correspond to the acidic chain of G1 glycinin, which has an estimated molecular weight of 40 kD and is resistant to proteolysis [12]. The allergenicity of glycinin fragments has been confirmed [25].

The Gly m Bd 30K major allergen is described as a 30- to 34-kD protein [10]. This protein retains its allergenicity in vitro after denaturation in 4 M urea [26]. It is found at various concentrations in various containing foods, and resists the various processing treatments as well as the action of trypsin and chymotrypsin in its native state [27]. The 33-kD protein that binds IgE in the extracts of soybean flour and Sojasun could be related to this allergen.

The different processings applied to alimentary proteins affect their allergenicity. Although neoallergens have been shown in some cases, this study confirms that certain technologies applied to soybean modify the relative proportion of the various proteins. The major modifications concern the native allergens: either we observed a decrease or an increase of the allergenicity. Compared with soy milk, the Modilac infant formula thus revealed no allergenicity although it contains proteins with molecular weights up to 28 kD.

Texturization leads to major modifications without IgE binding to the 30- to 34-kD proteins even if IgE binds to 30- to 34-kD proteins in flour and in soy milk. The processes used to texturize soy protein may lead to the disappearance of the major allergen Gly m Bd 30. However, a 38-kD allergenic band was observed which might correspond to a glycinin fragment. Further studies of texturized soy could lead to the development of modified technologies using a complementary enzymatic treatment in order to obtain a hypoallergenic product [17].

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