Identification of the Protein α-L-Fucosidase as the Possible Cause of Allergy to Cardamom

Bernal-Rubio L[1,2,3]*, Martínez-Alonso E[2,4]*, Fernández-Lozano C[3,5]*, Sola-Martínez FJ[1,2,3], Martínez-Botas J[5], Alcázar A[2,4], de la Hoz-Caballer B[1,2,3,6]

[1]*Allergy Department, Hospital Universitario Ramón y Cajal, Madrid, Spain*

[2]*Department of Research, Hospital Universitario Ramón y Cajal, IRYCIS, Madrid, Spain*

[3]*University Alcalá de Henares, Madrid, Spain*

[4]*Proteomics Unit, Hospital Universitario Ramón y Cajal, IRYCIS, Madrid, Spain*

[5]*Biochemistry Research Department, Hospital Universitario Ramón y Cajal, IRYCIS, Madrid, Spain*

[6]*Spanish Thematic Network and Co-operative Research Centres, ARADyAL, Spain*

**These authors contributed equally and should be considered first authors.*

J Investig Allergol Clin Immunol 2024; Vol. 34(3): 205-206
doi: 10.18176/jiaci.0957

Key words: α-L-Fucosidase. Cardamom. 2D PAGE. Slot blot. Spice allergy.

Palabras clave: Alfa-L-fucosidasa. Cardamomo. 2D PAGE. Slot blot. Alergia a especias.

Cardamom is the term used to name a group of aromatic spices of the Zingiberaceae family. The major species are *Elettaria cardamomum*, also known as true cardamom, which is distributed in Malaysia and India, and *Amomum* species, or large cardamom, which is distributed in Asia and Australia. A third species, *Aframomum*, is widely used in Africa [1].

Spice allergy seems to be rare, with an estimated prevalence of 0.04%-0.13% in the adult general population [2]. However, a series of spice allergens have been identified and characterized, as follows: Bet v 1, which is homologous in anise, fennel, coriander, and cumin [3]; 2S albumins, found in sesame seed and mustard; cysteine proteinase GP-I, found in ginger [4]; and 7S globulin, found in sesame and fenugreek [5]. Only 1 case of cardamom food allergy has been reported [6].

We report the case of a 38-year-old woman who presented with palmoplantar pruritus, diffuse erythema, palpebral and labial angioedema, nasal congestion and discharge, dyspnea, throat tightness, abdominal pain, and diarrhea 1 hour after eating a Sicilian mortadella (SM) sandwich with seedless white bread and a cola drink. No sauces were added. She took 2 mg of oral dexchlorpheniramine twice on the day of the reaction until she felt asymptomatic. The patient had eaten SM previously without incident. Later, she tolerated the cola drink and the same bread. No cofactors (exercise, nonsteroidal anti-inflammatory drugs, alcohol) were involved. The patient gave her informed consent for the publication of this report.

Skin prick tests (SPTs) performed with labeled ingredients of SM yielded negative results for pork meat, potato, and cow's milk proteins. SPTs performed with commercial extracts of aeroallergens, panallergens, and food (lamb and chicken meat, almond, walnut, hazelnut, cashew, peanut, chestnut, pistachio, and sunflower seed) also yielded negative results. Prick-by-prick tests performed with different parts of the implicated SM yielded negative results. Total serum IgE was 124 kU/L. Specific IgE to rPru p 3, black pepper, and cinnamon was 0 kU_A/L (UniCap System, Phadia).

In view of these results, an oral provocation test was performed. Within 5 minutes after ingestion of 0.8 g of SM (1 slice weighs about 14 g), the patient developed malaise, generalized pruritus, malar erythema, inferior labial angioedema, and dysphagia. Her vital signs were 120/84 mmHg, 87 bpm, and 97% oxygen saturation. Treatment with 0.5 mg of adrenaline, dexchlorpheniramine, and 40 mg of intramuscular methylprednisolone was administered; the patient's condition resolved in 15 minutes without sequelae. No prescription or admission was needed.

The manufacturer provided us with the names of nonlabeled additives: food color E-120, vitamin C, cinnamon, black pepper, rosemary, cardamom, and sherry. Prick-by-prick tests performed with all of them yielded a positive result of 4 mm to cardamom and negative results for the rest. Extracts of each of these additives of SM were obtained by homogenization in 20% phosphate-buffered saline (PBS), followed by centrifugation and dialysis in PBS. To determine the presence of IgE, we performed

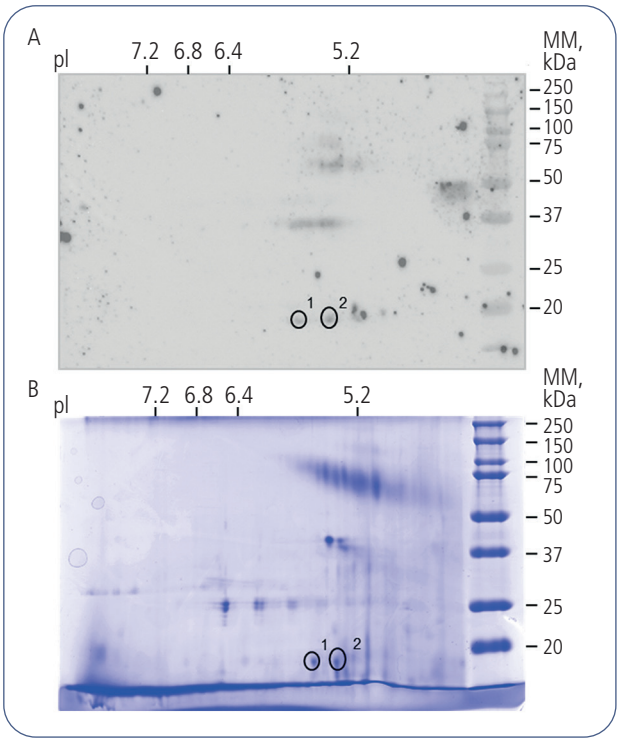

Figure. Detection of IgE-reactive cardamom proteins with serum from a cardamom-sensitive patient. A, Western blot performed after 2D gel electrophoresis of cardamom extract (~100 μg). B, Cardamom extract (~100 μg) analyzed using 2D gel electrophoresis and stained with Coomassie blue. The protein spots marked 1 and 2 were matched between the 2D western blot (A) and the stained 2D gel (B) before being excised and processed for identification by matrix-assisted laser desorption/ionization-time of flight (MALDI-TOF) mass spectrometry (MS) and MALDI LIFT-TOF/TOF (MS/MS) analysis. pI indicates isoelectric point; MM, molecular mass of protein markers in kDa.

a slot-blot assay against each of the extracts (Figure S1A). SDS-PAGE IgE immunoblotting assays revealed IgE reactivity in a <20-kDa band in the cardamom extract. Control sera showed no reaction (Figure S1B). To precisely determine the allergenic protein involved, cardamom extract was analyzed using 2D gel electrophoresis with a pH gradient of 3 to 10 in the first dimension. After the second dimension on SDS-PAGE, the gels were transferred onto a nitrocellulose membrane or stained with Coomassie blue. The membrane was incubated with patient serum (1:10 dilution) and developed with antihuman IgE secondary antibody (SouthernBiotech). IgE antibodies recognized 2 protein spots, with a molecular mass of <20 kDa and isoelectric point (pI) between 5.2 and 5.6 (Figure, A). To identify these proteins, spots matched between 2D immunoblot (Figure, A) and Coomassie blue–stained 2D gel (Figure, B) were excised from the stained gel and processed for identification by matrix-assisted laser desorption/ionization-time of flight (MALDI-TOF) mass spectrometry (MS). Spots were incubated for in-gel digestion, which was performed as described previously, and tryptic peptides were collected for peptide mass fingerprinting (PMF) analysis by MALDI-TOF MS [7]. A similar peptide mass spectrum was obtained in both spots, matching 10 peptides with masses (m/z) 1215, 1250, 1392, 1406, 1546, 1575, 1633, 1646, 1757, and 2119 (Figure S2A), indicating that they are the same protein. Next, the peptide with highest intensity in both spots (1646.9 m/z) was analyzed by MS in tandem MALDI LIFT-TOF/TOF (Figure S2B). MS data from PMF spectra and MS/MS data from MALDI LIFT TOF/TOF spectra were searched for in the SwissProt database using the Mascot database search algorithm for protein and peptide identification. The MS/MS analysis identified the peptide sequence AEGIGLGLYLSPWDR, which is characteristic of α-L-fucosidase in numerous plants (*Oryza* species, *Triticum* species) (Table S1). α-L-Fucosidase belongs to the glycoside hydrolase 29 family, which comprises proteins of 23 370-62 278 Da and 5.01-6.30 pI in the Zingiberales order, to which cardamom belongs, along with other well-known species such as banana. Thus, the analysis of the sequence in the Zingiberales order (Blast analysis at Uniprot database, https://www.uniprot.org/blast) yielded the sequence GIGLGIYLSPWDR (with a significant homology of 92.3% compared with the peptide identified in the proteomic analysis) found in α-L-fucosidase of banana (*Ensete ventricosum* or *Musa ensete*), a protein of 23 921 Da with a pI of 5.70. In summary, given the findings of the proteomic study and the analysis of the resulting sequence in the Zingiberales order, and since there is no database for cardamom, we can conclude that spots 1 and 2 correspond to α-L-fucosidase, a protein of cardamom (17 173 Da with a pI of 5.55 [spot 1] and 5.3 [spot 2]).

Composite foods are increasingly common, making it difficult to identify all the ingredients involved. Herbs and spices often act as hidden allergens [8].

Cardamom can be used alone or in seasoning mixes such as curry powder, thus making cardamom and other spices minor components in dishes and hampering recognition of their role in adverse reactions to food [2].

Although α-L-fucosidase has not been described as an allergen to date, other glycoside hydrolases, such as ß-glucosidase, ß-galactosidase, ß-mannanase, ß-xylosidase, and α-galactosidase, have been widely described as allergens [9].

To our knowledge, we present the first case of IgE-mediated allergy to cardamom. Our findings suggest that the causative allergen is α-L-fucosidase.

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. Govindarajan VS, Narasimhan S, Raghuveer KG, Lewis YS. Cardamom--production, technology, chemistry, and quality. Crit Rev Food Sci Nutr. 1982;16(3):229-326.
2. Chen JL, Bahna SL. Spice allergy. Ann Allergy Asthma Immunol. 2011;107(3):191-9.
3. Jensen-Jarolim E, Leitner A, Hirschwehr R, Kraft D, Wüthrich B, Scheiner O, et al. Characterization of allergens in Apiaceae spices: anise, fennel, coriander and cumin. Clin Exp Allergy. 1997;27:1299-306.
4. Gehlhaar P, Gonzalez-de-Olano D, Madrigal-Burgaleta R, Bartolome B, Pastor-Vargas C. Allergy to ginger with cysteine proteinase GP-I as the relevant allergen. Ann Allergy Asthma Immunol. 2018;121:624-5.
5. Breiteneder H, Radauer C. A classification of plant food allergens. J Allergy Clin Immunol. 2004;113:821-31.
6. Worm M, Sturm G, Kleine-Tebbe J, Cichocka-Jarosz E, Cardona V, Maris I, et al. New trends in anaphylaxis. Allergo J Int. 2017;26(8):295-300.
7. Martínez-Alonso E, Guerra-Pérez N, Escobar-Peso A, Regidor I, Masjuan J, Alcázar A. Differential association of 4E-BP2-interacting proteins is related to selective delayed neuronal death after ischemia. Int J Mol. Sci. 2021;22:10327.
8. Skypala IJ. Food-induced anaphylaxis: Role of hidden allergens and cofactors Front Immunol. 2019;10:273.
9. Carpio-Escalona L-V, Fernández-Lozano C, Peracho L, Martínez-Alonso E, Rita C-G, Martínez-Botas J, et al. Two new allergens in watermelon (Citrullus lanatus) allergy. J Investig Allergol Clin Immunol. 2023;33:59-61.

Manuscript received March 8, 2023; accepted for publication October 17, 2023.

Lorena Bernal-Rubio
Hospital Universitario Ramón y Cajal
Servicio de Alergia.
Carretera Colmenar Viejo, km 9
28034, Madrid, Spain
E-mail: lo.bernalrubio@gmail.com

Alberto Alcázar
Department of Research Hospital Universitario Ramón y Cajal, IRYCIS
Carretera de Colmenar Viejo
28034 Madrid, Spain
E-mail: alberto.alcazar@hrc.es

