

## Analysis of the heat stability of lipid transfer protein from apple

### *To the Editor:*

Lipid transfer protein (LTP), the major allergen in Rosaceae fruits in areas where the prevalence of birch pollen allergy is low,<sup>1,2</sup> is a potentially dangerous allergen. Because of its extreme resistance to pepsin digestion,<sup>3</sup> LPT probably reaches the intestinal tract in an almost unmodified form, inducing severe systemic reactions in allergic subjects.<sup>2-5</sup> Heat-stability is a less well known (but potentially relevant) feature of this protein. In this respect, existing data are few. In one study, treatment at 121°C for 30 minutes did not reduce the allergenicity of peach LTP,<sup>6</sup> and molecular biology analyses found that LTP extracted from cereals<sup>7</sup> and other plants<sup>8</sup> is heat-stable. To date, however, in patients sensitized to LTP, adverse reactions induced by thermally processed foods have been associated only with beer<sup>9</sup> and with maize.<sup>10</sup> We investigated the effect of heat processing on the IgE binding activity of apple LTP.

Fourteen patients sensitized to LTP, screened by means of positive skin prick test results with a commercial plum extract (Dome-Hollister/Stier 1:20 w/v),<sup>5</sup> were studied. All had peach allergy (repeated episodes of acute urticaria and/or oral allergy syndrome [OAS]); 4 of the patients had apple allergy as well. All scored strongly positive in skin prick testing (SPT) both with a peach peel extract (Lofarma, Milan; 440 µg protein/mL) and with fresh Golden Delicious apple peel. The following experiments were carried out:

- (a) Eight patients and 8 birch pollen-allergic control subjects with OAS induced by fresh apple underwent SPT with boiled (100°C for 10 minutes) apple peel extract (100 µg protein/mL).
- (b) IgE to LTP purified from peach peel by gel chromatography<sup>3</sup> was measured by ELISA, before and after absorption of 65 µL of serum from each of the 14 patients, with 55 µL of both raw and boiled (100°C for 30 minutes) Golden Delicious apple extract (300 µg protein/mL) for 150 minutes at 25°C.<sup>3</sup> Reductions of >75%, 50% to 75%, and <50% of reactivity to peach LTP were considered total inhibition, partial inhibition, and no inhibition, respectively.
- (c) Immunoblot to cooked apple peel (100°C for 30 minutes) was performed through use of sera from 3 patients, as previously described.<sup>3</sup>
- (d) Two patients monosensitized to LTP,<sup>3</sup> a 26-year-old woman with apple-induced OAS (patient 12) and a 21-year-old man with a history of severe abdominal pain and diarrhea induced by apple (patient 3), underwent SPT with raw, boiled (100°C for 10 and 30 minutes), and oven-cooked (180°C for 10 and 20 minutes) Golden Delicious apple peel as well as oral challenge tests with heat-processed apple. The woman was asked to chew approximately 5 to 10 g of oven-cooked apple, including peel, for 1 minute and then spit it out. Reactivity was assessed after 10 min-

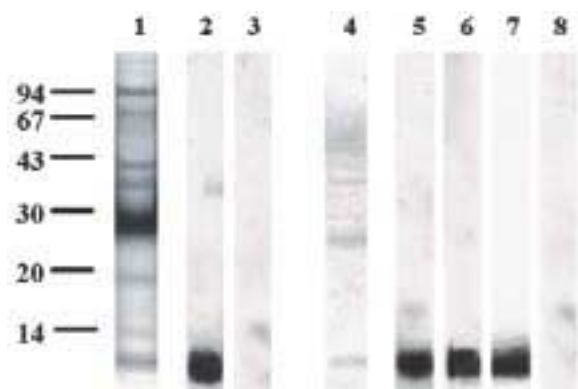


**FIG 1.** SDS-PAGE electrophoresis of raw (lane 1) and cooked (lane 4) apple peel extracts and immunoblot analysis. Lanes 2 and 3: IgE reactivity of patient 12 and of a normal serum to raw apple peel. Lanes 5-8: IgE reactivity of patients 12, 13, and 14 and of a normal serum to cooked apple peel. Heat processing does not cause any loss in IgE binding activity of LTP.

utes, and severity of OAS was graded by comparison with OAS induced by an equivalent amount of raw apple (considered 100% severity). The man was asked to ingest approximately 10 g of boiled apple (100°C for 30 minutes), including peel, and was kept under observation during the following 2 hours. SPT with both raw and heat-processed apple was carried out in 2 birch pollen-allergic patients with apple-induced OAS as control.

All of the 8 LTP-hypersensitive patients—versus none of 8 control subjects—showed significant skin reactivity to cooked apple peel extract. Sera from all 14 patients showed significant IgE reactivity to peach LTP. Preabsorption of patients' sera with raw apple caused a significant to total loss of IgE reactivity to peach LTP in 13 (100%) of 13 cases. Preabsorption of patients' sera with heat-processed apple totally abolished IgE reactivity to peach LTP in 12 (86%) of 14 cases and caused a >50% inhibition in the remaining 2 cases (Table 1). On immunoblot analysis, sera from LTP-allergic patients reacted to a single band at approximately 10 kd in both raw and heat-processed apple (Fig 1). In patient 12, SPT both with boiled apple and with oven-cooked apple induced a stronger skin reaction than the peel of the raw fruit (12 mm and 10 mm vs 7 mm, respectively). Oral challenge both with boiled apple and with oven-cooked apple induced OAS that the patient graded as "similar to that induced by raw apple." In patient 3, raw, boiled, and oven-cooked apple peel all induced strong skin reactivity (wheal diameter, approximately 7 mm in each case). Approximately 5 minutes after the ingestion of boiled apple, the patient reported gastric pain that he described as qualitatively identical to that induced by raw apple but less intense, probably because of the limited amount of apple ingested. Symptoms gradually disappeared within 45 minutes without any therapy. The 2 control subjects were positive on SPT with fresh raw apple but negative on SPT with heat-processed apple.

**TABLE 1.** IgE reactivity to peach LTP by ELISA and ELISA inhibition studies

| Patient no. | Reactivity to peach LTP | Percent inhibition of ELISA signal to peach LTP by . . . |              |           |
|-------------|-------------------------|----------------------------------------------------------|--------------|-----------|
|             |                         | Raw apple                                                | Cooked apple | Ovalbumin |
| 1           | 3174                    | 100                                                      | 97           | —         |
| 2           | 413                     | 100                                                      | 100          | —         |
| 3           | 3085                    | 99                                                       | 100          | —         |
| 4           | 859                     | 100                                                      | 57           | —         |
| 5           | 2897                    | 99                                                       | 100          | —         |
| 6           | 3236                    | 97                                                       | 79           | —         |
| 7           | 2555                    | 100                                                      | 100          | —         |
| 8           | 2909                    | ND                                                       | 71           | —         |
| 9           | 1701                    | 98                                                       | 97           | —         |
| 10          | 991                     | 100                                                      | 100          | —         |
| 11          | 3179                    | 100                                                      | 100          | —         |
| 12          | 1888                    | 91                                                       | 100          | —         |
| 13          | 2711                    | 100                                                      | 100          | —         |
| 14          | 1816                    | 100                                                      | 100          | —         |
| Pool        | 2256                    | ND                                                       | ND           | 24        |

Each value is expressed as optical density. Value is negative if <300. Inhibition: >75%, total; 50% to 75%, partial; <50%, none.

Popular foods such as juices, sweets, cakes, and jams are often prepared with Rosaceae fruits, including apple, that have been submitted to different thermal procedures. In this study, we investigated the stability of apple LTP after different methods of heat processing, reproducing cooking conditions that might be used in everyday life. Results show the extreme heat stability of this protein. Although clinical studies on a larger number of patients are needed to draw definitive conclusions, our findings suggest that LTP-hypersensitive patients with a history of severe reactions induced by apple should be advised to avoid unpeeled apple even after it has undergone thermal processing. The similarity between LTPs from different Rosaceae fruits<sup>11</sup> suggests that LTPs from other members of this botanical family might show a similar degree of heat resistance.

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