

Anaphylaxis to Sunflower Seed with Tolerance to Other Nuts. The Role of Lipophilic Allergens

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Sunflower (*Helianthus annuus*) belongs to the plants genus of *Asteraceae* family (*Compositae*). The consumption of sunflower seeds is widely extended overworld, being commonly known in clinical practice that allergic patients to other nuts usually tolerate them.

Sunflower seed's allergy is rare. Only a few cases of anaphylaxis after ingestion have been described[1-5],having most of cases an occupational origin in exposed workers[2,4].

Several allergenic proteins have been identified in sunflower seeds[1,3,4,6].There have already been documented by the WHO/IUIS allergen nomenclature sub-committee [www.allergen.org]: *Hel a1* (34kDa, a major and inhaled allergen without a specific protein family described yet); *Hel a2* (a 14.7kDa Profilin); *Hel a 3* (a 9kDa LipidTransferProtein (LTP), food allergen); and *Hel a 6* (a 42kDaPectateLyase, inhaled allergen). Others as *Hel a 2S albumin* (16 kDa), a 12kDa storage protein that appears to be the mature form from the previous, and a 13kDa LTP have been also described as potential allergens in others publications[1,5,6].

We present a case of anaphylaxis after ingestion of sunflower seeds with sensitization to other nuts, but with tolerance to all of them.

A 35-year-old man with a personal history of atopy (egg allergy overcome in childhood and seasonal rhinoconjunctivitis), experienced lingual and palmoplantar pruritus, generalized hives, facial angioedema, conjunctival injection, dyspnea and intense cough, 5minutes after eating a handful of roasted sunflower seeds (previously well tolerated). He went to the Emergency Department (blood pressure 128/65mmHg and baseline SatO₂of 95%), being treated with intravenous antihistamines and corticosteroids, improving progressively within the next hour. Not cofactors were associated.

The following complementary tests were carried out:

Commercial skin prick tests(ALK-Abelló) to peanut, walnut, pistachio, almond, hazelnut, chestnut, sunflower seed, peach LTP, profilin, mustard and mugwort. Positive only for sunflower seed (12mm).

Prick-Prick test to hazelnut, walnut, peanut, almond, pistachio. Positive only for hazelnut (9mm) and walnut (7mm). Sunflower seed hasn't been tested due to a 12mm skin prick test and a moderate initial reaction.

ImmunoCAP® (*ThermoFisher*): Total Immunoglobulin E (IgE) 124IU/ml, specific IgE against sunflower seed 3.18kU/L, Almond 0.11kU/L; Cashew 0.06kU/L; Hazelnut 0.27kU/L; Peanut 0.14kU/L; Walnut 0.27kU/L; Pistachio 0.18kU/L; and rPru p 3 LTP Peach 0.01kU/L.

ImmunoCAP® ISAC 112 (*ThermoFisher*). Moderate-high levels of Cup a1 Cypress allergen (10.30ISU); group 1 timothy grass pollen allergens (2.76ISU); and Alt a1 Alternaria (4.90ISU). Negative results for all proteins of nuts included.

Open oral challenges with roasted hazelnuts and walnuts: negative.

The patient tolerates peanuts, almonds, pistachios, cashews.

Proteins from hydrosoluble and liposoluble fractions of peanut, hazelnut, walnut and sunflower seed extracts were obtained by polyacrylamide-gel-electrophoresis with sodium-dodecyl-sulfate (SDS-PAGE). Briefly, proteins of each piece were obtained following the method described by Barbarroja-Escudero[7], after the homogenization and extract centrifugation processes, the lipid-soluble was separated from the hydro-soluble fraction, and they were treated independently to obtain the aqueous and oil-body extracts[7]. SDS-PAGE of extracts showed proteins ranging 10 to 50kDa in the hydrosoluble fraction, and 13 to 75 kDa in the liposoluble fraction.

Immunoblotting with patient's sera was performed according to the Laemmli method[1,3]. The patient's sera recognized two protein bands with respectively an estimated molecular mass of 12-14kDa in the hydrosoluble fraction, and 21-23kDa in the liposoluble fraction of sunflower seed extract. These bands weren't recognized in other nuts extracts.

Allergy to sunflower seeds with tolerance to other nuts is rare. Some allergenic proteins have been described as a possible cause of reactions.

According to previous publications, the 12-14kDa hydrosoluble protein band identified could correspond to a 12kDa storage protein[1,6], a 13kDa LTP[5] or to a 14.7kDa profilin (*Hel a 2*)[3,4,6]. Despite identifying a similar protein band in the hydrosoluble fraction after electrophoresis of peanut, hazelnut and walnut extracts, which might correspond to profilins[8] (*Ara h 5*, 14kDa; *Cor a 2*, 14kDa; and *Jug r 7*, 13kDa), it is less probable that *Hel a 2* is responsible for the reaction due to its severity, although it can not be rule out. Additionally, the ImmunoCAP ISAC test was negative for all storage proteins, LTPs, PR-10 and profilins of those nuts included, therefore a primary sensitization to only a sunflower seed's protein it is unlikely.

Because lipophilic allergens tend to elude standard protein purification procedures, they are not included in aqueous diagnostic extracts[9,10]. Consequently, more hydrosoluble than lipophilic allergens have been identified[3,9]. Both fractions in all extracts were analyzed for a more precise diagnose regarding the high lipid content reported in nuts and seeds[9,10].

Compare to other nuts analyzed by electrophoresis, significantly more lipoproteins in sunflower seed extract were observed. Few lipoproteins have been described, such as oleosins, structural proteins which stabilize oil bodies in seeds and nuts[7,9], which have been associated with severe allergic symptoms[3,9,10] as those presented by the patient.

In the liposoluble fraction, a protein band with an estimated molecular mass of 21-23kDa was identified, which was only recognized when immunoblotting with patient sera in sunflower seed extract. This lipoprotein could also be an oleosin responsible for the patient's anaphylactic reaction.

After electrophoresis, all extracts analyzed (peanut, hazelnut, walnut and sunflower seed) recognized a protein band of 16-17kDa in the liposoluble fraction. This band might correspond to already described oleosins such as *Cor a 12* (17kDa); *Cor a 13* (14-16kDa), *Ara h 10* (16kDa), *Ara h 14* (17.5kDa) or *Ara h 15* (17kDa)[8,9]. Although lipoproteins have been postulated as a possible cause of cross-reactivity[9], our patient tolerates the rest of nuts, despite probable oleosins being present.

These findings support the hypothesis of Barbarroja-Escudero[3] about the possible involvement of lipoproteins ranging 17-23.5kDa as probable allergens responsible for anaphylaxis in patients monosensitized to sunflower seeds. Based on this, we propose that the implication of lipoproteins in cases of allergy to sunflower seeds is probably related to a higher content of lipids compare to other nuts studied by electrophoresis.

This is the third case in the literature of a monoallergic patient suffering an anaphylactic reaction after ingestion of sunflower seeds, where the probable cause is due to sensitization to lipoproteins, specifically a 21-23kDa oleosin. As oleosins seem to be a relevant cause of severe allergic reactions to nuts and seeds, we emphasize the need to incorporate lipophilic allergens into routine diagnostics in order to improve risk assessment.

Conflicts of interest

Any author has any conflict of interest.

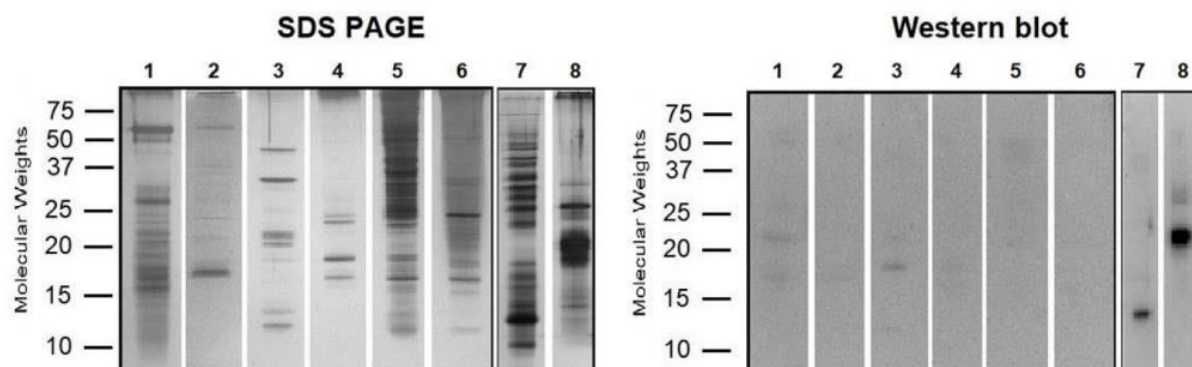
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Figure legend**Figure 1.** SDS PAGE and Western Blot.

1- peanut hydrophilic fraction; 2- peanut lipophilic fraction; 3- hazelnut hydrophilic fraction; 4- hazelnut lipophilic fraction; 5- walnut hydrophilic fraction; 6- walnut lipophilic fraction; 7- sunflower seed hydrophilic fraction; 8- sunflower lipophilic fraction.