
Case report

Identification of a 7S globulin as a novel coconut allergen

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Background: Coconut (*Cocos nucifera*) is a monocotyledonous plant of the Arecaceae family. Allergy to coconut is infrequent, with only 5 cases reported so far in the medical literature.

Objective: To identify coconut allergens in 2 patients allergic to this food.

Methods: We describe 2 patients allergic to coconut: an adult pollen-allergic patient monosensitized to coconut who presented with severe oropharyngeal symptoms and a child with a previous allergy to walnut, not allergic to pollen, who developed anaphylaxis on coconut ingestion. Both patients had positive skin prick test results and serum specific IgE (CAP) to coconut. IgE sodium dodecyl sulfate–polyacrylamide gel electrophoresis immunoblotting was performed to identify the allergens involved, and a strong IgE binding band detected in both patients was further analyzed by matrix-assisted laser desorption/ionization time-of-flight mass spectroscopy (MALDI-TOF MS). Stability to pepsin digestion of the coconut extract and its cross-reactivity with tree nuts were studied.

Results: An immunoblot showed an almost identical profile of IgE binding proteins in the coconut extract in both patients who reacted strongly to a band of approximately 29 kDa. The peptide analysis by MALDI-TOF MS of this band obtained the sequence GHGKREDPEKR. The protein with the highest correlation with this peptide was found to be a 7S globulin from *Elaeis guineensis*, another oil palm species also belonging to the Arecaceae family. The 29-kDa band was digested by pepsin in less than 1 minute. Cross-reactivity among coconut, walnut, and hazelnut was demonstrated by CAP inhibition in patient 2.

Conclusion: We have identified a 7S storage protein as a novel coconut allergen.

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INTRODUCTION

Coconut (*Cocos nucifera*) is a monocotyledonous plant of the Arecaceae family widely distributed in the tropical areas of Asia, Africa, Latin America, and the Pacific region. In the growing areas, coconut palm is a primary source of food, drink, fuel, and animal feed and shelter. In the occidental countries, coconut is rarely consumed. Only 5 cases of immediate hypersensitivity reactions to coconut after the consumption of this nut have been described in the medical literature so far.^{1–4} Three of these patients also presented with allergic symptoms to tree nuts, and these studies may demonstrate cross-reactivity between walnut and hazelnut allergens and coconut, which probably correspond to legumin-like (11S globulins) seed storage proteins. The only coconut allergen identified, although not well characterized, is an 11S

globulin named cocosin.⁵ Cocosin is a hexamer with a molecular weight of approximately 300 kDa, with each 54-kDa subunit comprising an acidic and basic chain linked by a disulfide bond. These bands migrate on electrophoresis to approximately 35 and 32 kDa (acidic chains) and a complex of 22 kDa (basic chains). We report 2 cases of coconut allergy and describe a novel coconut allergen belonging to the 7S globulin storage proteins.

METHODS

Patients

Patient 1 was a 30-year-old man, with a previous history of grass and olive pollen allergy, who 10 minutes after the intake of fresh coconut experienced severe oropharyngeal itching, throat hoarseness, and gastric pain. The reaction subsided spontaneously in 1 hour. Some weeks later the patient took a spoonful of a coconut dessert and experienced immediate oropharyngeal itching. The patient did not report any other adverse reactions to foods, including peanut and tree nuts. The results of skin prick testing (SPT) to fresh coconut were positive (11 mm wheal), and the serum specific IgE level (Pharmacia CAP System) for coconut was 18 kU/L. The results of SPT and serum specific IgE testing for almond, chestnut, hazelnut, walnut, peanut, and latex were negative.

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The results of serum specific IgE testing for rBet v 1 and rBet v 2 (birch profilin) were negative. The patient did not consent to an oral challenge with coconut.

Patient 2 was a 4-year-old girl first seen in our allergy unit at 2 years of age for an immediate reaction after taking a bite of a walnut. Her symptoms consisted of intense oropharyngeal itching and facial pruritic erythema. At 3.5 years she ate a piece of fresh coconut, and in less than 15 minutes she experienced intense oropharyngeal itching, followed by facial angioedema, cough, and wheezing dyspnea, which required emergency department treatment. The patient had experienced mild atopic dermatitis at 1 year of age, she had no respiratory symptoms, and she had avoided peanut and tree nuts since the walnut-induced reaction. Apart from this she had a normal diet. The results of SPT to fresh coconut were positive (11 mm wheal), and the serum specific IgE level for coconut was 18.3 kU/L. The results of SPT for almond (3 mm), hazelnut (11 mm), and walnut (4 mm) were positive, with negative results for peanut and latex. The serum specific IgE values for almond, hazelnut, and walnut were 2.40, 19.70, and 49.0 kU/L, respectively. The serum specific IgE test results for latex, rBet v 1, and rBet v 2 were negative. Because of the severity of the coconut adverse reaction, an oral challenge with coconut was discarded.

Coconut immunoblotting

Defatted coconut was extracted in phosphate-buffered saline (PBS) as previously described² and subjected to sodium dodecyl sulfate–polyacrylamide gel electrophoresis (SDS-PAGE) in reducing conditions.⁶ Ten micrograms (total protein) of coconut extract was applied per lane, and an IgE immunoblotting was performed according to Rodríguez-Pérez et al.⁷ Separated proteins were transferred to Immobilon-P membranes (Millipore) for 1 hour at 100 V with a Mini Trans-Blot Cell (Bio-Rad Laboratories, Hercules, CA). The membrane was blocked by 1-hour incubation at 40°C in Tris-buffered saline and 0.1% Tween 20 (TBS-T) that contained 5% skimmed milk powder (blocking buffer). After washings with TBS-T, the membrane strips were incubated with the patients' sera (undiluted, 500 µL) overnight at 4°C. Afterward, strips were washed with TBS-T before incubation with anti-human IgE conjugated with alkaline phosphatase (Sigma-Aldrich, St Louis, MO) at 1:100 in the blocking buffer for 3 hours at room temperature. Finally, the immune complex was detected by the addition of 5-bromo-4-chloro-3-indolyl phosphate/nitro blue tetrazolium chloride (Sigma-Aldrich).

Mass spectrometry analysis of protein bands

To identify the protein of interest by mass spectrometry (MS), the protein band was cut from the Coomassie blue–stained polyacrylamide gel. Protein selected for analysis was in-gel reduced, alkylated, and digested with trypsin according to Sechi and Chait,⁸ and the resulting peptide mixture was analyzed by matrix-assisted laser desorption/ionization time-of-flight (MALDI-TOF) MS. After chemically assisted frag-

mentation treatment of peptides with a chemically assisted fragmentation MALDI sequencing kit (Amersham Biosciences, Sunnyvale, CA), the suitable precursors for MS/MS sequencing analyses were selected and fragmentation was performed using the collision-induced dissociation. De novo sequencing from fragmentation spectra of peptides was performed using De Novo tool software (Applied Biosystems), and homology search of the sequences was obtained by the Basic Local Alignment Search Tool.

Digestion of coconut extract with simulated gastric fluid

The digestion of coconut extract was performed essentially as described by Díaz-Perales et al.⁹ Briefly, the crude coconut extract (120 µg of protein) was dissolved in 50 µL of pre-warmed 100-mmol/L hydrochloride, pH 1.2, with 30-mmol/L sodium chloride (controls) and in the same solution (150 µL) containing 0.32% (wt/vol) of pepsin A (Sigma). Digestion was performed with continuous shaking for 30 minutes at 37°C. Aliquots of this solution were withdrawn at 30 seconds and 1, 10, and 30 minutes and immediately frozen in liquid nitrogen, freeze-dried, redissolved in sample buffer, and separated by means of SDS-PAGE.

CAP inhibition assays

The serum of patient 2 was used for CAP inhibition assays. Duplicated samples of the serum were premixed with coconut or walnut extracts at a final concentration of 1.8 µg/µL in PBS buffer, pH 7.4, or with the PBS buffer alone (basal value). Serum samples were incubated overnight at 4°C, and afterward specific IgE levels for coconut, walnut, and hazelnut were determined following the standard CAP procedure.

RESULTS

With SDS-PAGE, 10 protein bands between 17 and 93 kDa were identified (Fig 1). The profile of IgE-binding proteins was almost identical in the 2 patients (Fig 1), and surprisingly, both patients reacted strongly to a protein band of 29 kDa approximately.

To identify this 29-kDa protein band, a peptide analysis by MALDI-TOF MS was performed. The MS spectrum showed multiple peaks ranging from 700 to 4,000 Da. Two prominent peaks, 1,270.4 and 1,426.5 Da, and another of lesser intensity, 1,308.7 Da, were selected for MS/MS sequencing analyses (Fig 2). The fragmentation spectra identified the peptides at 1,270.4 and 1,426.5 as the same peptide, with a sequence QQPFYDEGMR and QQPFYDEGMRR, respectively, but no sequence similarities with other peptides were found in databases. The third peak at 1,308.7 was identified as GHGKREDPEKR. The protein with the highest correlation with this peptide was found to be a 7S globulin storage protein from *Elaeis guineensis* (African oil palm), which presents 77% of identity. *E. guineensis* is an oil palm species, native to West Africa, and belongs to the Arecaceae family as coconut. Fruits and seeds of the African oil palm yield a valuable vegetable oil similar to coconut oil.

The effect of simulated gastric fluid on coconut extract was analyzed by SDS-PAGE separation followed by Coomassie

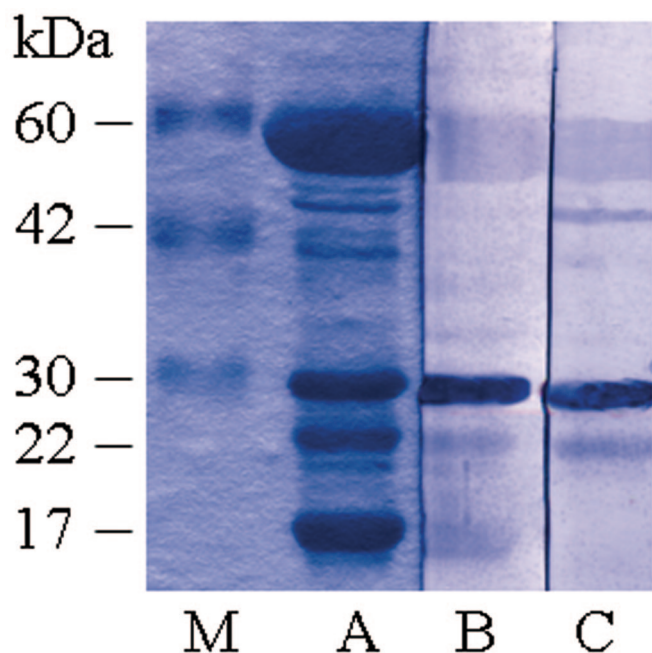


Figure 1. Sodium dodecyl sulfate–polyacrylamide gel electrophoresis (SDS-PAGE) and immunoblotting. A, SDS-PAGE of coconut extract; B, IgE immunoblotting of patient 1; and C, IgE immunoblotting of patient 2. M indicates molecular weight markers.

protein staining of the treated samples (Fig 3). The 29-kDa coconut allergen was extensively digested by pepsin in less than 1 minute. Unfortunately, immunoblot analysis of the treated samples was not possible because of the lack of serum samples from patients.

CAP inhibition assays were performed in patient 2 to investigate the existence of cross-reactivity among coconut, walnut, and hazelnut. When coconut was used as inhibitor, a 100% inhibition of coconut CAP was achieved, whereas the inhibitions of walnut and hazelnut CAPs were of 48% and 54%, respectively. Using walnut as an inhibitor, the walnut CAP was completely inhibited (100%), and almost total inhibitions of the CAPs to coconut and hazelnut were achieved (96% and 94%, respectively).

DISCUSSION

Allergy to coconut is uncommon, with only 5 reported cases in the medical literature. Three of these patients^{1,4} presented with a primary sensitization to tree nut allergens, and 2 patients^{2,3} were monosensitized to coconut. Teuber and Peterson¹ described the first 2 patients with coconut allergy and a primary sensitization to walnut who later developed systemic reactions to coconut ingestion due to cross-reactivity to legumin-like seed storage proteins. The sera IgE of these patients recognized proteins of 55 (only 1 patient), 36.5, and 35 kDa on a reduced coconut immunoblot. The authors suggested that the 35-kDa protein corresponded to the acidic polypeptides of the coconut 11S globulin.⁵ The study of a

patient with anaphylaxis to coconut and oral symptoms to tree nuts by Nguyen et al⁴ demonstrated the presence of cross-reactive IgE between hazelnut and coconut. Immunoblots showed IgE binding bands of 35 and 50 kDa in the coconut and hazelnut extracts. In contrast, the allergen profile found in the 2 patients monosensitized to coconut was different, with identification on IgE immunoblots of strong binding bands of 78 kDa² and 18 kDa,³ together with other weaker bands in both cases.

In this article we describe 2 patients allergic to coconut: an adult pollen-allergic patient monosensitized to coconut who presented with severe oral allergy syndrome, and a child with a previous allergy to walnut, not allergic to pollen, who developed a systemic reaction on coconut ingestion. Despite this different clinical presentation, our patients presented an almost identical profile on the IgE immunoblot (Fig 1) with a strong binding to a protein band of 29 kDa approximately identified as a 7S globulin. This protein was shown to be quickly digested by pepsin treatment (Fig 2). We have not found data about the resistance of 7S globulins from tree nuts to proteolysis. In contrast to our finding, the storage globulins of soybean seem to be resistant to pepsin digestion, since immunoreactive globulins have been detected in the ileum 4 to 6 hours after feeding calves on soya flour. Additionally, the degree of digestion of legume proteins is also dependent on the effect of previous processing (including heat treatment) on the globulins themselves.¹⁰

Stability to proteases has been postulated as a general characteristic of food allergens needed to induce sensitization through the oral route and systemic symptoms on oral exposure.^{11,12} Therefore, how can a labile allergen induce a systemic reaction as in the case of patient 2? One possible answer is that absorption takes place in the oral cavity, before the allergen reaches the stomach and is degraded. Another possibility (which can be combined with the previous one) is that the peptides that arise from proteolysis still contain IgE binding epitopes that can be absorbed in the intestine and stimulate the patient's immune system. This has been shown for the chitinase of avocado (Prs a 1),⁹ but unfortunately we did not have enough serum to test the IgE binding capacity of the digested coconut extract. However, the clinical presentation of a food allergic reaction does not only depend on the physicochemical characteristics of the allergens involved. For instance, the clinical presentation of patients allergic to chitinases¹³ or lipid transfer proteins¹⁴ varies from mild local oral allergy syndrome to severe anaphylaxis. Therefore, other factors related to the quality (affinity) of the IgE response, other foods taken in the same meal, and patient-related factors might determine the final clinical expression of a food allergic reaction.¹⁵

The 7S globulins have already been described as allergens in walnut (Jug n 2, Jug r 2) and hazelnut (Cor a 11),¹⁶ but this is the first description in coconut. Sensitization to 7S globulins can explain the associated walnut allergy of patient 2 and the sensitization to hazelnut in this child detected by means of SPT and CAP. The CAP inhibition results confirm the exis-

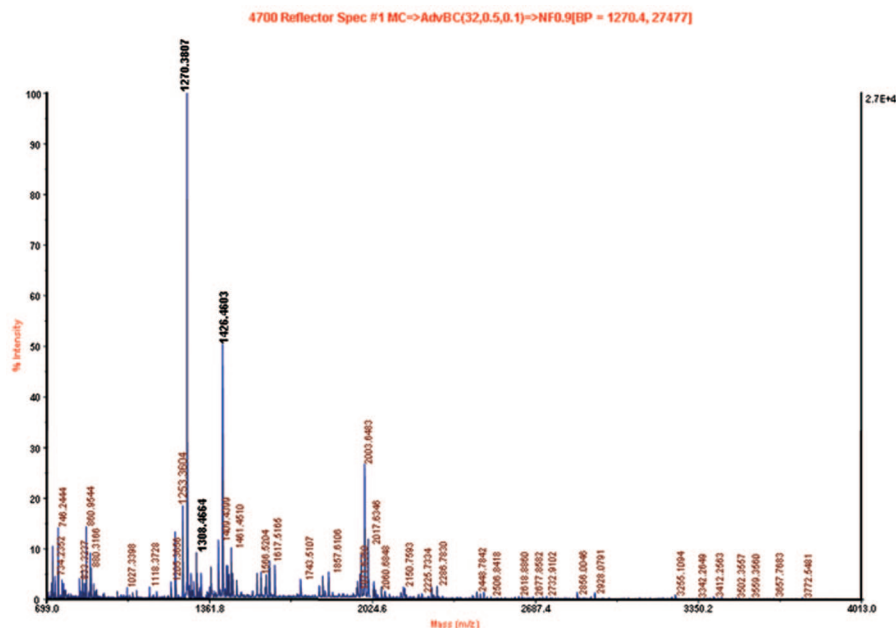


Figure 2. Matrix-assisted laser desorption/ionization time-of-flight mass spectroscopy profile of the 29-kDa protein band. The peaks 1,270.4, 1,308.7, and 1,426.5 were selected for fragmentation.

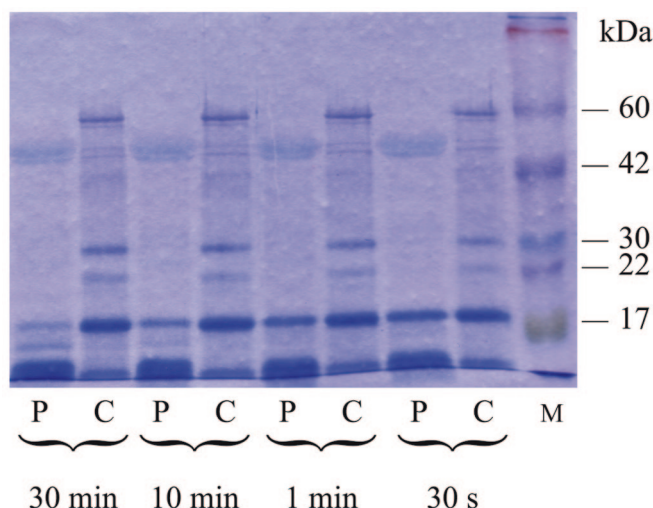


Figure 3. Effect of simulated gastric fluid on coconut extract (sodium dodecyl sulfate–polyacrylamide gel electrophoresis). M indicates molecular weight markers; C, control; and P, pepsin treated.

tence of cross-reactivity among these 3 nuts in patient 2. Altogether, the studies performed in patient 2 suggest a primary sensitization to walnut: this nut induced the first reaction, its CAP value was the highest, and it was the most potent inhibitor in the CAP inhibition assays. The lack of sensitization to tree nuts in patient 1, who was also sensitized to the 7S globulin in coconut, is however surprising. This finding is probably related to a different epitope recognition pattern related to the primary sensitizer, which might be

coconut 7S globulin in patient 1 and the walnut 7S globulin in patient 2. Further investigations are necessary to fully characterize this novel coconut allergen and to investigate its cross-reactivity pattern with tree nut allergens.

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