Mini-Review:
What about the Allergenicity of Vegetable Oils?

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SUMMARY
Cases of allergy to peanut, sunflower seed, soybean and sesame seed oils have been reported in the literature, although some authors have claimed that these oils are not allergenic.
The aim of this article is to review this subject, to describe the processing methods to which the seeds are subjected during the extraction of their oil, to recall that oils do not consist solely of triglycerides and to describe the findings of our studies.
The allergenicity of oils is a frequent subject of controversy and the bibliography constantly produces contradictory examples. This can be explained by the variability of the processes used in industry, and by the conditions under which proteins are extracted in the laboratory.

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KEYWORDS
edible oils
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protein content
processing

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INTRODUCTION

Soy oil is the vegetable oil consumed in the largest amounts worldwide (29%). In France, sunflower (40%) and oil-seed rape (26%) oils are the ones most commonly used, those of soy and peanut oils each corresponding to less than 5% of total consumption (Proléa-Documentation 1998, ONIDOL, Paris, France) (Table 1).

Vegetable oils are produced from the corresponding seeds after mechanical processing: shelling, flaking, in some cases cooking, pressing and possibly hexane-extraction. The crude oil that is obtained after these steps contains high levels of triglycerides, but also contains partial glycerides, free fatty acids, phospholipids, coloring agents, free sugars, glycolipids, waxes, metals, water … and may also contain traces of proteins. Some toxic substances such as gossypol, a brown polyphenolic compound of cotton oil, may be naturally present in certain oils. Moreover organophosphorus or organochloride pesticides, liposoluble substances often present on the top of the seeds, can be found in vegetable oils.

The purpose of refining is to produce a product that is neutral in flavor, attractive in appearance and devoid of unwanted substances. Each of the steps in the chemical refining process is carried out under specific conditions defined by each manufacturer. Briefly, demucilagigation corresponds to the treatment of the oil by phosphoric acid, to allow the elimination of phospholipids, glycolipids and gum. This step is followed by neutralisation using sodium hydroxide, to eliminate free fatty acids. Washing steps using water clear oil of vegetable wax, residual phospholipids and soap. After drying, the bleaching step conducted by adsorption on bleaching clay eliminates mainly pigments that have been partially destroyed by neutralization. At a low temperature, vegetable waxes are insolubles. It is possible to eliminate them by filtration or centrifugation. The last step is to get rid of odors and peroxides at a very high temperature under vacuum. An example of the refining process of sunflower oil is given in Table 2.

SPECIFIC VEGETABLE OILS

An overview of the studies conducted on peanut, sunflower, soy and sesame oil is given in Table 3.

Peanut oil

The high incidence of peanut allergy has led to investigations of peanut oil (Moneret-Vautrin 1998). In 1981 and 1997, Taylor et al. and Hourihane et al. respectively concluded that refined peanut oil was not allergenic to peanut-sensitive individuals, but in 1991 the team of Moneret-Vautrin reported two cases of allergy to peanut oil in infants fed with milk formulas. Immunochemical studies have identified allergens in cold-pressed crude oils (Hoffman & Collins-Williams 1994), in oils that have been refined at temperatures between 50 and 150°C (14 to 80 kDa) (Teuber et al. 1997), and in one oil processed at high temperature (18 kDa) (Olszewski et al. 1998).

<table>
<thead>
<tr>
<th>Oil</th>
<th>World</th>
<th>France</th>
</tr>
</thead>
<tbody>
<tr>
<td>Soybean</td>
<td>29%</td>
<td>5%</td>
</tr>
<tr>
<td>Palm</td>
<td>21%</td>
<td>9%</td>
</tr>
<tr>
<td>Oil-seed rape</td>
<td>15%</td>
<td>26%</td>
</tr>
<tr>
<td>Sunflower seed</td>
<td>11%</td>
<td>40%</td>
</tr>
<tr>
<td>Cotton seed</td>
<td>5%</td>
<td>-</td>
</tr>
<tr>
<td>Peanut</td>
<td>5%</td>
<td>4%</td>
</tr>
<tr>
<td>Sesame seed</td>
<td>0.7%</td>
<td>?</td>
</tr>
</tbody>
</table>

Table 1: Consumption of vegetable oils in France and throughout the world (ONIDOL 1998)
Table 2: Description of the steps of refining process of sunflower oil (from Zitouni et al. 2000)

<table>
<thead>
<tr>
<th>Step</th>
<th>Oil obtained</th>
<th>Treatment used</th>
<th>Temperature applied</th>
<th>Time</th>
<th>Removed components</th>
</tr>
</thead>
<tbody>
<tr>
<td>0-Expeller and chemical extraction</td>
<td>Crude oil (A)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1-Acidic and neutral treatment</td>
<td>Neutralized oil (B)</td>
<td>H₃PO₄ / NaOH</td>
<td>85 °C</td>
<td>15 min</td>
<td>phospholipids, free fatty acids, metals, pigments, products from oxidation, some contaminants</td>
</tr>
<tr>
<td>2-Pregumming by centrifugation</td>
<td>Pre-gummed oil (C)</td>
<td>Soap and water</td>
<td>10 °C</td>
<td>4 h</td>
<td>Plant wax</td>
</tr>
<tr>
<td>3-Washings</td>
<td>Washed oil (D)</td>
<td>Water</td>
<td>85°C</td>
<td>few minutes</td>
<td>Soap, phospholipids</td>
</tr>
<tr>
<td>4-Bleaching</td>
<td>Bleached oil (E)</td>
<td>Filters</td>
<td>85</td>
<td>20 min</td>
<td>Pigments, residues of soap, phospholipids, metals, products from oxidation</td>
</tr>
<tr>
<td>5-Gumming by filtration</td>
<td>Gummed oil (F)</td>
<td>Filters</td>
<td>10 °C</td>
<td>5 h</td>
<td>Plant wax</td>
</tr>
<tr>
<td>6-Deodorization</td>
<td>Refined oil (G)</td>
<td>Water steam</td>
<td>220°C-4 min under vacuum</td>
<td>2h30min</td>
<td>Peroxides, odors, some contaminants</td>
</tr>
</tbody>
</table>

**Sunflower oil**

There have been numerous studies of sunflower seeds. Noyes et al. (1979), Halsey et al. (1986), Iwaya et al. (1994) and Axelsson et al. (1994) have reported reactions such as generalized urticaria, dyspnea and anaphylactic shock after consumption of sunflower seed. Klurfeld & Kritchevsky (1987) and Halsey et al. (1986) extracted proteins from refined oil and virgin cold-pressed oil, and concluded that these oils were entirely safe. In 1994, Kanny et al. reported two episodes of anaphylactic shock in a female patient, occurring at an interval of two months, after she had eaten a meal containing sunflower oil and margarine. Skin tests and a positive oral challenge test with 5 ml of sunflower oil, demonstrated that the anaphylactic shock had been induced by sunflower oil. Our team identified proteins both in the crude oil (13.6 µg/mL) and after it had been refined by heat treatment (0.22 µg/mL) (Figure 1). One of these proteins (67 kDa) found in the refined oil was recognized by the serum IgE of the patient allergic to the oil (Figure 2) (Zitouni et al. 2000).
## Table 3: Overview of the studies conducted on peanut, sunflower, soy and sesame oil

<table>
<thead>
<tr>
<th>Seeds</th>
<th>Oil Type</th>
<th>Proteins</th>
<th>Allergenicity</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>Peanut</td>
<td>Refined</td>
<td>no protein</td>
<td></td>
<td>Tattrie et al. 1973</td>
</tr>
<tr>
<td>Peanut</td>
<td>Unspecified</td>
<td></td>
<td>Negative skin tests, negative DBPCFC (0/10)</td>
<td>Taylor et al. 1981</td>
</tr>
<tr>
<td>Peanut</td>
<td>Unspecified</td>
<td></td>
<td>Positive oral provocation test (2/2)</td>
<td>Moneret-Vautrin et al. 1991</td>
</tr>
<tr>
<td>Peanut</td>
<td>Cold-pressed Hot-pressed</td>
<td>0.2-3.3 in some cold-pressed oil</td>
<td>Dot blots: positive for some cold-pressed oil, negative for hot-pressed oils (pooled sera)</td>
<td>Hoffman et al. 1994</td>
</tr>
<tr>
<td>Peanut</td>
<td>Virgin peanut oil</td>
<td></td>
<td>Positive skin-prick tests (1/1), labial challenge (2/2) and SBOC (4/4)</td>
<td>Moneret-Vautrin et al. 1994</td>
</tr>
<tr>
<td>Peanut</td>
<td>Crude and refined peanut oil</td>
<td></td>
<td>Negative DBPCFC with refined oil, positive with crude oil (10/60)</td>
<td>Hourihane et al. 1997</td>
</tr>
<tr>
<td>Peanut</td>
<td>a) Unrefined b) Refined, bleached and deodorized</td>
<td>a) 10.5-10.7 b) 3.0-5.7</td>
<td>Slot Blot: strong IgE binding for unrefined oils, low or no IgE binding for refined oils, Western-Blot: same binding pattern for peanut extract and unrefined peanut oil extract</td>
<td>Teuber et al. 1997</td>
</tr>
<tr>
<td>Peanut</td>
<td>a) Crude b) Neutralized c) Refined</td>
<td>a) 3.4 µg/g b) 0.2 µg/g c) 0.1-0.2 µg/g</td>
<td>14-76 LHR positive with the protein extract (9/11) Western-Blot revealed a 18 kDa protein</td>
<td>Olszewski et al. 1998</td>
</tr>
<tr>
<td>Peanut</td>
<td>a) Peanut oil extract b) Peanut oil proteinic extract</td>
<td></td>
<td>a) Positive DBPCFC (14/62) b) Positive skin-prick tests (9/10)</td>
<td>Moneret-Vautrin et al. 1998</td>
</tr>
<tr>
<td>Sunflower</td>
<td>Cold-pressed and refined</td>
<td>2 to 8</td>
<td>Negative SPT (2/2), Negative DBPCFC (2/2)</td>
<td>Halsey et al. 1986</td>
</tr>
<tr>
<td>Sunflower</td>
<td>Refined</td>
<td>0.85</td>
<td></td>
<td>Klurfeld et al. 1987</td>
</tr>
<tr>
<td>Sunflower</td>
<td>Unspecified</td>
<td></td>
<td>Positive scratchs test (1/1)</td>
<td>Frazier et al. 1995</td>
</tr>
<tr>
<td>Food</td>
<td>Form</td>
<td>Properties</td>
<td>References</td>
<td></td>
</tr>
<tr>
<td>-----------</td>
<td>---------------</td>
<td>----------------------------------------------------------------------------</td>
<td>-----------------------------</td>
<td></td>
</tr>
</tbody>
</table>
| Sunflower | a) Crude      | a) 13.6  
        b) 11.5  
        c) 11.3  
        d) 2.7   
        e) 1.6   
        f) 1.4   
        g) 0.22 | 67 kDa (Western-Blot)  Positive SPT (1/1)  Positive DBPCFC (1/1) | Zitouni et al. 2000 |
|           | b) Neutralised| 67-145  
        67-145  
        67-132  
        67-132  
        67-132  
        67  
        67 | Non allergenic proteins | |
|           | c) Pregummed   | 67-145  
        67-132  
        67-132  
        67-132  
        67  
        67  
        67 | Proteins interacted with oxidized soy oil are allergenic to soybean-sensitive patients | Doke et al. 1989 |
|           | d) Washed      | 67-145  
        67-145  
        67-132  
        67-132  
        67-132  
        67  
        67 | Positive ELISA | |
|           | e) Bleached    | 67-145  
        67-145  
        67-132  
        67-132  
        67-132  
        67  
        67 | Positive EAST inhibition | |
|           | f) Gummed      | 67-145  
        67-145  
        67-132  
        67-132  
        67-132  
        67  
        67 | Non allergenic proteins | |
|           | g) Refined     | 67-145  
        67-145  
        67-132  
        67-132  
        67-132  
        67  
        67 | Anaphylactic shock (1/1) | Andersen et al. 1993 |
| Soybean   | Refined       | no protein  | Non allergenic proteins | Tattrie et al. 1973 |
| Soybean   | Refined and   | Non allergenic proteins  | Negative DBPCFC (7/7) | Bush et al. 1985 |
|           | cold pressed  |                                  |                           | Porras et al. 1985 |
| Soybean   | Unspecified   | 110-3300  | Non allergenic proteins |                                   |
| Soybean   | a) Crude      | 1.9  
        b) 0.72 | Positive patch test (1/1) and positive oral challenge (1/1) | Klurfeld et al. 1987 |
| Soybean   | b) Refined    | 0.01-0.04  
48-67 | Non allergenic proteins | Awazuhara et al. 1998 |
| Soybean   | Oxidized soy  | Positive ELISA | Proteins interacted with oxidized soy oil are allergenic to soybean-sensitive patients | Doke et al. 1989 |
|           | oil           |                                  |                           | |
| Soybean   | Soy oil emulsion (parenteral nutrition) | Anaphylactic shock (1/1) |                           | |
| Soybean   | Refined       | 0.01-0.04  
48-67 | Non allergenic proteins | Awazuhara et al. 1998 |
| Soybean   | a) Unrefined  | a) 0.09-0.14  
        b) 0.035 | Positive SPT (1/1)  Positive DBPCFC (1/1) | |
|           | b) Refined    | a) 14-94  
        b) 14-94 | Non allergenic proteins | Packe et al. 2001 |
| Soybean   | a) Crude      | 1.8  
        b) 0.3 | Positive patch test (1/1) and positive oral challenge (1/1) | Maneret-Vautrin et al. 2002 |
|           | b) Deodorized | a) 14-150  
        b) 14-150 | 56-57 kDa  Positive RAST | |
| Sesame    | Unspecified   | Positive RAST, positive BHR (1/1) | Positive skin-prick test (1/1) | Chiu et al. 1991 |
| Sesame    | Unspecified   | LHR positive in 1/3 case | Positive oral provocation test (1/2)  Positive labial provocation test (2/2) | Kanny et al. 1996 |

LHR leucocyte histamine release, BHR basophile histamine release, SBOC single blind oral challenge
Figure 1: SDS-PAGE of protein extracts from samples of sunflower oil taken at different steps during refining (from Zitouni et al. 2000) A: Crude oil, B: Acidification and neutralisation, C: Pregumming, D: Washing, E: Bleaching, F: Gumming, G: Deodorizing

Figure 2: Western blot of protein extracts from samples of sunflower oil taken at different steps during refining, using a serum from a female patient allergic to sunflower oil (from Zitouni et al. 2000) A: Crude oil, B: Acidification and neutralisation, C: Pregumming, D: Washing, E: Bleaching, F: Gumming, G: Deodorizing
Soybean Oil

Soybean allergy is considered as one of the five most common causes of food allergy among children. The prevalence of soybean allergy has been estimated at 1% among adults. Allergy to soy oil is therefore a crucial matter which demands investigation (Paschke et al. 2001). In 1985, Bush et al. concluded that refined soy oil was not allergenic. Indeed the authors enrolled seven soybean-sensitive patients in a double-blind crossover study and no reactions were observed with the commercially available soybean oils. However, Awazuhara et al. (1998) demonstrated the presence of proteins in soy oil, and the presence of allergens in soy lecithin by in vitro methods (SDS-PAGE and immunoblotting of soy oil proteins performed with the sera from 15 soybean-sensitive patients). Our team’s study of soy lecithins and egg lecithins confirmed the allergenicity of the proteins in these lecithins (Palm et al. 1999). In the case of soy lecithin, one patient had a negative prick test to soy lecithin and a positive double blind placebo controlled oral challenge at 100 mg of soy lecithin. Paschke et al. (2001) identified allergens in the unrefined oil, but not in the refined oil. More recently, our team has shown that the IgEs of the serum of soybean-sensitive patients specifically recognized 28 and 56 kDa proteins extracted from deodorized oil (Errahali et al. 2002).

Sesame Seed Oil

Sesame seed has been subjected to many clinical studies, but there has still been little attempt to characterize the allergens. From 1978 to 1991, Kägi & Wüthrich (1993) found only nine cases of allergy to sesame seed. However, the discovery in 1996 of 6 cases of allergy to seed and/or sesame oil underlines the growing risk of sesame consumption (Kanny et al. 1996). Chiu & Haydik (1991) report one case of anaphylactic shock to sesame oil. Kanny et al. (1996) found that the histamine release assays were positive in 1/3 cases with sesame oil, the labial provocation test was positive in 2/2 and the oral provocation test was positive in 1/2 at a dose of 3 mL. It should be noted first that sesame oil is supplied in unrefined form, and second that sesame oil is often a masked allergen, frequently labeled “vegetable oil” in a wide variety of products like margarine.

CONCLUSIONS

Food allergies to peanuts, sunflower, soybean and sesame seeds have been well established, although allergies to the oils of these seeds remain controversial. Various factors may account for this controversy:

- The process by which the oil is prepared. The oil can be obtained by mechanical pressing, or by an extraction process involving a solvent. It may be submitted to refining, under conditions that are chosen and developed by each manufacturer. These refining conditions are crucial, and may influence the concentration and denaturing of the proteins.
- The conditions under which the proteins are extracted from the oil in the laboratory. It is very difficult to extract amphiphilic molecules (proteins) from a hydrophobic medium (oil). As a result, the quantity and quality of the proteins extracted are directly determined by the extraction method used.
- The source of the allergenic proteins in the oil is not clear: they may be native proteins or may have been modified by the industrial process. The severity of the clinical reactions induced by the DBPCFC conducted using a few milliliters of oil (corresponding to a few micrograms of protein) suggest that an adjuvant effect may be involved.
REFERENCES


[Summary] [Abbreviations]