Allergy to White Perilla and Cross-reactivity With Sesame

Ramírez-Mateo E[1,2*], Elías-Sáenz I[1*], Fernández-Lozano C[3,4,5], Carrón-Herrero A[1], Solano-Solares E[1], González-De-Olano D[1,2]
[1]*Allergology Department, Hospital Universitario Ramón y Cajal, Madrid, Spain*
[2]*Instituto Investigación Sanitaria IRYCIS, Madrid, Spain*
[3]*Biochemistry-Research Department, Hospital Universitario Ramón y Cajal, Madrid, Spain*
[4]*Universidad de Alcalá de Henares, Madrid, Spain*
[5]*Nextmune S.L., Madrid, Spain*
**Both authors contributed equally.*

J Investig Allergol Clin Immunol 2024; Vol. 34(6)
doi: 10.18176/jiaci.1016

Key words: 2S albumin. Occupational. Perilla ocymoides Linn. Seed storage proteins (SSPs). Vicilin.

Palabras clave: 2S albúmina. Ocupacional. Perilla ocymoides Linn. Proteínas de almacenamiento. Vicilina.

Perilla is a flowering plant belonging to the Lamiaceae family that grows mainly in Asia. The most common among the many different perilla species are red perilla (*Perilla frutescens var frutescens*) and white perilla (*Perilla ocymoides Linn*). The use of seeds in our environment has grown in recent years, and, consequently, adverse reactions caused by their consumption have increased in frequency [1]. The most common reactions are to sesame and mustard [2]. Perilla seeds can be used as a source of nutrients for both humans and animals. Red perilla seed is used in oriental cuisine, especially in Japan and Korea, and was recently reported as a food allergen [3]. White perilla seed is commonly used for bird food because of its high protein content and low carbohydrate content. No adverse reactions to white perilla have been reported to date.

We present 2 cases of IgE-mediated hypersensitivity to white perilla. In the first, a 70-year-old man (patient 1) who kept birds at home for years developed itching in his hands when handling bird food. On one occasion, while eating white perilla seeds, he developed pharyngeal itching and foreign body sensation. He subsequently experienced self-limiting oral itching with the ingestion of foods containing sesame seeds. In the second case, a 4-year-old boy (patient 2) was referred to our clinic because he developed nasal congestion, facial urticaria, and eyelid angioedema after handling homemade birdseed containing white perilla. He previously tolerated contact with white perilla. He did not report symptoms after handling other seeds and, to date, has never eaten any seeds or nuts. Patient 1 and the legal representatives of patient 2 agreed to the testing and publication of the study data and signed the informed consent document. Both patients underwent skin prick tests with commercial common aeroallergens, including pollens, dust mites, molds, and dander. Prick-prick tests were carried out with all the seeds included in the mixture. All the results are shown in the Table. A *Perilla ocymoides Linn* extract (PE) was prepared by homogenization in phosphate-buffered saline followed by centrifugation, dialyzation, and lyophilization. SDS-PAGE IgE immunoblot assays were performed with both patients' sera, yielding IgE reactivity with 2 bands in patient 1 (15 and 10 kDa) and a further 2 bands in patient 2 (50 and 25 kDa) (Table and Supplementary figure 1). To identify these IgE-reactive proteins, bands from the Coomassie blue–stained gel were manually excised (gel slices) and processed for identification by matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MS) (MALDI-TOF). The gel pieces were incubated for in-gel digestion, and tryptic peptides were collected for peptide mass fingerprinting (PMF) analysis by MALDI-TOF MS. MS data from PMF spectra were searched for in the NCBI database by taxonomically narrowing Perilla when accessing the Mascot database search algorithm for protein and peptide identification. Two different proteins were identified, as follows: (*i*) hypothetical protein c2S53_005981 of *Perilla frutescens var. hirtella* and (*ii*) hypothetical protein c2S52_019628 of *Perilla frutescens var. hirtella*. Thus, the analysis of these sequences in other proteins (Blast analysis in the NCBI database, https://blast.ncbi.nlm.nih.gov) resulted in a 2S seed storage albumin with a significant homology of 79.5% for *Salvia hispanica* in patient 2 and 72.9% for vicilin in *Sesamum indicum*. In summary, from the analysis of the proteomic study and since there is no register for *Perilla ocymoides Linn*, we could conclude that bands 1, 2, and 3 (15, 10, and 50 kDa, respectively) correspond to a 2S seed storage albumin and that band 4 (25 kDa) corresponds to vicilin. In order to identify the most plausible route of sensitization, we performed an inhibition enzyme-linked immunosorbent assay (ELISA) with *Sesamum indicum* extract (SE) in the solid phase (0.1 µg/well in 0.1 M bicarbonate buffer, pH 9.6) and PE (0.04 µg/mL, 1 µg/mL, 25 µg/mL, and 625 µg/mL) in the inhibitory phase. A second inhibition ELISA was also performed with PE in the solid phase and SE in the inhibitory phase (0.04 µg/mL, 1 µg/mL, 25 µg/mL, and 625 µg/mL). The inhibition results are shown in the Table and Supplementary figure 2.

Seed storage proteins (SSPs) are the most abundant allergens from plant-derived sources. They comprise a group of proteins generated during seed production that accumulate in large quantities during the seed maturation period [4].

Table. Skin Tests Results and Analytical Determinations.

	Patient 1	Patient 2
SPT, mm[a]		
Inhalants[b]	None	None
Food[c]	None	None
Prick by prick test (larger diameter of the wheal, mm[a,d])		
White perilla	5	7
Total IgE, kU/L	568	231
sIgE sesame, kU_A/L	10.50	NP
SDS-PAGE IgE immunoblot assay, kDa	15 and 10	50 and 25
Inhibition ELISA, %[e]		
PE (0.04/1/25/625 µg/mL) - Coated SE	28/53/57/60	35/77/89/90
SE (0.04/1/25/625 µg/mL) - Coated PE	22/51/56/62	18/38/41/45

Abbreviations: NP, not performed; PE, perilla extract; PPT, prick prick test; sIgE: specific IgE; SE, sesame extract; SPT, skin prick test.
[a]Only positive (≥3 mm) results expressed.
[b]The inhalant series includes *Dermatophagoides pteronyssinus, Dermatophagoides farinae, Alternaria alternata, Aspergillus fumigatus*, dog dander, cat dander, mixed gramineous pollen, *Phleum pratense, Cynodon dactylon, Olea europaea, Platanus acerifolia, Artemisia vulgaris, Plantago lanceolata*, profilin, polcalcin, lipid transfer protein.
[c]The food series includes cow's milk, egg, white fish, blue fish, *Anisakis*, wheat, barley, rye, rice, soybean.
[d]Results for the remaining bird food components (oats, millet, sunflower seeds, buckwheat, flaxseed, quinoa, rapeseed) were negative in the prick-by-prick test.
[e]Supplementary figure 2.

They are the source of nitrogen for seed germination and are synthesized only by seeds. The main functions of SSPs are nutrient supply and seed defense [4,5]. They usually form aggregates inside vesicles surrounded by a membrane. SSPs often consist of a number of different polypeptide chains [6]. The main SSPs are globulins and prolamins. SSPs can be classified according to their sedimentation coefficient, which varies from 7S for vicilins (also known as the cupin superfamily) to 11S for legumins; globulins of the storage proteins comprise 11S globulins (legumins) and 7S globulins (vicilins), also known as the cupin superfamily [5,6]. The prolamins (2S albumins, 8-16 kDa) are water-soluble polypeptides (usually heterodimeric proteins) that possess a well-conserved cysteine supporting the stability of temperature, pH, and proteolysis [4-6]. Their 3D stability contrasts with their low amino acid sequence conservation, which could favor cross-reactivity between different 2S albumins [4]. Most have been described as major allergens from their sources [4]. Vicilins (150-220 kDa) are trimeric globulins comprising 45- to 53-kDa subunits with no interchain disulphide bonds [7]. They are heat- and protease-resistant proteins and are soluble in saline solution [8]. The resemblance between vicilins of different species, both at the amino acid sequence level and at the 3D structural level, could result in cross-reactivity [9].

We report the case of 2 patients with allergy to white perilla presenting with symptoms after contact with the seed or after its ingestion. We identify 2S albumin and vicilin as relevant allergens exhibiting cross-reactivity with sesame, one of the most consumed seeds worldwide. The authors hypothesize that the differences between the inhibition ELISA results for the 2 patients in inhibition ELISA could be because total IgE was higher in patient 1 than in patient 2, and, on the other hand, the inhibitions in patient 1 were similar because of the plateau effect, indicating that inhibition is saturated at the concentrations used. It is important to remember that, today, formerly local dietary habits and customs can spread rapidly, as can, therefore, the associated risks. In addition, some of the food humans eat also serves as food for animals. Handling such food, whether as a hobby in the case of pets or professionally, also entails risks that we should be aware of.

Funding

The authors declare that no funding was received for the present study.

Conflicts of Interest

The authors declare that they have no conflicts of interest.

References

1. Galleani C, Diéguez MC, Cabanillas B, Martín-Arriscado Arroba C, Enríquez-Matas A, Crespo JF. Sunflower (Helianthus annuus) Seed Allergy. J Investig Allergol Clin Immunol. 2023 Dec 11:0.
2. Patel A, Bahna SL. Hypersensitivities to sesame and other common edible seeds. Allergy. 2016;71(10):1405-13.
3. Jeong K, Lee S-Y, Jeon S-A, Gantulga P, Nam JY, Hong S-J, et al. Clinical and immunological characterization of Perilla seed allergy in children. J Investig Allergol Clin Immunol. 2023;33(1):14-20.
4. Bueno-Díaz C, Martín-Pedraza L, Parrón J, Cuesta-Herranz J, Cabanillas B, Pastor-Vargas C, et al. Characterization of Relevant Biomarkers for the Diagnosis of Food Allergies: An Overview of the 2S Albumin Family. Foods. 2021 May 29;10(6):1235.
5. Souza PFN. The forgotten 2S albumin proteins: Importance, structure, and biotechnological application in agriculture and human health. Int J Biol Macromol. 2020 Dec 1;164:4638-49.
6. Palomares O, Cuesta-Herranz J, Vereda A, Sirvent S, Villalba M, Rodríguez R. Isolation and identification of an 11s globulin as a new major allergen in mustard seeds. Ann Allergy Asthma Immunol. 2005 May;94(5):586-92.
7. Lauer I, Foetisch K, Kolarich D, Ballmer-Weber BK, Conti A, Altmann F, et al. Hazelnut (Corylus avellana) vicilin Cor a 11: molecular characterization of a glycoprotein and its allergenic activity. Biochem J. 2004 Oct 15;383(Pt 2):327-34.
8. Hirano H. Basic 7S globulin in plants. J Proteomics. 2021 May 30;240:104209.

9. Sanchez-Monge R, Lopez-Torrejón G, Pascual CY, Varela J, Martin-Esteban M, Salcedo G. Vicilin and convicilin are potential major allergens from pea. Clin Exp Allergy. 2004 Nov;34(11):1747-53.

Manuscript received February 4, 2024; accepted for publication May 15, 2024.

David González-de-Olano
https://orcid.org/0000-0001-6653-4900
Cta. Colmenar Viejo, km. 9, 100
28034 Madrid, Spain
E-mail: dgolano@yahoo.es