

Allergenicity of some isoforms of white sesame proteins

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Summary

Background Allergy to sesame seeds is often associated with particularly severe reactions, with a high risk of anaphylaxis. The increase in reports of allergic reactions to sesame is probably due to the growing use of sesame seeds or sesame oil in food.

Objective To determine the molecular weights of the proteins in three variety of sesame seeds and to study the isoelectric points and the allergenicity of white sesame proteins.

Methods Extracts of white, brown and black sesame seeds were prepared. The white sesame extract, mostly used in bakery, was run on SDS-PAGE and two dimensional electrophoresis. Six sera from patients sensitized or symptomatic to sesame seed were used for Western blotting.

Results The protein patterns of the white, brown and black sesame extracts showed major quantitative differences. The white extract had the higher protein concentration and contained 15 proteins of 12–79 kDa, some of them having several acidic isoelectric points. The lowest isoelectric point was 4.9 and the highest was 6.4, giving 35 isoforms. Ten of the 15 proteins (12–57.5 kDa) were recognized by specific IgE. The 12–13 kDa and 22–33 kDa proteins could correspond to the main allergens.

Conclusion White sesame seeds contain at least 10 allergenic proteins with acidic isoelectric points. In accordance with previous results, two of them seem to contain the major allergens.

Keywords sesame seed, food allergen, isoelectric points, variety

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Introduction

Sesame, *Sesamum indicum* L., is a plant originally from tropical Africa that is now universally cultivated for its seeds. There has been a great increase in the consumption of sesame seeds in Europe, and the number and severity of cases of allergies to dietary sesame has also increased dramatically over the past few years [1, 2]. There have also been reports of allergy to sesame oil [3], but few studies have been done on the allergens in sesame seeds.

The preliminary results of Western blots after sodium dodecyl sulphate polyacrylamide gel (SDS-PAGE) of an extract of brown sesame identified three allergens [4].

This study involves three fresh cases of sesame sensitization and three definite cases of symptomatic reactions. It involves a Western blot of white sesame, a variety with a high protein content, after two dimensional electrophoresis. The use of modified extraction conditions made it possible to detect quantitative differences in the protein content of three varieties of sesame and to identify 10 allergens with isoelectric points between 5.1 and 6.4.

Materials and methods

Patients

The sera from six patients allergic or only sensitized to sesame seed were used, separately or pooled (Table 1). Allergy is defined as a hypersensitivity reaction initiated by immunologic mechanisms, and is characterized by clinical reactions [5]. Three sera were from allergic patients who had presented with an episode of anaphylaxis after ingesting sesame seed (patient 1) or with positive challenge tests (labial challenge and double-blind, placebo-controlled food challenge) which complied with both American and European standards (patients 2 and 3) [6–10]. The other three were from sensitized patients, without clinical reaction (patients 4, 5 and 6), and were assessed by a positive skin prick test and the presence of specific IgE measured by RAST (Pharmacia, Uppsala, Sweden). The skin prick tests were done using white, brown and black sesame seed flour [11]. Oral challenges were performed with crushed sesame (white or brown varieties) hidden in mashed potatoes. Placebo was mashed potatoes including a same quantity of crushed crumb to obtain the same aspect.

Protein extraction

Brown, black and white sesame seeds were purchased from a seed retailer and ground in the laboratory. The ground seeds were crushed in 20 mM phosphate buffer (pH 6.0) containing 1 M NaCl, extracted on a rocking shaker for 60 min at 4 °C and then centrifuged. The supernatant was filtered, dialysed and lyophilized [12].

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Table 1. Characteristics of the six patients presenting sesame allergy (sensitized or symptomatic)

Patient	Age	Sex	Symptoms	Prick test (mm) Codeine	White sesame	Brown sesame	Black sesame	RAST (kU/L)	Food challenge to white or brown sesame
1	38	M	Anaphylactic shock	3	5.5	3.5	9.5	15.4 (cl 3)	ND
2	10	F	Atopic dermatitis and asthma	2	5	2	3	10.7 (cl 3)	LC positive DBPCFC positive (965 mg)
3	4	M	Atopic dermatitis and asthma	3	4	2	6	11.5 (cl 3)	LC positive
4	8	M	Atopic dermatitis and asthma	3	3	0	3	23.6 (cl 4)	ND
5	20	M	Atopic dermatitis and asthma	2.5	3	4	3	7.74 (cl 3)	DBPCFC negative (7110 mg)
6	4	F	Atopic dermatitis	2	1	0	1.5	1.03 (cl 2)	ND

ND: Not Done, DBPCFC: Double Blind, Placebo-Controlled Food Challenge, LC: Labial Challenge.

Protein assay

The protein concentration of each extract was measured using the Bradford assay (Bio-Rad, Hercules, CA, USA) [13].

SDS-PAGE

SDS-PAGE was carried out by the method of Laemmli in a vertical slab gel (Bio-Rad) [14]. An acrylamide concentration of 12% or a 4–20% gradient was used. Protein samples were boiled for 5 min in sample buffer (0.125 M Tris-HCl, 4% SDS, 20% glycerol, 10% β -mercaptoethanol, 0.004% bromophenol blue, pH: 6.8). Sesame protein extract (from 5 to 60 μ g) was loaded in each slot and run at constant voltage (150 V). The proteins were transferred to polyvinylidene difluoride paper (Millipore, St Quentin, France) using a semidry apparatus (Bio-Rad), 1 h at 0.3 A.

2D electrophoresis

The extracted sesame protein was first separated by isoelectric focusing (IEF) and then by SDS-PAGE. The sesame extract was incubated for 15 min at room temperature in sample buffer (5.7 g urea in 2 mL 10% Triton X-100, 0.5 mL of β -mercaptoethanol, 100 μ L ampholytes (3/10) and 100 μ L ampholytes (5/7).

The isoelectric focusing gels used N, N'-methylene-bisacrylamide as cross-linking agent, and contained 5.5 g urea, 6.5 mL deionized water and 1.33 mL acrylamide/bis (30% T and 5.4% C), 100 μ L ampholytes (3/10) and 400 μ L ampholytes (5/7). Pre-focusing was performed in a mini Protean II 2-D cell (Bio-Rad) for 10 min at 200 V, 15 min at 300 V and 15 min at 400 V. The sample was then loaded on the basic side of the capillary tube and isoelectric focusing was performed for 10 min at 500 V, then for 3 h 30 min at 750 V.

Strips of IEF gel were incubated for 10 min in equilibration solution containing 12.5 mL Tris-HCl (pH: 6.8), 23 mL 10% SDS, 5 mL β -mercaptoethanol, 8 mL 10% glycerol and 2.5 mL 0.05% bromophenol blue. Proteins were separated by SDS-PAGE on a 12% gel at 150 V for 1 h. Standards of known isoelectric point (pH 4.5–8.5) and molecular weight (17.5–76 kDa) were used (Bio-Rad). The proteins were transferred as described previously.

Western blotting

Western blotting was done on the blots obtained after SDS-PAGE, using each of the six sera, or on the blot obtained by 2D-electrophoresis, using pooled sera, since a large volume is required. The blots were probed with diluted serum (1/5) in PBS buffer containing 150 mM NaCl and 1% bovine serum albumin

with 0.1% Tween-20 (BSA-PBS-T) overnight on a rocking mixer at 4°C. The blots were washed two times for 15 min with PBS-0.1% Tween 20 and incubated with anti-human IgE antiserum conjugated to alkaline phosphatase (Sigma, St Quentin, France) (diluted 1/1000) for 2 h at room temperature. The blots were then washed two times and revealed with 5-bromo 4-chloro-3-indoyl phosphate/Nitro blue tetrazolium.

Two negative controls were included. One strip of white sesame seed extract was incubated with the secondary antibody without any serum to check for non-specific binding. Another strip was incubated with the serum of an individual not sensitized to sesame seed protein.

Results

The extracts contained, respectively, 182, 145 and 28 mg protein per gram of white, brown and black sesame seeds. The electrophoretic profiles of 50 μ g protein from the three varieties showed mainly quantitative differences (Fig. 1). The 22–23.5 kDa band was very abundant in all three types of seeds; the 29 kDa band was intense in the black sesame extract, clearly visible in the brown sesame extract, but very faint in the white sesame extract. In contrast, the 32–36 kDa proteins were abundant in the white and brown sesame extracts, but less evident in the black sesame

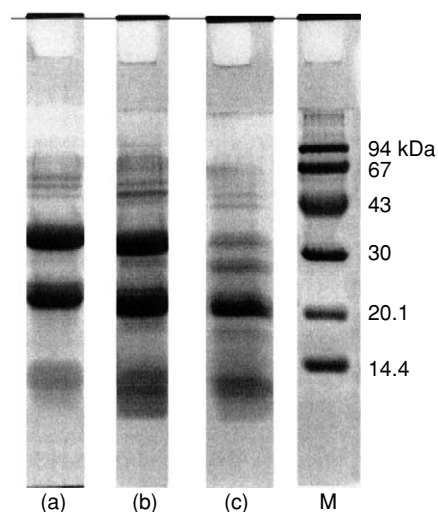
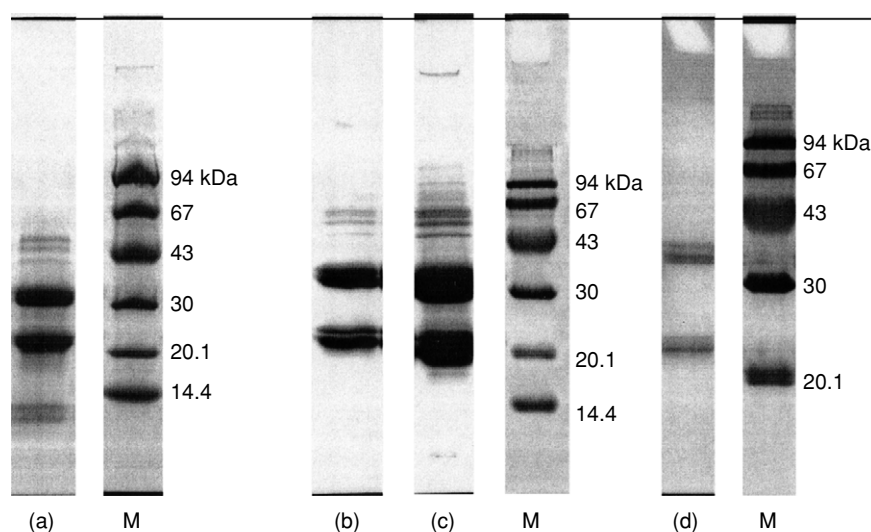


Fig. 1. 12% SDS-PAGE patterns of white (a), brown (b) and black (c) sesame seeds showing quantitative differences within the three varieties (50 μ g protein was placed in each well) (M: Molecular weight standard).

Fig. 2. Test of several SDS-PAGE conditions of white sesame extract. The main proteins are 22–23.5 kDa and 32–36 kDa, while the less concentrated are 12, 13, 16, 19, 29, 47, 53, 57.5, 67.5, 79 kDa (M: Molecular weight standard). (a) 30 µg extract loaded on a 4–12% polyacrylamide gel (b) 20 µg extract loaded on a 12% polyacrylamide gel (c) 60 µg extract loaded on a 12% polyacrylamide gel (d) 5 µg extract loaded on a 20% polyacrylamide gel



extract. A protein of 53 kDa was particularly abundant in the brown sesame extract.

We used different quantities of white sesame extract (5, 20, 30 or 60 µg), and different conditions of electrophoresis (PAGE 4–12%, 12% or 20%) to describe at least 15 proteins of 12–79 kDa (Fig. 2). The main proteins had molecular weights of 22–23.5 and 32–36 kDa, while the less concentrated proteins were 12, 13, 16, 19, 29, 47, 53, 57.5, 67.5, 79 kDa.

The Western blots obtained after SDS-PAGE showed quite similar results with all six sera (Fig. 3). Thus 10 of the 15 proteins (12–57.5 kDa) were recognized by specific IgE in each serum.

Using 2D-electrophoresis, we determined the isoelectric point of proteins up to 36 kDa (Fig. 4a). The lowest isoelectric point was 4.9 and the highest was 6.4, giving a total of 35 isoforms.

The Western blot obtained after 2D-electrophoresis shows 22 allergenic isoforms. Their isoelectric points were between 5.1 and 6.4 (Table 2, Fig. 4b).

Neither of the two negative controls showed any binding (data not shown).

Discussion

An anaphylactic reaction to sesame seed was first reported in 1950 in a patient who had ingested a Turkish honey-based confectionary (halvah), which contains sesame seeds and sesame oil [15]. Since then, there have been many reports of anaphylactic shock [1, 2, 16] or generalized skin rash [17, 18] in people who have eaten sesame seeds. Sesame has also been implicated in asthma in certain professions [19–21]. Allergy to sesame seeds is often associated with particularly severe reactions, with a high risk of anaphylaxis [1, 22]. The increasing consumption of sesame products may account for the growing incidence of cases of immediate-type systemic reactions after the ingestion of sesame seed, as has been reported from Australia, where sesame allergy is now more common than nut allergy [23]. Common foods that contain sesame seeds are vegetarian burgers, oriental food, bread, cakes, pastries, toppings, Parsi seed (tahini), spice mixtures, salad dressings and diet foods.

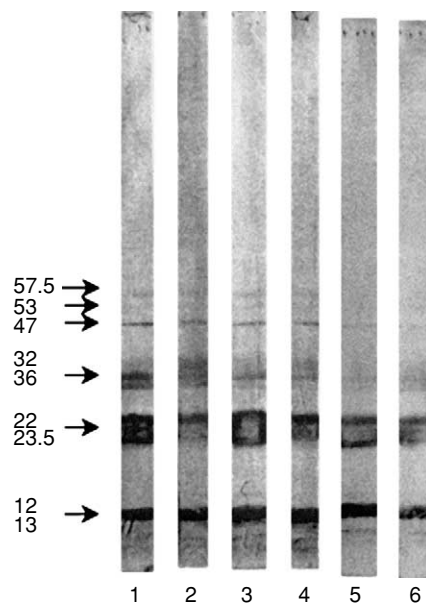


Fig. 3. Western blot of SDS PAGE with serum from each of the six patients sensitized to sesame seed. The number are the individual patients.

Sesame seed oil may also contain allergens, and two cases of anaphylaxis to sesame oil have been described [3, 15].

Little is known about sesame allergens. Certain allergenic structures are common to sesame seeds, hazelnut, rye grain, kiwi, and poppy seeds [24]. Some patients allergic to sesame seeds also have positive prick tests with soybean and peanut [11]. In 1981, Malish et al. studied four sera. They identified nine allergens of 8–62 kDa using relative RAST inhibition after density-gradient ultracentrifugation of sesame seed extract and crossed electrophoresis [25].

Three other recent studies have investigated sesame allergens. The first involved just one serum and described a single 14 kDa IgE-binding component in crushed sesame seeds, but did not give any information about the variety of seeds or on the extraction buffer [20]. In 1997, Kolopp-Sarda et al. made

Table 2. Number of isoproteins and isoelectric points of allergens

MW(kDa)	Number of isoproteins	Isoelectric points of allergens
12	1	6.0
13	3	6.0
16	2	—
19	4	—
22	2	5.9, 6.1
23.5	3	5.6, 5.9, 6.1
29	3	—
32	2	5.5, 5.7
34	4	5.8, 6.0, 6.1, 6.3
36	11	9 from 5.1 to 6.4

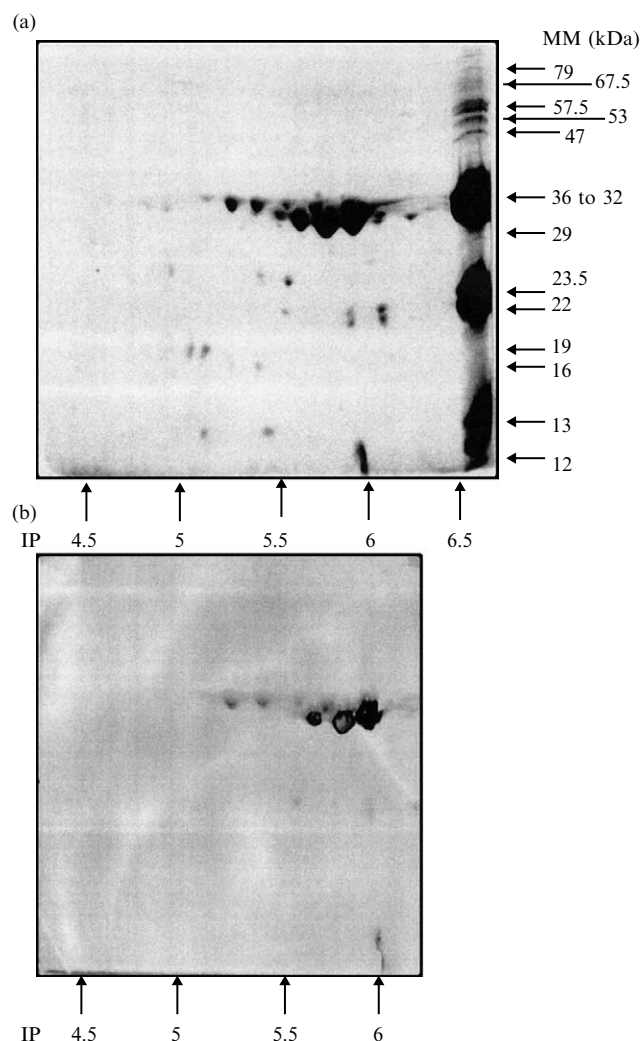


Fig. 4. (a) 2D-PAGE electrophoresis of white sesame seed extract. We used the same extract in the place of the molecular weight standard. Isoelectric points are comprised between 4.9 and 6.4 (vertically: molecular mass, MM; horizontally: isoelectric points, IP) (b) Western blot of 2D-PAGE electrophoresis with pooled sera from all six patients sensitized to sesame seed, showing 22 allergenic isoforms. Their isoelectric points are between 5.1 and 6.4.

extracts of brown, black and white sesame seeds and found the same SDS-PAGE patterns in the three varieties [4]. They identified 19 protein bands with molecular weights of 13.5–96 kDa. Western blot analysis was done using brown sesame extract and the sera of 10 sesame-sensitized individuals. Six of the sera recognized a 25-kDa protein which the authors indicated as a major allergen, two sera recognized a 30-kDa protein, and one recognized a 14-kDa protein.

Another study based on a single serum, reported several bands of 10–97 kDa in a sesame seeds extract, with intense IgE binding activities at about 10, 15–20 and 30–67 kDa [2]. There were bands with less activity at about 12 and 25 kDa. The authors suggested that the 10–12 kDa band was an allergen common to sesame seed and poppy seed extracts.

White seeds have a much higher total protein content than black seeds (182 mg/g vs. 28 mg/g). The SDS-PAGE patterns of brown, black and white sesame seed extracts also show quantitative differences in the proteins they contain, particularly in the 32–36 kDa range. White sesame seeds were used for further studies, because of their use in baking, and because of their high protein content. Differences observed in different studies may be due to the variety [2, 4, 26] and/or to the extraction conditions [27] used. The extraction buffer is described in one paper: 0.1 M Tris-HCl (pH 7.4) with 0.14 M NaCl containing deoxycholate, Triton X100 and Nonidet P40 [4], but no information is given about the extraction methods used in the other papers. We used ground white seeds crushed in 20 mM phosphate buffer (pH 6.0) and extracted with 1 M NaCl for 60 min at 4 °C. By using different SDS-PAGE conditions (quantity of protein loaded, pore size of the gel), we were able to optimize the resolution of the gel for the low or high molecular weight proteins separately.

Numerous IgE binding proteins were identified by this study: 12, 13, 22, 23.5, 32, 34, 36, 47, 53 and 57.5 kDa. The 12–13 kDa proteins seem to be very important, as the binding obtained with six sera was intense, although SDS-PAGE did not give a very marked band. Other intensely immunoreactive proteins were found at 22–23.5 kDa. These findings correlate closely with those of the previous study [4]. Major allergens could be present in these two bands.

Numerous isoforms were detected by 2D electrophoresis, and the 32–36 kDa proteins gave a total of 17 isoforms with pI of 5.1–6.4. Only the less acidic isoforms displayed IgE binding potential.

Conclusion

The total protein and allergen contents were shown to vary with the variety of sesame.

Using white sesame seeds, which are currently used in bakery products, the study indicated two main bands at 12–13 kDa (pI 6.0) and 22–23.5 kDa (pI 5.6, 5.9, 6.1) as likely to contain major allergens. However, the 2D-electrophoresis completed by Western blot indicates few isoallergens in these bands, but highlights the presence of 15 isoallergens (pI from 5.1 to 6.4) in the 32–36 kDa band. This suggests that a thorough assessment of the allergenicity of foods calls for a study of the Western blot based on combined SDS-PAGE and isoelectrophoresis.

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