Identification of alpha-L-fucosidase protein as the possible responsible of cardamom food allergy

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Accepted Article

This article has been accepted for publication and undergone full peer review but has not been through the copyediting, typesetting, pagination and proofreading process, which may lead to differences between this version and the Version of Record. Please cite this article as doi: 10.18176/jiaci.0957

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doi: 10.18176/jiaci.0957

Key words: Alpha-L -fucosidase. Cardamom. 2-D PAGE. Slot blot. Spice allergy.

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

Cardamom is a term used to name a group of aromatic spices of the Zingiberaceae family. *Elettaria cardamomum,* also called "true cardamom" distributed in Malaysia and India, and *Amomum* sp or "large cardamom" distributed in Asia and Australia, are the major species of cardamom. A third specie, *Aframomum* sp, 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, some spice allergens have been identified and characterized: Bet-v-1 homologous in anise, fennel, coriander, and cumin [3], 2S albumins in sesame seed and mustard, cysteine proteinase GP-I in ginger [4] or 7S globulin in sesame and fenugreek [5]. Only one case of cardamom food allergy has been reported [6].

We report a case of a 38-year-old woman who presented with palmoplantar pruritus, diffuse erythema, eyelids and lips angioedema, nasal congestion and discharge, dyspnea, throat tightness, abdominal pain and diarrhea one hour after eating a Sicilian mortadella (SM) sandwich with white bread without seeds and a coke, no sauces were added. She took 2 mg of oral dexchlorfeniramine every 12 hours during this day until she felt asymptomatic. The patient had eaten this SM previously without incidents. Later, she

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doi: 10.18176/jiaci.0957

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tolerated coke and the same bread. No cofactors (exercise, AINES, alcohol) were involved.

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

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

We asked the manufacturer for non-labeling additives: food color E-120, vitamin C, cinnamon, black pepper, rosemary, cardamom and Sherry. Prick by prick were performed with all of them, with 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 developed a slot-blot assay against each of the extracts

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doi: 10.18176/jiaci.0957

(Figure S1A). SDS-PAGE IgE immunoblotting assays revealed IgE reactivity in a <20 kDa-band in the extract of cardamom, where control sera showed no reaction (Figure S1B).To determine precisely the allergenic protein involved, cardamom extract was analyzed by two-dimensional (2-D) gel electrophoresis using a 3–10 pH gradient in the first dimension. After second dimension on SDS-PAGE , 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 anti-human IgE secondary antibody (Southern-Biotech). Two protein spots, with a molecular mass less than 20 kDa and isoelectric point (pI) between 5.2 and 5.6, were recognized by IgE antibodies (Figure 1A). To identify these proteins, spots matched between 2-D immunoblot (Figure 1A) and Coomassie blue-stained 2-D gel (Figure 1B) 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 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 were obtained in both spots , matching ten peptides with masses (m/z) 1215, 1250, 1392, 1406, 1546, 1575, 1633, 1646, 1757 and 2119 (Figure S2A), which would indicate that they are the same protein. Next, the peptide with higher 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 in the SwissProt database using the Mascot database search algorithm for protein and peptide identification. The MS/MS analysis identified the AEGIGLGLYLSPWDR peptide, sequence characteristic of alpha-L-fucosidase in numerous plants (*Oryza spp*, *Triticum spp*) (Table S1). Alpha-L-fucosidase belongs to the *glycoside hydrolase 29 family*, proteins of 23,370–62,278 Da and 5.01–6.30 pI in the Zingiberales order, to which

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cardamom belongs along with other well-known species such as banana. Thus, the analysis of that sequence in the Zingiberales order (Blast analysis at Uniprot database, https://www.uniprot.org/blast) gave the GIGLGIYLSPWDR sequence —with a significant homology of 92.3% compared with the peptide identified in the proteomic analysis— existing in alpha-L-fucosidase of banana (*Ensete ventricosum* or *Musa ensete*), protein of 23,921 Da and pI of 5.70. In summary, from the analysis of the proteomic study and the analysis of its sequence in the Zingiberales order, and since there is no cardamom database, we can conclude that spots 1 and 2 would correspond to alpha-L-fucosidase, protein of 17,173 Da and pI of 5.55 (spot 1) and 5.3 (spot 2) of cardamom.

Nowadays, eating composite foods is common, turning into a difficult task to discover all the ingredients involved. Herbs and spices often act as hidden allergens [8].

Cardamom can be used alone or in seasoning mixes like curry powder. This fact transforms cardamom and other spices into minor components in dishes, which makes it easy to ignore its role in adverse reactions to food [2].

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

To our knowledge, we present the first case of IgE-mediated allergy to cardamom that suggests an alpha-L-fucosidase as allergen.

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doi: 10.18176/jiaci.0957

Conflicts of interest

The authors declare no conflicts of interest.

Funding sources

None

Informed consent to publication of the patient was obtained.

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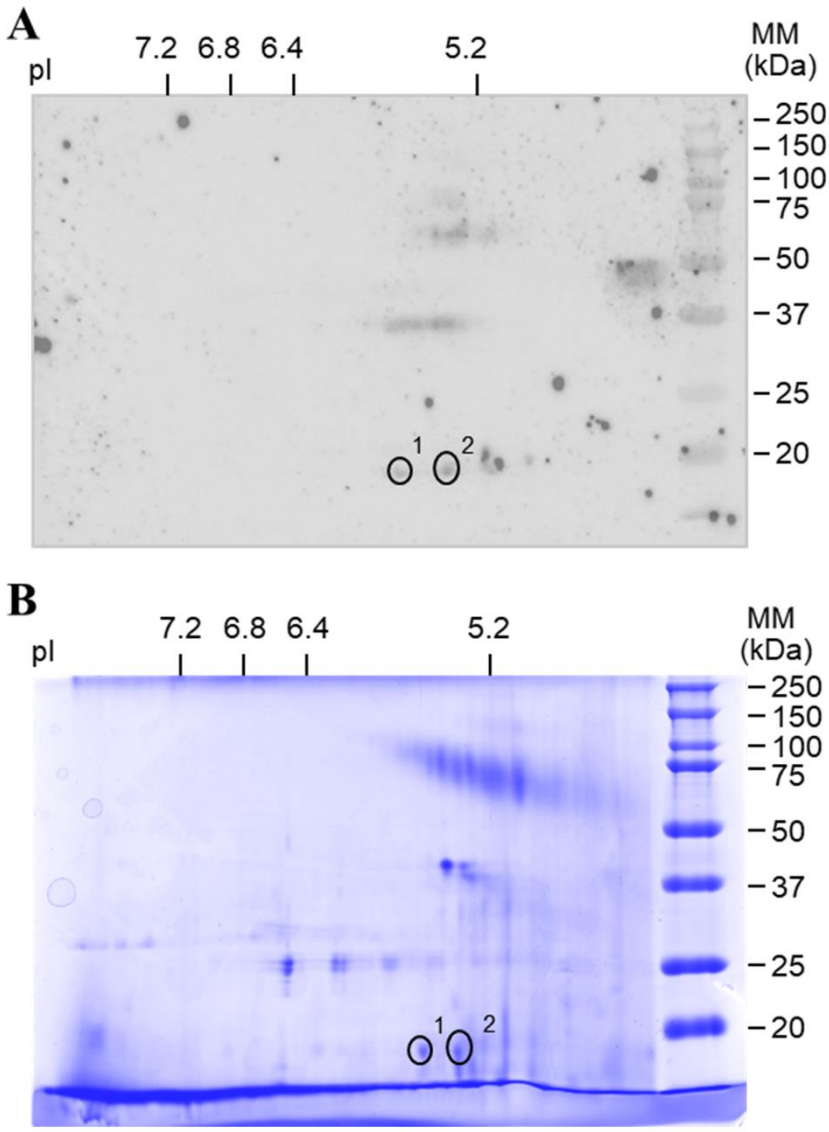
doi: 10.18176/jiaci.0957

Figure 1. Detection of cardamom IgE-reactive proteins with serum of cardamom-sensitive patient. (A) Western blot performed after two-dimensional (2-D) gel electrophoresis analysis of cardamom extract (~100 µg). (B) Cardamom extract (~100 µg) analyzed by 2-D gel electrophoresis and stained with Coomassie blue. Protein spots marked 1 and 2 were matched between the 2-D western blot (A) and the stained 2-D gel (B), and were then excised and processed for identification by MALDI-TOF MS and MALDI LIFT-TOF/TOF (MS/MS) analysis. MM indicates molecular mass of protein markers in kDa.



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