JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY

*J. Agric. Food Chem.* **2004,** *52,* 7997−8000 **7997**

# Presence of Allergenic Proteins in Different Peach (*Prunus persica*) Cultivars and Dependence of Their Content on Fruit Ripening

ORESTE V. BRENNA,*,† ELIDE A. PASTORELLO,‡ LAURA FARIOLI,‡ VALERIO PRAVETTONI,‡ AND CARLO POMPEI†

Department of Food Science and Microbiology, University of Milan, Milan, Italy, and Allergy Center, Department of Internal Medicine, Ospedale Maggiore IRCCS, Milan, Italy

It has been reported that various cultivars of fruits and vegetables may present a different pattern for the contained allergens. Here, we report on the different content in allergenic proteins for different peach (*Prunus persica*) cultivars, sampled during two consecutive harvest seasons. Fruits from six cultivars of peaches were harvested fully ripe, and the proteins extracted from whole or chemically peeled fruits were analyzed by SDS−PAGE and immunoblotting. All the protein extracts from whole fruit contained a 9 kDa protein. This protein proved to be absent in the extracts taken from chemically peeled fruit. In four cultivars, this protein corresponds to the allergen Pru p3, a lipid transfer protein that causes the oral allergy syndrome (OAS) in sensitized people. In the following year, fruits from four of the six cultivars of peaches studied previously were harvested at different times, at one and two weeks before the commercial ripening time and when fully ripe, to ascertain whether the presence of the 9 kDa allergen might be related to the ripening process. Two cultivars out of four produced an intense allergenic band corresponding to a 9 kDa protein already two weeks before the commercial ripening date, while the others showed a progressive increment of the 9 kDa allergen during ripening.

**KEYWORDS:** ***Prunus persica*; cultivars; allergen; fruit ripening; oral allergy syndrome (OAS)**

## INTRODUCTION

Food allergies are caused by a wide variety of foods. Following the ingestion of the offending food, people show different manifestations of IgE-mediated food allergies, which may range from mild to life-threatening events. The management of food allergies suffers at present from poor diagnostic methods and a lack of therapeutic options. Allergic reactions to fresh fruits and vegetables are mostly associated with the oral allergy syndrome (OAS) (*1*). The easiest way to prevent OAS is a voluntary avoidance of certain foods, which may result in a nutritionally unbalanced diet.

The food industry could offer a real alternative solution, if it were able to deliver hypo- and/or anallergenic foods, thus benefiting those who suffer from food allergies or those who are at risk of developing them. This goal might be achieved by finding new analytical and processing strategies to ensure detection and maximum reduction in allergen content with minimal processing input.

We reported on the feasibility of introducing some easy and well-known steps in the usual flowchart of fruit derivatives production, focusing on peaches (*2*). While working on the feasibility of extending these results to other kinds of prunoidee, like apricots (work in progress), we wished to explore the possibility that some peach cultivars might have low allergenic protein content, so that people suffering from this particular allergy could also enjoy fresh fruits with impunity. Indeed, different degrees of allergenicity have also been reported for apples (*3*), dates (*4*), avocados (*5*), bell peppers (*6*), and soybeans (*7*), according to the cultivars and agronomic practices employed.

We investigated the allergens contained in six peach cultivars by means of SDS−PAGE and immunoblotting. To determine whether the main allergen causing OAS, Pru p3, is always confined to the epicarp (*2*, *8*, *9*), we also analyzed the protein content in fruits from the six cultivars after they had been chemically peeled, a step that was shown to completely eliminate the allergen in question (*2*). To find out whether this allergen could be synthesized during fruit ripening, in the following season, fruits of four out of the six cultivars previously checked were harvested at three different ripening stages.

Although many papers have reported on the differential gene expression in ripening fruits (*10*−*12*), only one paper reported on the variation in allergenic proteins in an apple variety during ripening (*13*). While our work was in progress, a paper dealing with the dependence of two lipid transfer proteins from peach fruits appeared (*14*).

* To whom correspondence should be addressed. Phone: +39-02-503-16633; fax: +39-02-503-16632; e-mail: Oreste.Brenna@unimi.it.
† University of Milan.
‡ Department of Internal Medicine.

10.1021/jf0491052 CCC: $27.50 © 2004 American Chemical Society
Published on Web 11/25/2004

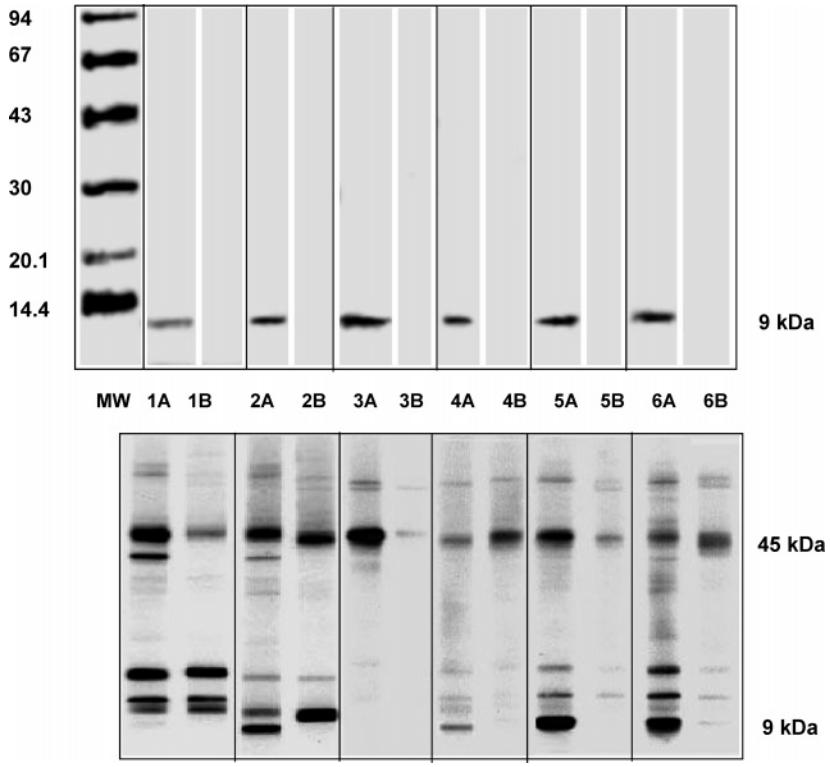


**Figure 1.** SDS–PAGE (top) and immunoblotting (bottom) of the different peach cultivars analyzed. (1) Luisa Berselli; (2) Springcrest; (3) Maria Bianca; (4) Maria Serena; (5) Red Haven; and(6) Maria Laura. Sample A: protein extracts from whole (unpeeled) fruits and B: protein extracts from chemically peeled fruits.

## MATERIALS AND METHODS

**Samples.** The fruits of six cultivars were harvested when fully ripe (i.e., at the date on which they possessed the required commercial features). Two were white-fleshed cultivars (Maria Bianca and Luisa Berselli), and four were yellow-fleshed: a percoca (Maria Serena), mostly used for the production of peaches in syrup, two downy peaches (Springcrest and Red Haven), and a nectarine (Maria Laura). Four of these cultivars, the two white-fleshed varieties, the percoca, and the nectarine, were also considered during the following season to ascertain whether the Pru p3 content could be related to the ripening process. Consequently, during that season, the fruits were harvested at three different ripening stages, that is, two weeks before and one week before the commercial ripening and at the commercial ripening date. The fruits were harvested in the orchard of the Faculty of Agriculture, University of Milan, during the 2002 and 2003 seasons. Fruits were treated within 24 h of gathering.

**Chemical Peeling.** The procedure described by Brenna et al. (*2*) was followed, with minor changes. Briefly, the peaches were immersed in 10% NaOH (w/v) at 60 °C for 2 min, then chilled and gently rubbed under tap water, then dipped again in 2% NaOH at 60 °C for 2 min. The peeled fruits were finally chilled in tap water and briefly rinsed in 1% citric acid.

**Protein Extracts.** The fruits of the six cultivars mentioned previously, whole or peeled, were pitted, cut in small pieces, and homogenized with distilled water (2:1 w/v); the diluted puree was centrifuged, and the supernatant was collected and dialyzed versus distilled water, containing 40 mg/L NaF, using a membrane with 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 through filter paper. 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 Corporation, Danvers, MA). In the following year, the protein extracts from the fruits of four out of the six cultivars were prepared as described previously, starting from a 4 mm layer cut from whole fruits, including the epicarp and some of the flesh.

**Table 1.** Characteristics of the Patients Included in the Study[a]

| pt | age (years) | sex | symptoms | peach CAP (kU/L) | peach SPT | birch CAP (kU/L) |
|---|---|---|---|---|---|---|
| 1 | 30 | M | OAS | 11.0 | +++ | <0.35 |
| 2 | 34 | F | urticaria AE | 16.2 | ++ | <0.35 |
| 3 | 35 | F | urticaria | 3.22 | +++ | <0.35 |
| 4 | 22 | F | OAS | 27.8 | ++++ | <0.35 |
| 5 | 18 | M | urticaria AE | 56.4 | ++++ | <0.35 |
| 6 | 31 | F | OAS | >100 | ++++ | 38.7 |
| 7 | 33 | F | OAS | 64.8 | ++++ | 1.20 |
| 8 | 23 | F | OAS | 7.74 | +++ | 24.9 |
| 9 | 45 | F | OAS | 3.42 | +++ | 21.0 |
| 10 | 26 | M | OAS GI | 11.2 | +++ | >100 |

[a] SPT: skin prick test; CAP: CAP System; AE: angioedema; OAS: oral allergy syndrome; GI: gastrointestinal symptoms.

**SDS−PAGE and Immunoblotting.** The procedure followed was as described by Brenna et al. (*2*). Briefly, the proteins separated in SDS−PAGE were electrophoretically transferred onto a nitro-cellulose sheet (Amersham, Buckinshire, U.K.) and incubated with a pool of sera from patients having a peach allergy. Patients were selected on the basis of a history of allergic reaction to peaches (such as OAS), Skin Prick test positive to fresh peach, and circulating IgE specific antibodies positive for peach (Pharmacia CAP System, Uppsala, Sweden). **Table 1** shows the characteristics of the 10 patients whose pooled sera were used to perform the immunoblottings. As demonstrated in other studies (*15*, *16*), it is important to mix birch pollen positive and birch pollen negative patients so to detect not only the 9 kDa band, which is the major allergen of peach.

The sheets were then washed and incubated overnight with $^{125}$I-labeled anti-IgE (CAP RAST IgE RIA, Pharmacia & Upjohn, Uppsala, Sweden). Following final washings, the sheets were dried and left in contact with a photographic plate (Hyperfilm, Amersham, Buckinshire, U.K.) at −70 °C for times varying from one to 14 days.

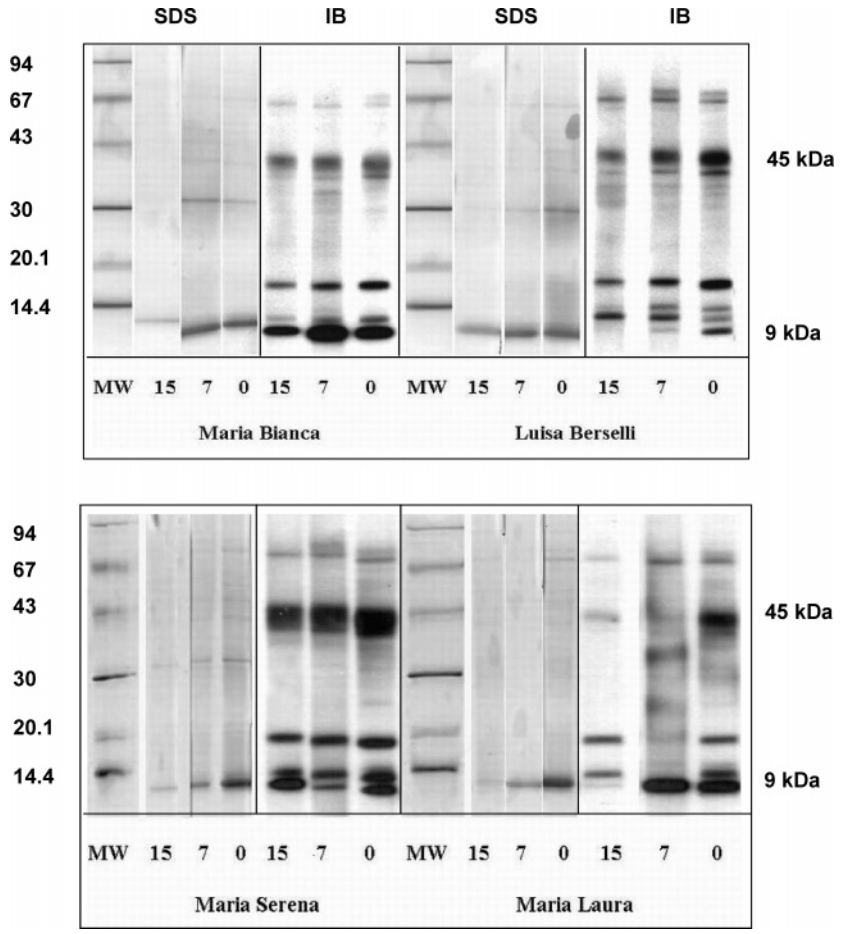


**Figure 2.** SDS–PAGE and immunoblotting of the protein extracts from fruits of the four peach cultivars, harvested at 15 and 7 days before their commercial ripening date, 0.

## RESULTS AND DISCUSSION

**Figure 1**(top) shows the SDS−PAGE of the six cultivars initially studied. The presence of the protein corresponding to Pru p3 in all the samples prepared from whole fruits is evident, while this band is absent in the chemically peeled samples of the same cultivars. The corresponding immunoblottings (**Figure 1**, bottom) show for the first time that the protein band, corresponding with that of the Pru p3 in the whole samples, is totally free of allergenic activity in the white-fleshed cultivars, Maria Bianca and Luisa Berselli. For all the yellow-fleshed cultivars analyzed, a band of allergenic activity corresponding to the protein stain of Pru p3 was evident in the extracts prepared from the whole fruits. As reported in our previous paper (*15*, *16*), the Pru p3 allergen has been fully characterized; immunoblot inhibition carried out using purified Pru p3 completely abolished the radiostaining of the 9 kDa protein, so confirming that protein as the LTP (data not shown).

In all the cultivars, the presence of an allergenic active band at ∼45 kDa that is slightly revealed by the protein stain can be observed.

**Figure 2** shows the SDS−PAGE and the relative immunoblottings of the extracts prepared from fruits of the four cultivars of peaches harvested in the following year at different stages of maturation. The SDS−PAGE shows that the protein band corresponding to Pru p3 is present in all four cultivars, with increasing intensity during ripening. This increase is not surprising since the protein in question, a lipid transfer protein, acts as a first line of defense for the fruit against microbial attacks, and these are more probable at the ripening stage, when the sugar content is increasing and the fruit is gradually softening. Indeed, the induction of other pathogen-response proteins, like chitinases and thaumatin-like proteins, has been reported in ripening fruits (*17*−*19*).

As occurs with the protein band, the allergenic activity of Pru p3 also increases, and a high activity was present in two of the analyzed cultivars two weeks before commercial ripening.

While for the Maria Serena and Maria Laura cultivars the identification of Pru p3 by the pool of sera from allergic patients gave results identical to those obtained with the fruits picked in the previous season, discordant results were obtained for the other two cultivars.

In the extract prepared from fruits of the Maria Bianca cultivar, Pru p3 proved to be present and highly active already 15 days before full ripening, unlike the previous year, when no allergenic activity was associated with the corresponding protein band. In the Luisa Berselli cultivar, an increase in the content (in terms of protein and allergenic activity) of the Pru p3 was found at the three stages examined, with a massive presence in the fully ripened fruits.

One of the reasons for these different behaviors could be the difference in climatic conditions since it is known that natural stress conditions, such as heat, cold, and drought, cause the synthesis of new sets of proteins in plants (*20*). Indeed, the 2003 season was characterized by a very hot and dry summer that shortened the harvesting period. Another cause could be the agronomic cultivation conditions, as in the case of treatments with phytochemicals, which might have influenced the kind or the content of proteins. In fact, since microbial elicitors induce

the expression of the pathogenesis-related proteins (*21*), it would be arguable that inhibition of microbial growth might negatively influence their expression.

In our case, different mixtures of phytochemicals were used in the two years considered, which could perhaps have influenced the expression of the main peach allergen Pru p3. To check this possibility, it would be of interest to treat different rows of the same peach cultivars with the different phytochemicals employed.

From the results of SDS−PAGE and immunoblotting analysis of the proteins extracted from whole and chemically peeled peaches belonging to six different cultivars, we can draw the following conclusions.

All the protein extracts prepared from whole (unpeeled) fruits contained the protein Pru p3. In the cases of the cultivars Maria Bianca and Luisa Berselli (white fleshed), this protein proved to be allergenically inactive in the first season studied and active in the second, while all the remaining cultivars studied possessed the allergenic activity in both seasons.

The chemical peeling process confirms that the Pru p3 allergen is situated in the skin (*2*, *7*, *8*); therefore, peeling the fruits removes it, making the preparation of hypoallergenic purée possible for every peach cultivar. Other allergens, such as that observed at ∼45 kDa, are mostly present in the flesh of the fruit and therefore cannot be removed by chemical peeling. However, a clear anallergenic juice from peaches can be obtained by introducing into the production process, in addition to the chemical peeling of the fruits, an ultrafiltration through membranes with a 10 kDa cutoff, via which the allergens contained in the flesh are wholly removed from the permeate, as reported in our previous work (*2*).

These intermediate products with reduced or no allergenic activity can be employed as starting material for the preparation of hypo- and anallergenic foods, thus widening the range of products for people allergic to peaches since very recently the frequency of peach allergy has been proven to have increased (*22*).

## LITERATURE CITED


(1) Ortolani, C.; Ispano, M.; Pastorello, E.; Bigi, A.; Ansaloni, R. The oral allergy syndrome. *Ann. Allergy* **1988**, *61*(6 Pt 2), 47−52.

(2) Brenna, O.; Pompei, C.; Ortolani, C.; Pravettoni, V.; Farioli, L.; Pastorello, E. A. Techological processes to decrease the allergenicity of peach juice and nectar. *J. Agric. Food Chem.* **2000**, *48*, 493−497.

(3) Vieths, S.; Jankiewicz, A.; Schoning, B.; Aulepp, H. Apple allergy, the IgE-binding potency of apple strains is related to the occurrence of the 18-kDa allergen. *Allergy* **1994**, *49*, 262−271.

(4) Kwaasi, A. A.; Harfi, H. A.; Parhar, R. S.; Collison, K. S.; Al-Sedairy, S. T.; Al-Mohanna, F. A. Cultivar-specific IgE-epitopes in date (*Phoenix dactylifera* L.) fruit allergy. Correlation of skin test reactivity and ige-binding properties in selecting date cultivars for allergen standardization. *Int. Arch. Allergy Immunol.* **2000**, *123*, 137−144.

(5) Blanco, C.; Carrillo, T.; Castillo, R.; Quiralte, J.; Cuevas, M. Avocado hypersensitivity. *Allergy* **1994**, *49*, 454−459.

(6) Jensen-Jarolim, E.; Santner, B.; Leitner, A.; Grimm, R.; Scheiner, O.; Ebner, C.; Breiteneder, H. Bell peppers (*Capsicum annuum*) express allergens (profilin, pathogenesis-related protein P23 and Bet v 1) depending on the horticultural strain. *Int. Arch. Allergy Immunol.* **1998**, *116*, 103−109.

(7) Codina, R.; Ardusso, L.; Lockey, R. F.; Crisci, C.; Medina, I. Allergenicity of varieties of soybean. *Allergy* **2003**, *58*, 1293−1298.

(8) Fernandez-Rivas, M.; Cuevas, M. Peels of *Rosaceae* fruits have a higher allergenicity than pulps. *Clin. Exp. Allergy*. **1999**, *29*, 1239−1247.

(9) 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.

(10) Clendennen, S. K.; May, G. D. Differential gene expression in ripening banana fruit. *Plant Physiol*. **1997**, *115*, 463−469.

(11) Dopico, B.; Lowe, A. L.; Wilson, I. D.; Merodio, C.; Grierson, D. Cloning and characterization of avocado fruit mRNAs and their expression during ripening and low-temperature storage. *Plant Mol. Biol.* **1993**, *21*, 437−449.

(12) Ledger, S. E.; Gardner, R. C. Cloning and characterization of five cDNAs for genes differentially expressed during fruit development of kiwifruit (*Actinidia deliciosa* var. deliciosa). *Plant Mol. Biol*. **1994**, *25*, 877−886.

(13) Hsieh, L. S.; Moos, M., Jr.; Lin, Y. Characterization of apple 18 and 31 kDa allergens by microsequencing and evaluation of their content during storage and ripening. *J. Allergy Clin. Immunol*. **1995**, *96*, 960−970.

(14) Botton, A.; Begheldo, M.; Rasori, A.; Borghi, C.; Tonutti, P. Differential expression of two lipid transfer protein genes in reproductive organs of peach (*Prunus persica* L. Batsch). *Plant Sci*. **2002**, *163*, 993−1000.

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

(16) 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*. **1999**, *103*, 520−526.

(17) Atkinson, R. G.; Perry, J.; Matsui, T.; Ross, G. S.; Macrae, E. A. A stress-, pathogenesis-, and allergen-related cDNA in apple fruit is also ripening-related. *NZJ Crop Hortic. Sci*. **1996**, *24*, 103−107.

(18) Fils-Lycaon, B. R.; Wiersma, P. A.; Eastwell, K. C.; Sautiere, P. A cherry protein and its gene, abundantly expressed in ripening fruit have been identified as thaumatin-like. *Plant Physiol*. **1996**, *111*, 269−273.

(19) Tattersall, D. B.; van-Heeswijck, R.; Hoj, P. B. Identification and characterization of a fruit-specific thaumatin-like protein that accumulates at very high levels in conjunction with the onset of sugar accumulation and berry softening in grapes. *Plant Physiol.* **1997**, *114*, 759−769.

(20) Ho, T. H. D.; Sachs, M. M. Stress-induced proteins, characterization, and the regulation of their synthesis. In T*he Biochemistry of Plants, A Comprehensive Treatise*; Stumpf, P. K., Conn, E. E., Eds.; Academic Press: New York, 1989; Vol. 15, p 347.

(21) *Pathogenesis-Related Proteins in Plants*; Datta, S. K., Muthukrishnan, S., Eds.; CRC Press: Boca Raton, FL, 1999.

(22) Fernandez-Rivas, M.; Gonzales-Mancebo, E.; Rodriguez-Perez, R.; Benito, C.; Sanchez-Monge, R.; Salcedo, G.; Alonso, M. D.; Rosado, A.; Tejedor, M. A.; Vila, C.; Casas, M. L. Clinically relevant peach allergy is related to peach lipid transfer protein, Pru p 3, in the Spanish population. *J. Allergy Clin Immunol*. **2003**, *112*, 789−795.


**Received for review June 2, 2004. Revised manuscript received September 22, 2004. Accepted October 5, 2004.**

JF0491052