

Review

Implications of Allergic Reactions to Soybean Oil

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Abstract

Soybeans oil both cold-pressed and fully refined oils have been shown to contain proteins. Although most publications suggest that refined oils do not induce allergic reactions in sensitive individuals, adverse reaction to soy oil in an infant has been reported. Its used in cooking and food formulations. The presence of protein in soy oil depends on the degree of refining, as for other seed oils.

There seems to be agreement that fully refined soybean oils are not allergenic but our groups recently have found cases of anaphylaxis due to soybean oil presented in generic drugs and anaphylaxis during the night that was caused by an unexpected exposure to soybean oil included in their pillows. Skin prick tests and determination of specific Immunoglobuline IgE to soy were negative but molecular analysis by microarrays resolved the diagnosis.

Keywords: Allergic reactions; *Glycine max*; Soy bean; Soy bean oil

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Introduction

Soy (soybean) (*Glycine max*) is an edible legume belonging to the Fabaceae family. The seed contains around 20% oil and 38 to 40% protein. Consumption of soy, widespread in Asia and the US, has increased in Europe during the past years particularly [1,2].

More than 90% of food allergies are caused by eight foods: cow's milk, hen's egg, soy, peanuts, tree nuts, wheat, fish, and shellfish. The diagnostic workup for a patient with suspected food allergy includes a detailed medical history, physical examination, food allergy screening tests, and responses to an elimination diet and an oral food challenge. None of the screening tests, alone or in combination, can definitely diagnose or exclude a food allergy [3]. Novel microarrays diagnostic methods including those that focus on immune responses to specific food proteins or epitopes of specific proteins are under active study.

Soy bean is the world's most important legume. Protein and soy oils are widely used in animal and human nutrition, pharmaceutical industry and in different manufactures textiles [1,2]. Soybean oil is used in cooking and food formulations. The presence of protein in soy oil depends on the degree of refining, as for other seed oils.

There is a certain consensus that fully refined soybean oils are not allergenic but our groups recently have found cases of anaphylaxis due to soybean oil presented in generic drugs and anaphylaxis during the night that was caused by an unexpected exposure to soybean oil included in their pillows.

Clinical Properties of Soy Bean Oil

Soy is a leguminous but it is also an oilseed with a high fat content, 20% dry matter, the second highest content among all food legumes (the highest oil content is found in peanut, 48%; then chickpea, 5%, and other food legume species in the range of 1-3.6%) [4]. thus, the average composition of the soybean is (% dry weight): fat 21% (located mainly in the cotyledon and in the hypocotyls), protein 40.3%, carbohydrates 33.9% and ash 4.9% [5].

Table 1 shows the composition of crude and refined soybean oil. Soybean oil consist primarily of triglycerides (>99% in refined oils), some phospholipids and some minor compounds such as phytosterols and tocopherols. Triglycerides are formed mostly by three unsaturated fatty acids (65.3% of all TG, wt) or two unsaturated FA (31% wt) [6]. Thus, soy oil stands out by a low content in saturated fats (15.5%, mainly palmitic acid) and high in unsaturated fats (85.5%) (Particularly oleic acid, 21% and polyunsaturated acids, 58.5%). Among the polyunsaturated fats, soy oil is rich in n-6 like linoleic acid (53% in weight) and in n-3 like linolenic acid (8% in weight). There are some variations in the composition because of varietal and seed maturation temperature effects, and feed of animal (Table 2) [7].

Phospholipids like lecithin are present in crude soybean oil (1.5-2.5%) and are eliminated during oil refining (during degumming). In fact, lecithin is a co-product of soybean and corn oil processing (Table 1) [7].

The unsaponifiable fraction of soy oil is 0.3-1.6%. The main phytosterols (0.13-0.33%) in soy oil are β -sitosterol (1317 mg/kg), campesterol and stigmasterol (with around 50% of the β -sitosterol each) [8]. Phytosterols have healthy effects in the diet, decreasing cholesterol absorption in the intestine [9-12].

Soy oil has tocopherols, minor components with antioxidant activities, with the γ being in the highest concentrations, close to 1000 mg/kg (crude oil), followed by δ (around 400 mg/kg) and α (66.5-90.7 mg/kg, according to the variety) [5]. Tocopherols are decomposed during vegetable oil processing but a large proportion is left in the finished oils. The content of mixed tocopherols is quite high and range between 550 and 1000 $\mu\text{g/g}$ in Refined, Bleached and Deodorized (RBD) oils [12]. This value is above the optimum range of 400-600 $\mu\text{g/g}$ for antioxidant activity [10]. The tocopherol content in several oils, including soybean, may be adequate to protect the oils against oxidation under ambient conditions, while in others like olive and peanut oils the levels (110 and 200 $\mu\text{g/g}$, respectively) are not sufficient [11].

Components	Crude oil	Refined oil
Triglycerides (%)	95-97	> 99
Phospholipids (%)	1.5-2.5	0.003-0.045
Unsaponifiable matter (%)	1.6	0.3
Phytosterols	0.33	0.13
Tocopherols	0.15-0.21	0.11-0.18
Hydrocarbons	0.014	0.01
Free fatty acids (%)	0.3-0.7	< 0.05
Trace metals		
Iron (ppm)	1-3	0.1-0.3
Copper (ppm)	0.03-0.05	0.02-0.06

Table 1: Average compositions for crude and refined soybean oil.

Adapted from Pryde [12].

Soy oil has a light yellow color and a soft flavor [8]. The fatty acid profile of soybean oil results in low oxidative stability that limits the uses of soybean oil in food products and industrial applications, and can be modified easily by genetic engineering, thus obtaining oil with a low content of saturated fats and high of unsaturated fats like oleic or linoleic acid, for example [9].

Soy oil has also a low stability to frying comparing to other edible fats. In addition, due to the presence of furanic acids, this oil oxidizes rapidly in the presence of light. For this reason, the storage in the dark is recommended [8].

Soy Bean Oil as Source of Allergens

Both cold-pressed and fully refined oils have been shown to contain proteins (0.35-0.78 mg/kg) [3]. Although there is a certain consensus that fully refined soybean oils are not allergenic and most publications suggest that refined oils do not induce allergic reactions in sensitive individuals, an adverse reaction to soy oil in an infant has been reported [13-15].

IgE-binding proteins with MWs of 53 and 58 kDa were identified in three unrefined soybean oils [16]. A 56 kDa allergenic protein was also found in cold-pressed and deodorised soybean oils, which was later identified as soybean β -amylase (7S globulin), together with the 20 kDa allergen Kunitz Trypsin Inhibitor (KTI) [17,18].

The protein profile of the cold-pressed soy oil was similar to that of soy flour, with seven bands in a wide molecular range (94-14 kDa) [19]. The soy lecithin seed maturation protein P34 from the 7S globulin fraction (35 kDa) and β -amylase (56 kDa) were identified.

Hypersensitivity Reactions to Generic Drug-Containing Soybean Oil

Over the last years the use of generic drugs has increased in Spain and in the European Union due to their minor economic cost. The main regulatory request to market these products is their equivalence to the original compounds.

	Soy (<i>Glycine max</i>)	Sunflower (<i>Helianthus annuus</i>)	Peanut (<i>Arachis hypogaea</i>)	Canola (<i>Brassica napus</i>)
Oil content of the seed (% in weight)	18-23	25-30	42-52	Aprox. 40
Solidification point (°C)	-8(-18)	-18(-20)	-2-(+3)	0(-2)
Saturated fatty acids:				
Palmitic acid (16:0)	10	6.5	10	4
Stearic acid (18:0)	5	5	3	1.5
Eicosanoic acid (20:0)	0.5	0.5	1.5	0.5
Docosanoic acid (22:0)	0	0	3	0
Unsaturated fatty acids:				
Oleic acid (18:1)	21	23	41	63
Linoleic acid (18:2, n-6)	53	63	35.5	20
Linolenic acid (18:3, n-3)	8	<0.5	0	9
Eicosenic acid (20:1) and Eicosadienoic acid (20:2)	3.5	1	1	1
Erucic acid (22:1)	0	0	-	0.5

Table 2: Composition in fatty acids (% in weight) and properties of some edible oils.

Adapted from Belitz [8].

Soy bean is an additive of some drugs. Hypersensitivity reactions to soy bean oil as a result of drug intake have been scarcely reported [20-22]. Recent studies on analytic investigation on protein content in refined seed oils suggest that fully refined seed oils should be taken into account in the context of allergic reactions and would benefit of further toxicological studies [22].

The observations suggest that an immediate hypersensitivity to drug-containing soy oil may cause anaphylaxis reaction in patients previously sensitized to this legume. The first report of possible anaphylaxis after a drug containing soybean oil implicated the drug propofol [20]. Since propofol contains both egg lecithin and soybean oil, its use is contraindicated in patients with hypersensitivities to these component. Several other drugs may have a food component, resulting in contraindications and warnings in product labelling.

Several commercially important refined vegetable oils are derived from plants which are recognized as potent food allergens (peanut, soy). Full refining of oils results in the almost complete removal from oils of protein, which is responsible for allergic reactions. However, it is uncertain whether the minute amounts remaining could provoke allergic reactions in highly susceptible individuals. This has led to a vigorous debate about the safety of refined oils and specifically whether to label each oil individually because of the potential risk of allergenicity [23-25].

Until active principles are clearly indicated in drug labellings, excipients and other minor additives are not included and only defined as excipients. Since the introduction of generic drugs to the pharmaceutical market a debate exists whether they are well-investigated and of high quality. There is some uncertainty about whether evidence of bioequivalence is enough to guarantee efficacy and safety of generic drugs [25]. Food allergy consumers depend on ingredient labels for allergen avoidance [23]. However the drug labelling may not indicate the form or source of the allergen, and individuals who currently avoids foods may presented severe allergic symptoms after intake of drugs with minimal protein content such as soy oil, soy lecithin or lysozyme [26]. There are now reliable assays for the determination of soybean proteins in processed foods that may be applicable in drugs [27, 28].

Over the last years the use of generic drugs has increased in the European Union due to their minor economic cost. The main regulatory request to market these products is their equivalence to the original compounds. We described two women that presented anaphylaxis after intake of generic omeprazole. The two patients had previously tolerated original non-generic omeprazole. Dot-blot assay revealed that the serum of the two patients was positive to generic omeprazole and soy. The diagnosis of soy allergy should not be excluded in cases of drug hypersensitivity. We suggest to tests soy in all hypersensitivity reaction to generic drugs [29].

Although incidences of anaphylactic reactions induced by proton pump inhibitors of H_2 are rare, they can life threatening. Skin prick tests and oral challenge tests may be useful for the diagnosis, but immunological test never confirm the presence of specific IgE antibodies to active principles of these drugs [23].

Soy is a clinical relevant allergenic source. The diagnosis of soy allergy should not be excluded in cases of drug hypersensitivity. We suggest to tests soy in all hypersensitivity reaction to generic drugs [28].

New studies described new protein involve in oil bodies fractions of several seed and nuts mainly, which are recognized by IgE antibodies from patients sensitized to these, and which cause allergic reactions ranging from oral allergy syndrome to anaphylaxis. These works used standard prick test and specific IgE determinations, which provided negative results. Although isolated reports have shown sensitization to the agent tested using prick-by-prick tests prepared with saline, in most cases specific techniques to conserve the lipid fractions of the extracts were required [29].

In addition, they have not only identified oleosins as sensitizing lipid fractions (fractions Ara h 10 and 11 of peanuts) but also establish that these are gastro-resistant allergens. This might explain the cases of anaphylaxis reported in the literature and the existence of cross-reactivity with other plant foods such as buckwheat, which has been tested by RAST inhibition techniques [30].

We hypothesize that negative results might be due to the lipid nature of the allergen in question and, therefore, the difficulties inherent in its processing. Interestingly, most allergenic extracts are obtained by water-soluble methods that could eliminate these lipids derivatives of the allergenic extract. The role of lipid derivatives in food allergies requires deeper investigation.

Soy Bean Oil as a Hidden Allergen: Anaphylaxis Due to Pillows

Protein and soy oils are widely used in animal and human nutrition, pharmaceutical industry and very recently in manufacturing of pillows. We studied cases of anaphylaxis during the night that was caused by an unexpected exposure to soy included in their pillows. The patients suffered anaphylaxis during their sleep, near daily. They presented nocturnal episode of dyspnoea, erythema and facial edema, scattered wheals, malaise, palpitations and intense palmar itching. Emergency treatment with adrenaline was necessary. The symptoms did not appear when the patients sleep in hotels or in other houses.

Today, pillows are stuffed primarily with materials such as polyester (a synthetic), feathers, down, or a combination of the latter two. There are recently made generations of pillows, soy-based. There are also internationally patented the *Aloe vera* injected in the pillow core, the use of natural soy oils in our compositions, and many other innovative products, most of them used in babies pillows.

The only common finding among these patients was that they have bought a viscoelastic pillow before their symptoms began. We obtained industrial information of soy-oil composition in the matrix of these pillows. Consequently, we performed a later allergic study. All patients presented Skin Prick Tests (SPT) pricks and IgE determination (ImmunoCAP assay, Phadia, Uppsala, Sweden) to conventional aeroallergens and foods (soy included) negative [29,30].

Component-resolved diagnosis and microarray technology have been recently introduced into clinical allergy practice, and may be particularly useful in food-sensitized allergic patients and in patient with anaphylaxis of hidden allergens. After informed consent, new SPT with nut, hull and oil soy and microarrays (ISAC[®]) were performed. The results of SPT to soy different nut extracts were negative in all patients. Only oil spy revealed a small wheal (12 mm², histamine 19.6 mm²). Nevertheless microarray-based IgE detection assay (ISAC[®]) revealed in all patients hypersensitivity to β -conglycinin (nGly m 5). Their symptoms disappear after elimination of their

pillows [29]. They eliminated all source of soy in their room (pillows) and the ingestion of any food that could be manufactured with this oil. They have been revised each six months during a period of 10 years without relapse.

Exposure to soy antigens has been associated with asthma in community outbreaks and in some workplaces. Recently, 135 Soy Flake Processing Workers (SPWs) in a Tennessee facility were evaluated for immune reactivity to soy. Allergic sensitization to soy was common and was five times more prevalent than in Health Care Worker controls (HCWs) with no known soy exposure. The prominent proteins that bound SPW IgE were identified by nanoUPLC MS/MS analysis to be the high molecular weight soybean storage proteins, β -conglycinin (Gly m 5), and Glycinin (Gly m 6) [31].

High molecular weight soybean storage allergens, Gly m 5 and Gly m 6, may be respiratory sensitizers in occupational exposed workers but in other patients no professionally exposed. Soy protein has shown great potential for use in biobased adhesives. β -Conglycinin is a major component of soy protein; it accounts for 30% of the total storage protein in soybean seeds. β -Conglycinin was isolated and purified, and its subunits' (β , α') physicochemical and adhesive properties were characterized [32].

Soy is also used in pharmaceutical products as soymorphins or drug-excipients [14,22]. Oral allergy syndrome to soy milk is classified as a phenotype of Pollen-Food Allergy Syndrome (PFAS). As causative antigens, Gly m 4 (Bet v1 homolog, 17 kD) and oleosin (23 kD), have been reported. Recently two cases of Pollen Food Allergy Syndrome (PFAS) to soy milk have been reported [15]. Both cases showed positive reactions to soy milk in Skin Prick Tests (SPT) and to Gly m 4 in specific serum immunoglobulin E antibody. When they measured specific serum IgE antibody of soy-related proteins using microarray analysis, both cases showed a positive reaction for Bet v1. Based on the array results, the authors diagnosed the two cases as PFAS to Gly m 4.

We performed protein microarray analysis and found it useful as a screening test for immediate allergy, such as PFAS. The reason of negative SPT and IgE to soy in the tests performed to our patients may be due to the acidic subunits of glycinin and β -conglycinin, major soybean storage proteins, appear to be absent or present in much reduced amounts in these techniques. Immunoblots with soy-allergic sera indicate alteration, reduction, or loss of IgE-binding in the commercial extracts as compared to extracts of soy flour. Preparation methods appear to be partially responsible for the variable allergen content in commercial soybean skin test extract [33].

In summary, soy is recognized as one of the "big 8" food allergens. IgE antibodies from soybean-sensitive patients recognize more than 15 soybean proteins [34]. Some of these food allergens can be found as a hidden allergen like pillow composition. Sensitization to the soybean allergens Gly m 5 or Gly m 6 is potentially indicative for severe allergic reactions to soy, like our patients suffered from [35-37].

Recent Advances in the Control of Allergic Reaction to Soybean

Recently, some global regulatory agencies started requiring quantitative analysis of individual allergens, including unproven allergens, as part of the risk assessment for genetically engineered soy beans

[38]. Identification of allergenic globulins as percutaneously sensitized soybean allergens have been demonstrated through epidermal application of crude soybean extract [39]. Glycinin and β -conglycinin can induce intestinal damage [40].

Measures as fermentation with microorganisms boiling and pressure cooking genetical modification and other measures can degrade major allergens and achieve a reduction of soybean hypersensitivity. Removal of soybean oil in intralipid for total parenteral nutrition have been also proposed [41-45].

Conclusion

Soy by itself is not usually an important food in the diet, however, it is used in many products. The elimination of all foods that include soy is essential for allergic patients. It is recognized as one of the major food allergens. IgE antibodies to soy appear in sensitive patients and have been recognized up to more than 15 soy proteins. Some of these food allergens can be found as a hidden allergen, as is the case with the composition of the pillows and their inclusion in certain pharmaceutical products. Sensitization to soy allergens Gly m 5 or Gly m 6 is potentially indicative of severe allergic reactions to soy.

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