Technological Processes To Decrease the Allergenicity of Peach Juice and Nectar

Oreste Brenna,[†] Carlo Pompei,*[,†] Claudio Ortolani,[‡] Valerio Pravettoni,[§] Laura Farioli,[§] and Elide A. Pastorello[§]

Department of Food Science and Microbiology, University of Milan, Milano, Italy; Bizzozzero Division, Niguarda Cà Granda Hospital, Milano, Italy; and The Allergy Center, III Department of General medicine, Ospedale Maggiore IRCCS, Milano, Italy

Among vegetable foods peach (*Prunus persica*) has been recognized as a significant cause of allergy. The protein, which is considered to be the major peach allergen, has been named Pru p 1. Because peaches are consumed both as fresh fruits and after processing to obtain peach juice, nectar, jam, syrupy peach, etc., research was carried out to identify a technological process for production of hypo- or nonallergenic peach-based products. SDS−PAGE and immunoblotting analysis of extracts prepared from four commercial peach nectars showed that the Pru p 1 was not removed, and neither was its allergenic activity decreased by technological treatments carried out for nectar production. Some treatments oriented toward a removal of or, at least, a decrease in the allergenic power were assumed and verified at laboratory scale. A variable considered was heat treatment at 121 °C for 10 and 30 min: this treatment was not able to decrease the allergenicity of the Pru p 1 protein. Furthermore, the protein band was still present even after 60-min reaction with two different acidic proteases. The two technological treatments that were found to decrease the major allergen of peach were chemical lye peeling of fruits and ultrafiltration of juice through membranes with suitable cutoff. On the basis of the results obtained from this research, a processing flow sheet was defined to obtain hypoallergenic or probably nonallergenic limpid juices and nectars. These products may represent, besides finished foods, intermediates to obtain various products after addition of further ingredients such as pectins, sugars, and fiber.

Keywords: *Allergy; fruit juice; nectar; peach*

INTRODUCTION

There is growing concern for food allergy as a result of its increased incidence in the population (Ortolani and Pastorello, 1997). Hence, it is necessary that the food industry be able to meet requirements of consumers with allergies to various foods. Among vegetable foods, peach (*Prunus persica*) has been recognized as a significant cause of allergy (Malet et al., 1988; Cuesta-Herraz et al., 1988; van Ree et al., 1995). Symptoms experienced by sensitized subjects upon consumption of peach and peach-based products include oral allergy syndrome (OAS). The definition of OAS is related to the appearance of oral itching, mucosal edema, labial itching, and papulas upon contact with the offending food or gastrointestinal or systemic symptoms within a few minutes after the food consumption. Recently OAS was classified in four grades: I, oral mucosal symptoms only; II, oral mucosal and gastrointestinal symptoms; III, oral mucosal and systemic symptoms (urticaria, rhinoconjunctivitis, or asthma); IV, oral mucosal symptoms plus life-threatening symptoms (laryngeal edema, anaphylactic shock) (Pastorello et al., 1999b).

Immunochemical analyses, carried out using sera from allergic subjects, have shown responses to >10 different proteins, of molecular masses ranging from 13 to 70 kDa, present in fresh peach fruit extract (Pastorello et al., 1994). The protein, which is considered to be the major peach allergen and to which 90% of allergic subjects react, has been named Pru p 1. It is a lipid transfer protein (LTP1), and its amino acid sequence, which has been registered recently, is represented by 91 amino acids showing a molecular mass of 9178 and an isoelectric point >9 (Pastorello et al., 1999a).

Annual world peach production is estimated to be $\sim 1.13 \times 10^{10}$ kg, a third of which is in the European Community alone (source: FAO). Because peaches are consumed both as fresh fruits and after processing to obtain peach juice, nectar, jam, syrupy peach, etc., research was carried out to identify a technological process for production of peach-based products able to decrease considerably their potential allergenicity. To this end, some industrial technological steps were evaluated in a laboratory, modified, and integrated to obtain a flow sheet for production of hypo- or nonallergenic peach-based products.

MATERIALS AND METHODS

Patients. All of the in vitro tests were performed using two different pools of sera from patients presenting positive skin prick tests (graded +++ or more according to the Nordic Guidelines for skin tests) and RAST for peach. Among the six patients in the first group, three presented only grad I OAS, and three presented systemic symptoms; all of them were birch negative. Among the six patients of the second group, only one had systemic symptoms and all were birch positive.

* Address correspondence to this author at the Department of Food Science and Microbiology, Sezione Tecnologie Alimentari, Via Celoria 2, 20133 Milano, Italy (telephone 39-02-70602063; fax 39-02-70638625; e-mail Carlo.Pompei@unimi.it).
[†] University of Milan.
[‡] Niguarda Cà Granda Hospital.
[§] Ospedale Maggiore IRCCS.

10.1021/jf9906681 CCC: $19.00
Published on Web 12/30/1999

Materials. The following materials were used in this work: four commercially available peach nectars produced by leading companies; two commercially available syrupy peaches produced by two different companies; Redhaven variety peach fruits; and an intermediate taken from an industrial production line for concentrated limpid juice. This sample was taken after both fruit sieving and fluidifying treatment carried out with pectinolytic enzymes.

Methods. *Preparation of Samples for SDS−PAGE Electrophoretic Analysis and Immunoblotting.* Commercial peach nectars were treated essentially according to the method of Björksten (1980). Peach nectar was centrifuged at 10000*g* for 30 min, and the supernatant solution was dialyzed versus distilled water containing 1 mL/L of 4% NaF solution, using a membrane with a 6−8 kDa cutoff (Spectra/Por 1, Spectrum, Laguna Hills, CA). The dialysate was successively treated with a 50% suspension of PVPP (BASF-AG, Ludwigshafen, Germany) in water (2 mL/100 mL of dialysate), gently stirred for 15 min and then filtered on Whatman grade 42 paper (Whatman International, U.K.). The limpid filtrate was concentrated to $^1/_{10}$ of the initial volume by ultrafiltration through a 10 kDa nominal molecular mass cutoff membrane (YM-10, Amicon Corp., Danvers, MA). The same procedure was applied to heat-treated peach nectar samples, fresh fruit homogenate, and the sample drawn from an industrial plant.

Heat Treatment of Peach Nectar. A commercial peach nectar sample was homogenized and poured into 50 mL glass bottles provided with screw caps. The bottles were subjected to autoclave treatment at a temperature of 121 °C for 10 and 30 min.

Preparation of Samples from Fresh Fruit and Syrupy Peach. Two different samples were prepared from fresh fruits: whole or following chemical peeling. The last was carried out by dipping the fruits in 10% NaOH at 60 °C for 90 s, washing in cold tap water, removing the peel by rubbing, and finally briefly washing in 1% HCl. The depitted fruits were homogenized in a blender and diluted 1:1 (v/v) with distilled water. Syrupy peaches were rinsed quickly with tap water, homogenized, and diluted 1:1 with water. These samples were then extracted as described above.

Semipurified Extract. A concentrated and semipurified preparation of the main allergen present in peach products was prepared to better follow the effects of the protease treatment to be applied.

The intermediate of limpid peach juice production was used as a starting material. This product (1 L) was centrifuged at 10000*g* for 30 min, and the supernatant solution was dialyzed versus 0.01 M phosphate buffer, pH 6.9, using a 6−8 kDa cutoff membrane (Spectra/Por 1). The dialysate was then treated with a 50% PVPP suspension in water (2 mL/100 mL) for 15 min and finally filtered on Whatman No. 42 filter paper. Fifty milliliters of S Sepharose Fast Flow (Amersham Pharmacia Biotech, Uppsala, Sweden), equilibrated with the same buffer used for the dialysis, was added and maintained by gentle shaking for 30 min, and the supernatant was then drawn by decantation. The resin was packed in a chromatographic column (2 × 20 cm) and eluted with 2 volumes of the 0.01 M phosphate buffer, pH 6.9. The Pru p 1 protein was easily eluted with 0.1 M borate buffer, pH 9.2, and further concentrated to $^1/_{10}$ of the volume by ultrafiltration on a Y10 membrane (Amicon).

Treatment with Proteolytic Enzymes. Enzymes were dissolved in distilled water (owing to their specific activities, 1.16 and 0.4 mg/mL, for the *Rhizopus* species and *Aspergillus saitoi* protease, respectively, both from Sigma Chemicals, St. Louis, MO). As usual, a ratio of 1:40 was used for the enzymes and the protein to be hydrolyzed (titrated to pH 3.4). Hydrolysis was carried out at 50 °C and pH 3.4, and the samples were drawn at 0, 20, and 60 min, immediately diluted 1:2 (v/v) with the sample buffer for SDS−PAGE, and heated at 100 °C for 5 min. The samples were then analyzed by SDS−PAGE, as described below (procedure a).

Ultrafiltration. Dialyzed and PVPP-treated samples were concentrated on a Y10 membrane, and the ultrafiltrate was newly concentrated on a 5 kDa nominal molecular mass cutoff membrane (YM-5, Amicon Corp.) to ascertain the feasibility of adding an ultrafiltration step to the established flow sheet of limpid fruit juice.

Electrophoretic Analysis. a. For screening, SDS−PAGE gels were prepared at 7.5% in 0.2 M phosphate buffer, pH 7.1, containing 1% SDS. Samples were diluted 1:1 (v/v) with the same buffer, containing 2% 2-mercaptoethanol and 2% SDS, and heated in a boiling water bath for 5 min. Runs were performed at 7 °C and 200 mA.

b. Immunoblotting. To test the immunological activity of the samples, SDS−PAGE was also run in a discontinuous buffer system by using the method of Neville (1991), with a separation gradient gel of 7.5−20% and 6% stacking gel. Proteins were then electrophoretically transferred on a nitrocellulose sheet, pore size 0.2−0.45 μm (Amersham, Buckinshire, U.K.). The unoccupied protein binding sites of the membrane were then saturated by incubation in blocking solution (phosphate-buffered saline, pH 7.4, with 0.1% Tween 20) for 30 min at room temperature. The sheet was then incubated for 6 h with a pool of sera diluted 1:4 in blocking solution overnight. The sera were obtained from patients having an allergy to either peach or peach and birch. Sheets were then washed and incubated overnight with ^{125}I-labeled anti-IgE (CAP RAST IgE RIA, Pharmacia & Upjohn, Uppsala, Sweden) diluted 1:4 in blocking solution. Following final washings, sheets were dried and contacted with a photograph plate (Hyperfilm, Amersham, Buckinshire, U.K.) at −70 °C for times varying from 1 to 14 days.

c. Immunoblotting Inhibition. To demonstrate the presence of Pru p 1 in the samples under study, immunoblotting inhibition was carried out as described by Pastorello et al. (1999a).

RESULTS AND DISCUSSION

The technology used for fruit nectar production includes a first step to obtain an intermediate (puree). After fruit washing and sorting for removal of unsuitable fruit, the following steps are carried out: depitting; heat treatment at 100 °C for a few seconds to soften the pulp and to inactivate the microbial charge, proteolytic enzymes, and oxidases; sieving to remove peel, possible pit fragments and other coarse solid impurities; deaeration to remove oxygen; sterilization at temperatures on the order of 115 °C for 30−40 s; and packaging in aseptic tanks. These steps are carried out during the time when fresh raw material is available. During subsequent months the second processing step, which leads to nectar production, is carried out. Puree is diluted 1:1 with demineralized water, supplemented with citric and ascorbic acid and sugars to obtain a soluble solids value of ∼15 °Brix, and then subjected to sterilization at ∼100 °C for ∼30 s and cooling to 20 °C. The sterilization step also includes degassing and homogenization. The nectar is then packed aseptically in flexible composite structure packages.

Extracts were prepared as described under Materials and Methods using four peach nectars, selected from the most important brands available on the Italian market, and subjected to SDS−PAGE and immunoblotting. From Figure 1 it can be observed that the Pru p 1 protein band was present in the nectars, and all of the nectars were therefore markedly allergenic. The reactivity of the two pooled sera revealed the presence of both Pru p 1 and Bet v 1 homologous allergens in the tested nectars, even though the IgE binding was clearly higher for the first protein, thus demonstrating a higher amount of this allergen. Bet v 1 is the major allergen of birch pollen, one of the most important causes of respiratory allergy. It belongs to group 10 of the pathogenesis-related protein, a family of plant

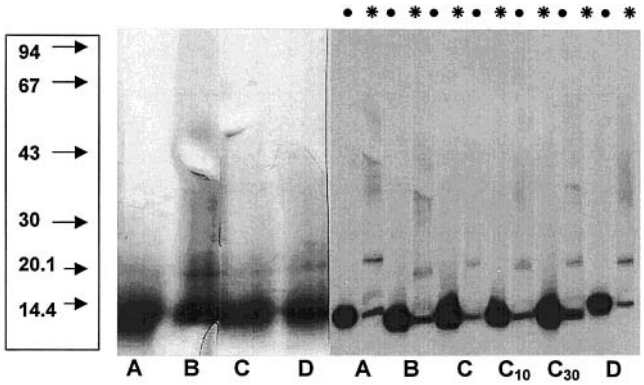

Figure 1. SDS−PAGE (left lanes) and immunoblotting (right lanes) of four commercial peach nectars. In the latter, blotted strips were incubated with two different pools of sera, one (●) from patients allergic to peach and not to birch pollen, reactive only to Pru p 1, and one (∗) from patients allergic to both peach and birch pollens, reactive to Pru p 1 and Bet v 1 homologue. Sample C was incubated as native or following heating to 121 °C for 10 and 30 min.

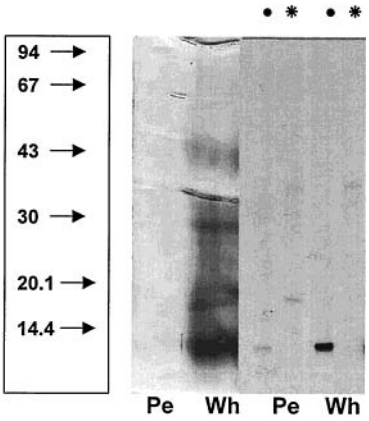

Figure 2. SDS−PAGE (left lanes) and immunoblotting (right lanes) of homogenate prepared from either chemically peeled fresh peaches (Pe) or whole fruits (Wh). Immunoblotting was done as in Figure 1.

defensive proteins present as homologous forms in many vegetables of different botanical families such as apple, pear, hazelnut, celery, and also peach (Cuesta-Herraz et al., 1988). This allergen is responsible of cross-allergic reactions between pollens and foods (Ebner et al., 1991), but patients allergic to foods not sensitized to birch do not became sensitive to Bet v 1 homologous. The complete inhibition of the IgE binding of pooled sera to the low molecular weight band after preincubation with purified Pru p 1 gave a definitive demonstration of the presence of this protein in the tested nectars (data not shown). The results of immunoblotting and immunoblotting inhibition with the different commercial preparations showed for the first time that the major allergen of peach was not removed and neither was its allergenic activity decreased by technological treatments carried out for nectar production.

The technology briefly described above includes the following two steps, during which epicarp (peel) and mesocarp (pulp) come into close contact: depitting, carried out by pitting machines that cause fruit crushing, and, in particular, sieving, carried out by paddle pulpers and finishers.

It has been shown that the Pru p 1 protein is found in the epicarp of peach (Lleoonart et al., 1992), as well as in the epicarp of other fruits (Martinez et al., 1997). In the two above-mentioned technological steps the allergenic protein, having an isoelectric point >9.0 (Pastorello et al., 1999a), is solubilized by the acids present in the pulp, pH 3.7−3.8, transferred from the peel to the pulp, and, consequently, found in the intermediate (pulp).

Because the Pru p 1 protein was found in commercial ready-to-drink peach nectars, as demonstrated above, some treatments oriented toward a removal of or, at least, a decrease in the allergenic power were assumed and verified in a laboratory.

The first process variable considered was heat treatment. As described under Materials and Methods, a commercial peach nectar was subjected to heat treatments in an autoclave at 121 °C for 10 and 30 min. From Figure 1 it can be seen that immunoblotting on extracts from fresh nectar and heat-treated samples clearly showed that even a heat treatment carried out under severe conditions, which is not feasible because of the relevant effects on the sensory characteristics of the product, was not able to decrease the allergenicity of the Pru p 1 protein.

The allergenicity of a protein molecule depends on a brief, either conformational or linear, amino acid sequence (Craig et al., 1998). Conformational epitopes depend on the tertiary structure of the protein, whereas linear epitopes depend on the amino acid sequence. Heat denaturation is able to modify the tertiary structure. Because even a severe heat treatment was not able to decrease the protein allergenicity, it was inferred that the epitope of the Pru p 1 protein was of a linear type.

Due to the fact that the allergen was contained in the epicarp, or rather was contained mainly in the epicarp, the effect of fruit peeling was verified.

Because manual peeling could not be proposed for industrial purposes, chemical lye peeling was carried out. This technology is traditionally used for peeling various fruits when the fruit or fruit half shape should be maintained and, more rarely, in industries for which fruits are processed for nectar and jam production.

Figure 2 shows the results from SDS−PAGE and immunoblotting carried out on extracts from whole homogenate from fresh peach and homogenate from previously lye-peeled peach. In SDS−PAGE analysis for peeled peach extract the absence of the Pru p 1 protein band, as well as other bands for proteins present in the extract from whole homogenate, should be noted. Immunoblotting analysis showed a very weak IgE binding band, developed after 96 h, for the extract from lye-peeled peach.

Disappearance of the Pru p 1 protein band and the very weak response in immunoblots demonstrate that this protein is chiefly found in the epicarp. The considerable decrease in allergenicity may depend on both peel removal and protein denaturation under highly alkaline conditions, as it has been shown for allergens present in *Hevea brasiliensis* latex (Baur et al., 1997). This result suggests that lye peeling of whole fruit may be applied to the nectar production process, prior to depitting and after sorting, to obtain a considerable decrease in allergenicity. In addition, lye peeling does not require the fruit-washing step.

Because lye peeling is generally used in the technology for syrupy peach production, the presence of the Pru p 1 protein was verified in two commercial syrupy peach samples by SDS−PAGE. From Figure 3 it can be observed that in both samples the relevant protein band

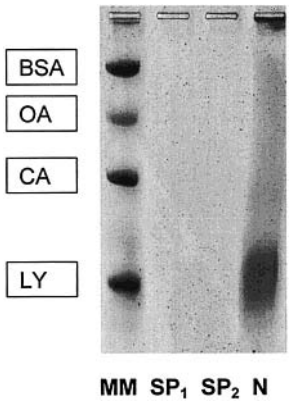

Figure 3. SDS−PAGE of extracts from nectars prepared from two different commercial syrupy peaches (SP_1 and SP_2). A partially purified preparation of the major peach allergen, prepared from the intermediate drawn from a plant for limpid peach juice production, was also included (N). As MM, a mixture of 0.2 mg/mL each of bovine serum albumin (BSA), egg albumin (OA), bovine carbonic anhydrase (CA), and egg lysozyme (LY) was loaded.

is absent, thus demonstrating that lye peeling is an effective means to decrease allergenicity.

The technology for limpid juice production, unlike that for nectar production, includes, after the sieving step, an enzymatic treatment with pectinolytic enzymes at 50 °C, followed by centrifugation and/or filtration after addition of filter aids. Regarding peach, this technology is applied to produce concentrates as intermediates to be used, after dilution, for the production of limpid mixed-fruit juices, soft drinks, and wine coolers.

Availability of a nonallergenic or hypoallergenic limpid peach juice may allow us to obtain several various peach-based foods such as jam, jelly, and preparations for baked product filling.

The availability of a hypoallergenic commercial product is the necessary proemial step to perform in vivo tests, which will confirm the lack of danger of these manufactured products.

Because limpid juice production requires an enzymatic treatment with pectinolytic enzymes, it was verified whether, in this step, it was possible to use a proteolytic enzyme able to hydrolyze the protein and, in particular, to break the amino acid sequence of the epitope. A semipurified extract from the Pru p 1 protein, prepared as described under Materials and Methods, was used to verify the action of two acid proteases, in accordance with peach pH, from *Aspergillus* and *Rhizopus*. From Figure 4 it can be observed that the Pru p 1 protein band was still present even after 60 min of reaction with both proteases. Hence, the enzymatic treatment with acid proteases did not appear to be useful for our purposes.

The technology for limpid peach juice production may also include, prior to concentration by evaporation, an ultrafiltration step through 50 kDa cutoff membranes. This step is carried out when bright, decolorized juices should be obtained. It was verified whether the allergenic protein could be removed by ultrafiltration of an industrial intermediate, taken from a limpid peach juice production line, through a 10 kDa cutoff membrane (Figure 5).

The two technological treatments, which were found to decrease the major allergen of peach, were chemical lye peeling and ultrafiltration through membranes with suitable cutoff. On the basis of the results obtained from

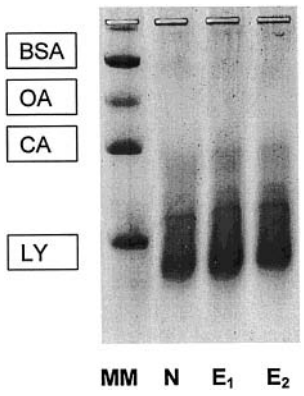

Figure 4. SDS−PAGE of sample N, as obtained, and subjected to the action of two different proteases at 50 °C for 60 min: E_1, from *Aspergillus* sp.; E_2, from *Penicillum saitoi*; MM as in Figure 3.

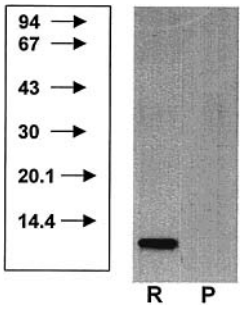

Figure 5. Immunoblotting of an experimental limpid peach juice subjected to an ultrafiltration step (10 kDa nominal cutoff membrane): (R) retentate; (P) permeate.

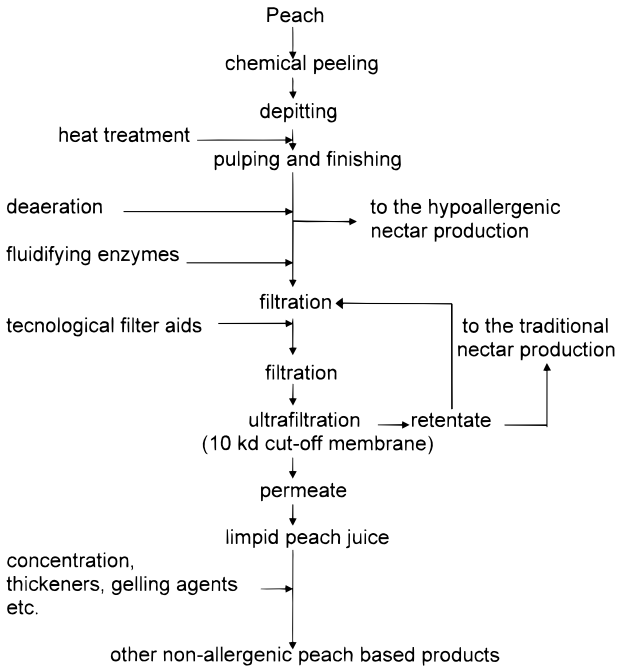

Figure 6. Proposed flow sheet for the production of a hypoallergenic peach nectar and a nonallergenic limpid peach juice.

this research, the processing flow sheet reported in Figure 6 was defined to obtain probably nonallergenic or undoubtedly hypoallergenic limpid juices and nectars. Obtainable products may include, besides finished foods, intermediates to obtain various products after the addition of further ingredients such as pectins, sugars, and fiber.

LITERATURE CITED

Baur, X.; Rennert, J.; Chen, Z. Latex allergen elimination in natural latex sap and latex gloves by treatment with alkaline potassium hydroxide solution. *Allergy* **1997**, *52*, 306−311.

Björksten, F.; Halmepuro, L.; Hannuksela, M.; Lahti, A. Extraction and properties of apple allergens. *Allergy* **1980**, *35*, 671−677.

Craig, L.; Sanschagrin, P. C.; Rozek, A.; Lackie, S.; Kuhn, L. A.; Scott, J. K. The role of structure in antibody cross-reactivity between peptides and folded proteins. *J. Mol. Biol.* **1998**, *281*, 183−201.

Cuesta-Herranz, J.; Lazaro, M.; de las Heras, M.; Lluch, M.; Figueredo, E.; Umpierrez, A.; Hernandez, J.; Cuesta, C. Peach allergy pattern: experience in 70 patients. *Allergy* **1988**, *53*, 78−82.

Ebner, C.; Birkner, T.; Valenta, E.; Rumpold, H.; Breitenbach, M.; Scheiner, O.; Kraft, D. Common epitopes of birch pollen and apples. Studies by Western and Northern blot. *J. Allergy Clin. Immunol.* **1991**, *88*, 588−95.

Lleoonart, R.; Cisteró, A.; Carreira, J.; Batista, A.; Moscoso del Prado, J. Food allergy: identification of the major IgE-binding component of peach (*Prunus persica*). *Ann. Allergy* **1992**, *69*, 128−130.

Malet, A.; Sanosa, J.; Garcia-Calderon, P. A. Diagnosis of allergy to peach. A comparative study of "in vivo" and "in vitro" techniques. *Allergol. Immunopathol.* **1988**, *16*, 181−4.

Martinez, A.; Fernandez-Rivas, M.; Martinez, J.; Palacios, R. Improvement of fruit allergenic extracts for immunoblotting experiments. *Allergy* **1997**, *52*, 155−161.

Neville, D. M. Molecular weight determination of protein-dodecyl sulfate complexes by gel electrophoresis in a discontinuous buffer system. *J. Biol. Chem.* **1971**, *246*, 6328−6334.

Ortolani, C.; Pastorello, E. A. EUR 16893—Study of nutritional factors in food allergies and food intolerances. *Eur. Commission Sci. Res. Dev.* **1997**, 58−71.

Pastorello, E. A.; Ortolani, C.; Farioli, L.; Pravettoni, V.; Ispano, M.; Borga, A.; Bengtsson, A.; Incorvaia, C.; Berti, C.; Zanussi, C. Allergenic cross-reactivity among peach, apricot, plum and cherry in patients with oral allergy syndrome. *J. Allergy Clin. Immunol.* **1994**, *94*, 699−707.

Pastorello, E. A.; Farioli, L.; Pravettoni, V.; Ortolani, C.; Ispano, M.; Monza, M.; Baroglio, C.; Scibola, E.; Ansaloni, R.; Incorvaia, C.; Conti, A. The major allergen of peach *(Prunus persica)* is a lipid transfer protein. *J. Allergy Clin. Immunol.* **1999a**, *103*, 520−526.

Pastorello, E. A.; Pravettoni, V.; Farioli, L.; Ispano, M.; Fortunato, D.; Monza, M.; Giuffrida, M. G.; Rivolta, F.; Scibola, E.; Ansaloni, R.; Incorvaia, C.; Conti, A.; Ortolani, C. Clinical role of a lipid transfer protein that acts as a new apple-specific allergen. *J. Allergy Clin. Immunol.* **1999b**, *104* (in press).

van Ree, R.; Fernandez-Rivas, M.; Cuevas, M.; van Wijngaarden, M.; Aalberse, R. C. Pollen-related allergy to peach and apple: an important role for profilin. *J. Allergy Clin. Immunol.* **1995**, *95*, 726−34.

Received for review June 18, 1999. Revised manuscript received October 25, 1999. Accepted November 2, 1999. Financial support was provided by EU (FAIR CT97-3224).

JF9906681