

The Role of Lipid Transfer Proteins in the Almond Tree and Almond Fruit as Contact and Airborne Allergens

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The almond, which is a member of the Rosaceae family, is the fruit of the almond tree (*Prunus dulcis*). It has a hard endocarp that contains a seed covered by a rough, brownish skin. Unlike other prunoid fruits, which have an edible mesocarp and an outer skin known as the epicarp, the almond fruit has an epicarp and a mesocarp that are difficult to separate and have no nutritional or commercial value (Figure 1).

We report the case of a 17-year-old atopic patient referred for consultation due to eyelid angioedema and rash in exposed areas related to exposure to or contact with almond tree leaves and almond fruit epicarp/mesocarp on the family farm. Symptoms appeared when he touched or was near almond tree leaves and/or epicarp/mesocarp. He reported good tolerance to contact with and ingestion of both peeled and unpeeled almonds. He also had a history of oral pruritus following the ingestion of unpeeled peach and rhinoconjunctivitis due to house dust mite and cat dander.

Almond fruit extracts (seed, skin, endocarp, and epicarp/mesocarp) (20% w/v) and almond tree leaf extracts derived from raw material were prepared for in vitro analysis and glycerinated at 50% for in vivo analysis. Commercial extracts of almond seed and peach skin and pulp (Bial Aristegui, Bilbao, Spain) were also used for in vitro analysis.

Skin prick tests (SPTs) performed with airborne allergens, food, fruits (including peach skin and pulp), nPru p 3, almond leaf, seed, skin, endocarp, and epicarp/mesocarp were positive for peach skin, nPru p 3, almond leaf and epicarp/mesocarp, among others. They were negative for the 3 types of almond

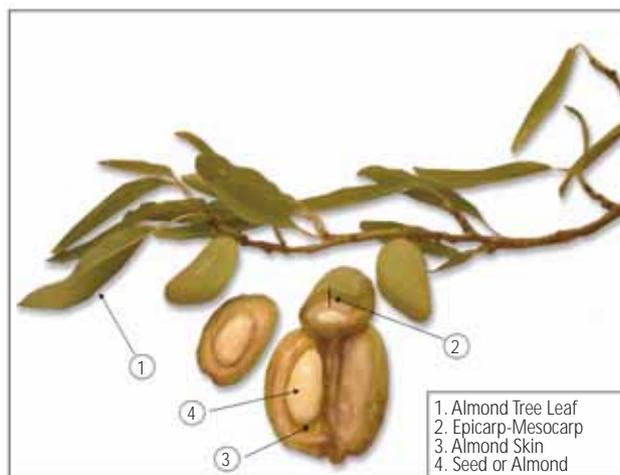


Figure 1. Parts of almond tree and fruit.

seeds used and for the almond seed, skin, and endocarp extracts. Serum specific immunoglobulin (Ig) E (CAP System; Pharmacia, Uppsala, Sweden) was negative for almond fruit and positive for peach (4.63 kU/L).

To aid diagnosis, we performed basophil activation tests (BAT) [1] and assessed antigen-specific sulphidoleukotriene production using the cellular antigen stimulation test (CAST) [2] with epicarp/mesocarp (5.8 µg/µL and 1.45 µg/µL), almond leaf (1.1 µg/µgL and 0.3 µg/µL), peach pulp (1.25 mg/mL and 0.31 mg/mL), and peach skin (2.09 mg/mL and 0.52 mg/mL). In vitro stimulation was positive for epicarp/mesocarp in BAT and for epicarp/mesocarp, peach skin, and peach pulp in CAST. BAT was negative for almond leaf, peach skin, and peach pulp and CAST for almond leaf. Negative SPTs and in vitro results were also obtained in 5 nonatopic control patients.

Proteins from epicarp/mesocarp and almond tree leaf

were separated by electrophoresis, which revealed a protein band pattern with molecular weights of between 9 kDa and 63 kDa.

Immunodetection performed on epicarp/mesocarp and tree leaf extracts with an anti-Pru p 3 monoclonal antibody revealed an uptake band of approximately 9 kDa for both structures but which was more intense in the case of the leaf extracts. When immunodetection was performed using the same extracts with the patient's serum and anti-IgE as a secondary antibody, a band of approximately 9 kDa was observed for the epicarp/mesocarp extract (Figure 2).

Our study found that both almond tree leaves and almond fruit epicarp/mesocarp were allergenic sources, indicating the existence of lipid transfer proteins (LTPs) with allergenic potential. This is indeed plausible as LTPs are mainly located in the external part of plants and vegetables.

Although basophils were poorly reactive, evidenced by the negative response to anti-IgE (positive control), a positive result was obtained for epicarp/mesocarp in BAT and sLT. This might explain the negative response to almond tree leaves in both these tests.

Although allergies due to members of the Rosaceae family and LTPs following inhalation [3], contact [4], and ingestion [5] have been well documented, we did not find any reports of airborne urticaria or angioedema caused by either of these sources in the literature.

Prevailing sensitization to LTPs was evidenced by the positive SPT for nPru p 3 and the presence of a protein weighing approximately 9 kDa in epicarp/mesocarp and almond leaf extracts recognized by the anti-Pru p 3 monoclonal antibody in both cases and by IgE-detection in the case of epicarp/mesocarp.

The close taxonomic relationship between peach and

almond (both members of the Rosaceae family) and the existence of previous sensitization to peach LTPs could account for the allergy to LTPs in different almond tree sources, with peach LTPs acting as the primary sensitizer through a mechanism of cross-reactivity.

The only almond fruit allergens registered in the official allergen nomenclature of the International Union of Immunological Societies are Pru du 4 (profilin) [6] and Pru du 5 (60S acidic ribosomal protein, unpublished).

Recently, other allergens such as 2S albumins [7], conglutinin γ [7], and the almond major storage protein, amandin (legumin-like protein) [8] have been described, as has an LTP in *Prunus dulcis* sequenced at the RNAm level [9]. Our findings constitute the first explicit report of the presence of LTPs (Pru du LTP) in almond fruit and almond tree leaves.

We conclude that the LTPs present in nonpollinic structures such as almond fruit epicarp/mesocarp and almond tree leaves may behave as contact or airborne allergens and that these panallergens are present and relevant in the almond tree and almond fruit.

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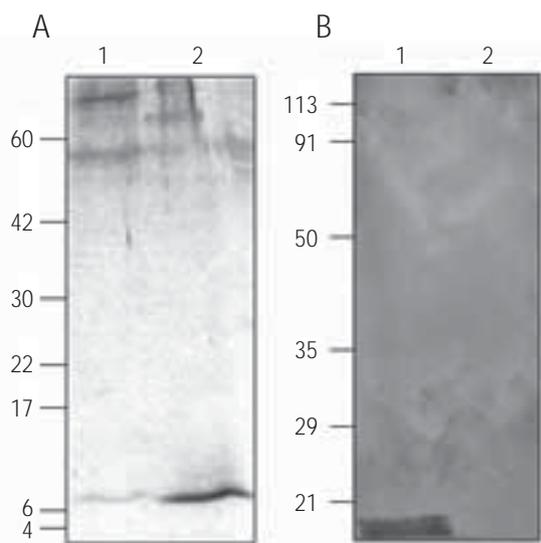


Figure 2. A, immunodetection with anti-Pru p 3 monoclonal antibody in epicarp/mesocarp extract (lane 1) and almond leaf extract (lane 2). B, immunoglobulin E detection. Epicarp-mesocarp extract (lane 1), almond leaf extract (lane 2).

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