R-mandelonitrile-lyase, homolog to Pru d 10, is a major peach allergen in peach allergic Spanish population

Short title: Identification of a new peach allergen

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Summary

Background: Peach allergy is a prevalent cause of food allergy. Despite the repertoire of allergens available for molecular diagnosis, there are still patients with undetectable IgE levels to peach allergens but presenting symptoms after its ingestion. The objective of this study was to investigate the allergenic profile in a patient population with symptoms produced by peach.

Materials and Methods: An exploratory retrospective study was performed with patients presenting symptoms after the ingestion of peach. Forty-two patients were included in the study. The allergenic profile of individual patients was investigated by immunoblot. A serum pool was prepared with the sera that recognized a 70 kDa band. This pool was used to detect this protein in peach peel and pulp and to identify the 70 kDa protein in 2D immunoblot. Spots recognized in the 2D immunoblot were sequenced by LC-MS/MS. Inhibition studies were performed between peach peel and almond.

Results: Twenty-two patients (52.4%) recognized the 70 kDa protein in immunoblot. This protein was recognized in peel and pulp. Two different spots were observed in 2D-PAGE, both were identified as (R)-mandelonitrile lyases (RML) with high amino acid similarity with Pru du 10. Peach RML were partially inhibited with an almond extract. No association was found between any reported symptom and sensitization to RML. RML-sensitized patients were older and reported pollen associated respiratory symptoms more frequently than negative patients.

Conclusions: A new peach allergen, a RML, homologous of Pru du 10, recognized by 52% of the population has been identified.

Key words: Peach allergy. Peach allergen. Mandelonitrile lyase. Food allergy. Pru du 10 homologous.

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Resumen

Antecedentes: La alergia a melocotón es una causa frecuente de alergia alimentaria. A pesar de que hay varios alérgenos disponibles para el diagnóstico molecular, existen pacientes con niveles indetectables de IgE a estos alérgenos pero que presentan síntomas tras la ingesta de melocotón. El objetivo de este estudio fue investigar el perfil alergénico en una población de pacientes con síntomas producidos por el melocotón.

Materiales y Métodos: Se realizó un estudio exploratorio retrospectivo con pacientes que presentaron síntomas tras la ingesta de melocotón. Se incluyeron en el estudio cuarenta y dos pacientes. El perfil alergénico de cada paciente se investigó mediante inmunoblot. Se preparó un pool con los sueros que reconocían una banda de 70 kDa. Este pool se utilizó para detectar esta proteína en piel y pulpa de melocotón y para identificar la proteína de 70 kDa en un inmunoblot 2D. Los puntos reconocidos en el blot 2D se secuenciaron mediante LC-MS/MS. Se realizaron estudios de inhibición entre piel de melocotón y almendra.

Resultados: Veintidós pacientes (52,4%) reconocieron la proteína de 70 kDa en el inmunoblot. Esta proteína fue reconocida tanto en piel como en pulpa. Se observaron dos puntos en 2D-PAGE, ambos se identificaron como (R)-mandelonitrilo liasas (RML) con alta similitud de aminoácidos con Pru du 10. Las RML de melocotón se inhibieron parcialmente con un extracto de almendra. No se encontró asociación entre ningún síntoma y la sensibilización a RML. Los pacientes sensibilizados a RML tuvieron mayor edad y reportaron síntomas respiratorios asociados al polen con mayor frecuencia que los pacientes negativos.

Conclusiones: Se ha identificado un nuevo alérgeno del melocotón, una RML, homóloga de Pru du 10, reconocida por el 52% de la población.

Palabras clave: alergia a melocotón, alérgeno de melocotón, mandelonitrilo liasa, alergia a alimentos, homólogo de Pru du 10.

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Summary box

- **What do we know about this topic?**

Five peach allergens have been associated with the symptoms produced by the ingestion of this fruit. However, the symptoms are not related with any of these allergens in some patients. There has to be more allergens involved in peach allergy.

- **How does this study impact our current understanding and/or clinical management of this topic?**

The identification of a new peach allergen improves the knowledge of this allergy, allowing a better diagnosis. It is also important the fact that the new allergen has cross-reactivity with almond to make recommendations to the patients sensitized to it.

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Introduction

The prevalence of food allergies has increased significantly over the last decades, and peaches are one of the most important causes in European countries, particularly in the Mediterranean area [1].

Five distinct food allergens have been described in peach: Pru p 1 (pathogenesis-related protein, PR-10); Pru p 2 (thaumatin-like protein); Pru p 3 (non-specific lipid transfer protein 1, nsLTP1); Pru p 4 (profilin); and Pru p 7 (gibberellin-regulated protein). Two other components have been described as respiratory allergens: Pru p 9 (pathogenesis-related protein PR-1); and Pru p 10 (polygalacturonidase) [2]. Pru p 3 is the main sensitizer allergen in Mediterranean countries [3, 4] and is involved in primary food allergy, producing symptoms from oral allergy syndrome (OAS) to anaphylaxis [5]. On the contrary, Pru p 1 is the main sensitizer in Central Europe, related with cross-reactivity to birch pollen [4]. Pru p 2, Pru p 4 and Pru p 7 are further involved in secondary sensitization to peach by cross-reactivity with pollen allergens [6].

Despite the repertoire of allergens already identified, which are often available for molecular diagnosis, some patients present a wide range of symptoms after the ingestion of peach despite having undetectable serum IgE levels against any previously described allergens [7]. In these patients specific diagnosis remains elusive, although it is crucial to offer an appropriate recommendation for treatment and to prevent future allergic reactions. It is also relevant to discard cross-reaction with other fruits and/or vegetables. The objective of the present study was to investigate the allergenic profile in a population with peach allergy. This study discovered a new peach allergen recognized by the patients' sera and identified it by mass spectrometry.

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Materials and Methods

Patient Population

An exploratory retrospective study from February 2020 to September 2021 was performed at Hospital La Paz (Madrid, Spain) with patients of any age who were diagnosed with peach allergy. Diagnosis was made on the basis of a suggestive clinical history (oral allergy syndrome, urticaria/angioedema, rhinoconjunctivitis/asthma, gastrointestinal symptoms, or anaphylaxis) occurring within 2 hours after peach intake or contact in addition to positive SPT and/or sIgE against peach extract. The study was approved by Local Ethics Committee (PI-4513); demographic and clinical data were extracted from medical records.

Skin prick-test (SPT) with commercial peach peel and pulp, profilin (Pho d 2, ALK Abello, Madrid, Spain), birch pollen extract (LETI Pharma S.L, Madrid, Spain) and purified Pru p 3 (10,000 DPU/mL, Roxall) was performed. A wheal of 3 mm or greater was considered positive. Total IgE and specific IgE to peach extract, Pru p 1, Pru p 3, Pru p 4 and Pru p 7 were determined by ImmunoCAP (Thermo Fisher Scientific).

Extract manufacturing

Peaches were purchased at a local market and carefully peeled to prepare peel and pulp extracts according to internal manufacturing procedures (LETI Pharma). In short, peach peels and pulps were homogenized separately and extracted for 4 hours in phosphate-buffered saline (PBS)/polyvinylpolypyrrolidone buffer under continuous magnetic stirring at 4°C. Extracts were then centrifuged for 30 min at 15,000 *g* and supernatants were collected, dialyzed, filtered, frozen and freeze-dried. Protein content and protein profile were analyzed by Bradford and SDS-PAGE, respectively.

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Allergenic profile

The allergenic profile of individual patients was investigated by immunoblot. Briefly, the proteins contained in 100 µg of lyophilized peach peel extract were separated according to their molecular weight in Invitrogen NuPAGE PreCast 4-12% BisTris gels (ThermoFisher Scientific) under reducing conditions, electrotransferred onto a PVDF membrane using a Trans-Blot® Turbo™ Transfer Pack (Bio-Rad) and dried at room temperature. Thereafter, membranes were incubated overnight with the individual sera diluted 1/2 in PBS-0.1% Tween. After two hours of incubation with monoclonal anti-human-IgE-PO (Southern Biotech), the reaction was developed with ECL™ Prime Western Blotting Detection (Amersham) and visualized by chemiluminescence. A pool of peach-positive plasma sera (Plasmalab International, Everett, WA, USA) was used as positive control for the assay. A serum pool was prepared from twenty-two individual sera that showed a 70-kDa band during immunoblot testing. This pool was used to determine if the same protein was present in pulp extract. ImmunoCAP to peach extract and cross-reactive carbohydrate determinants (CCDs) (MUXF3, ThermoFisher) was performed to characterize the pool of sera.

2D electrophoresis and immunoblot

Peach peel extract was purified and concentrated with ammonium sulphate in two different steps until a saturation percentage of 40% and 80% was attained, then maintained at 4°C overnight. The sample was centrifuged at 10,000 *g* for 5 min at 4°C, and the pellet was collected and reconstituted in ultra-pure water. Concentrated extract was cleaned with ReadyPrep 2-D Cleanup Kit (BioRad), and proteins were separated according to their isoelectric point (pI) on ReadyStrip IPG Strips (BioRad) in a pH 3-10 range, using Protean IEF Cell (BioRad). Two strips were processed simultaneously; after the first dimension run, they were equilibrated with ReadyPrep 2-D Kit buffers (Bio-Rad).

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Then proteins were separated in the second dimension according to their molecular weight. After the second dimension one of the gels was developed with Oriole fluorescent gel stain (BioRad) to study the spot protein profile, and the second gel was used to determine the allergenic profile by 2D immunoblot as previously described.

Allergen identification

Spots recognized in the 2D immunoblot were excised from the gel, digested with trypsin, analysed by LC-MS/MS (liquid chromatography mass spectrometry/mass spectrometry) in a Q Exactive HF spectrometer (Thermo Fisher Scientific) and identified with Proteome Discoverer software (Thermo Fisher Scientific) in the Proteomic Unit of the Complutense University (Madrid, Spain).

Immunoblot inhibition

Inhibition studies were performed between peach peel extract and almond extract using the pool of sera. Briefly, 100 µg of lyophilized peach peel extract corresponding to approximately 6 µg of protein were electrophoresed and electrotransferred as explained in the Allergenic profile section. Almond extract (30 µg of protein, LETI Pharma) or peach peel extract (30 µg of protein, positive control) was preincubated with the pool of sera (dilution 1/2) for two hours. Afterwards, the inhibition mixtures were incubated with the membrane for 2 hours, washed and developed by chemiluminescence. Percentage of recognition of the 70 kDa band was calculated by densitometry with the software ImageQuant TL 8.1 (Cytiva).

Statistical analysis

Descriptive statistical analyses (median and range) were used for the analysis of numerical variables (age and kU/L of total or specific IgE). Chi-squared or Mann-Whitney tests were used to compare groups of individuals (those reactive to the new allergen *vs* those negative).

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Results

Patient Population

Forty-two patients (22 females, 52.4%), with ages ranging from 1 to 46 years old (median 9.59; IQR 5.90-14.50) with symptoms, from OAS to anaphylaxis, after the ingestion of peach were included in the study. Table 1 shows the population characteristics. To note, none of the patients reported symptoms after ingestion of almond.

Allergenic profile of patient sera

There was great variation in the recognition profiles as can be observed in Figure 1, although 22 of 42 individual serum samples (52.4%) recognized a protein of approximately 70 kDa in immunoblots (Figure 1A). Only one patient (patient 42) was monosensitized to this protein (Figure 1).

The pool of patient sera recognized the band of approximately 70 kDa in peach peel and pulp (Figure 1B). However, the protein recognized in the pulp was slightly smaller than that recognized in the peel.

The pooled sera had an sIgE of 5.4 kU/L to peach extract and 0.1 kU/L (negative) to the molecular component MUXF3 (marker of sensitization to CCDs).

2D electrophoresis and immunoblot

The pool of sera recognized an area corresponding to a pI of 5-6 at ~70 kDa (Figure 2B). This area corresponds to two different spots (M1 and M2) in the 2D-PAGE (Figure 2A), which were identified as (R)-mandelonitrile lyases (RML) by LC-MS/MS analysis. Fifteen peptides from M1 corresponded to the protein A0A251QUN8 with a sequence coverage of 25.9%, and sixteen peptides from M2 corresponded to the protein A0A251QUN1 with a sequence coverage of 31.5% (Figure 3 and Table 2). Both are isoforms of the peach RML with amino acid identity to the almond allergen Pru du 10 of 70% and 70.5%, respectively.

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Immunoblot inhibition

Due to the high identity of RML with Pru du 10, we performed an immunoblot inhibition assay to study their cross-reactivity. The band corresponding to RML detected in the peach peel extract were inhibited a 58% approximately with an almond extract (Figure 4).

(R)-mandelonitrile lyase sensitized patients

Of the 22 patients whose serum reacted with the newly identified peach allergen RML, 13 were women (59%) with a median age of 11.5 years (range 5.2 to 46.9). Ten were children aged ≤10 years, seven were adolescents aged between 11 and 18 years and five were adults >18 years.

More than half of patients reported OAS as the only symptom (13, 61.9%). Interestingly, 9 patients reported systemic reactions (42.9%), but no association was found between any specific reported symptom and sensitization to RML allergen (data not shown).

Thirteen patients were sensitized to Pru p 3 (59.1%), 2 to Pru p 1 (9.1%), 4 to Pru p 4 (18.2%) and 3 to Pru p 7 (13.6%) (Table 3). Only one patient was monosensitized to RML allergen. Seventeen patients (77.3%) reported respiratory symptoms upon pollen exposure.

Comparative results between RML-sensitized and negative patients are summarized in Table 1. Of note, RML-sensitized patients were older (median 11.5 years *vs* 7.0 years old, p=0.014) and reported pollen-associated respiratory symptoms more frequently (77.3% *vs* 30%, p=0.005). RML-sensitized patients reported anaphylaxis more frequently than the RML-negative ones. SPT with peach peel and Pru p 3 resulted positive in all the patients who tested negative for RML. Conversely, SPT with peach pulp, profilin and birch, although not statistically significant, resulted positive in a higher proportion of patients in the RML-sensitized group. Accordingly, levels of total and specific IgE for

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peach and its components were higher in RML-sensitized patients, except for Pru p 3 (Table 3).

Patients whose serum reacted with RML also reported symptoms upon ingestion of Cucurbitaceae fruits more frequently than those who tested negative (36.4% vs 0%, p=0.04).

Discussion

A deeper knowledge of peach allergens is essential in order to improve the products used for allergy diagnosis, and to better understand the source of symptoms in patients. Although there are molecular *in vitro* diagnostic assays for four peach allergens (Pru p 1, Pru p 3, Pru p 4 and Pru p 7), we found some patients who had symptoms after consuming peaches despite testing negative to all peach allergens. We identified and characterized a new peach allergen, RML, which reacted with serum from 22 of 42 tested patients (52.4%) with symptoms after peach ingestion. To the best of our knowledge, this is the first time that this protein has been described in peach and, the high prevalence of sensitization to RML in the study population indicates that this protein could act as a major allergen with high incidence in our area. The prevalence of RML was even higher than that of Pru p 1 (4.8%), Pru p 4 (9.5%) and Pru p 7 (9.5%) and was only surpassed by that of Pru p 3 (76.2%).

Mandelonitrile lyases belong to the family of hydroxynitrile lyases involved in cyanogenesis, which is the process plants use to degrade α-cyanohydrins into hydrocyanic acid and the respective aldehyde or ketone. This reaction is catalyzed by hydroxynitrile lyases, and the release of hydrocyanic acid functions as a defense mechanism against herbivores and microbial attack [8, 9]. In fact, ingestion of cyanogenic plants can produce

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acute cyanide poisoning in animals and in humans [10]. Though RMA is a major seed protein, we found this protein both in the peel and pulp of peaches.

An allergen from the RML family has been previously described in almond, named Pru du 10 [11]. The new peach allergen has a similar molecular weight (~70 kDa) and was inhibited with an almond extract, indicating that this allergen is homologous to Pru du 10. Although Pru du 10 is a glycoprotein, the authors demonstrated that IgE bound to protein and not glycosides [11]. In the case of the new peach allergen, we measured the sIgE against MUXF3 (a marker of sensitization to CCDs) in the pool of sera, with a negative result, indicating that the patients recognized the protein and not the glycoside residues.

Patients reactive to RML were older than those negative to RML, indicating a late sensitization to RML. This data is corroborated by the presence of only one monosensitized patient to RML. Our hypothesis is that most of the patients that were previously sensitized to different allergens were sensitized later to RML, though the relatively small number of adult patients included in the study population made it difficult to confirm here. Further studies with a greater number of adults are needed to replicate these results. The greater incidence of pollen-associated respiratory symptoms in the RML-sensitized patients could indicate a secondary sensitization to peach, as described for Pru p 1 in relation to Bet v 1 [4, 12, 13], in Pru p 7 with Cupressaceae pollinosis [14-16], although this relationship has not been found in the Mediterranean area [17-18], or the panallergens Pru p 2 [19] and Pru p 4 [20]. As this study was not intended to cover specific pollen sensitization, not all patients were systematically tested against the same pollen extracts, so no hypothesis can be drawn from the possible relationship between any given pollen and RML sensitization. Nevertheless, the fact that RML-sensitized patients reported pollen allergy symptoms more frequently, and were older than the RML-negative ones, suggests the possibility that RML sensitization comes with time and

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accompanies pollen sensitization, although to date, no homologue to this allergen in pollens is known.

No association could be established between any given symptom reported by patients and RML sensitization, but RML-sensitized patients reported anaphylaxis more frequently that the RML-negative ones, although it did not reach statistical significance. Of note, the RML-monosensitized patient reported anaphylaxis upon peach ingestion. One of the drawbacks of the study is the lack of systematic OFC to all patients, so we cannot ensure clinical relevance of this allergen, but in light of our data, it is a potentially highly relevant allergen. Further studies are needed to confirm its clinical relevance.

Interestingly, patients sensitized to RML reported significantly greater frequency of symptoms upon ingestion of fruits belonging to the Cucurbitaceae family. To the best of our knowledge, no allergen belonging to mandelonitrile lyase family has been described in these fruits. Whether this association is truly related to RML sensitization or not is not known. It is well known that Cucurbitaceae allergy is related to profilin sensitization in pollen allergic patients [21], and the higher incidence of allergy to these fruits can be attributed to the fact that these patients are pollen allergic. However, the prevalence of profilin sensitization in RML-sensitized patients was not higher than that of the RML-negative ones. The prevalence of profilin sensitization in peach-allergic patients has been previously reported to be around 34% [22]. Conversely in our population, the frequency of profilin sensitization was lower (9.5%), accordingly to previously reported data in pediatric peach allergic population [23]. These differences may be related to the age of patients, given that the first study [22] was performed in adults The frequency of *Cucurbitaceae* allergy in our population was much higher (36.4%) than that reported to profilin, hence higher than usually reported in pollen-allergic patients. Larger studies should be done to clarify this possible association.

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In conclusion, we newly identified a peach protein that could act as an allergen in peach-allergic patients, corresponding to the enzyme RML, homologous to almond allergen Pru du 10. The new allergen was recognized by 52.4% of the study population, therefore, RML could represent a major peach allergen. More studies are necessary to fully understand the role of this allergen in the symptoms after peach ingestion.

Conflicts of interest

M. A. López-Matas, F. Álvarez, and J. Carnés are employees of LETI Pharma.

The remaining authors declare that they have no conflicts of interest.

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Previous presentation

Clinical results of this study were presented as an oral communication to the EAACI Congress, Hamburg 2023. The identification and characterization of the new allergen was presented as poster in the EAACI Congress, Hamburg 2023.

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Table 1. Description of the study population[a].

	Total (N=42)	RML-sensitized (N=22)	RML negative (N=20)	P value
Sex (female)	22 (52.4)	13 (59.1)	9 (45)	0.361¥
Age (y) Median (range)	9.59 (0.86-46.91)	11.5 (5.22-46.91)	6.98 (0.86-38.6)	0.014*
Anaphylaxis	12 (31.6)	9 (42.9)	3 (17.6)	0.096¥
OAS only	27 (71.1)	13 (61.9)	14 (82.4)	0.153¥
Symptoms w/other fruits				
Kiwi	9 (21.4)	6 (27.3)	3 (15)	0.460¥
Melon/Watermelon	8 (19.0)	8 (36.4)	0 (0)	0.004¥
Banana	3 (7.1)	3 (13.6)	0 (0)	0.233¥
Rosacea (other)	8 (19.0)	6 (27.3)	2 (10)	0.243¥
Nuts/Peanut	13 (31.0)	7 (35)	6 (27.3)	0.741¥
Pollen related respiratory symptoms	23 (52.3)	17 (77.3)	6 (30)	0.003¥
SPT positive				
Peach peel	34 (81.0)	16 (76.2)	18 (100)[b]	0.027¥
Peach pulp	8 (19.0)	2 (10)	6 (33.3)	0.078¥
LTP	34 (81.0)	16 (76.2)	18 (100)	0.027¥
Profilin	6 (14.3)	4 (19)	2 (11.8)	0.540¥
Birch	11 (26.2)	7 (38.9)	4 (22.2)	0.278¥
Total IgE (kU/L) Median (range)	97.8 (1.40-4112)	229 (14.8-4112)	47.1 (1.40-403)	0.002*

RML: R-mandelonitrile lyase

[a]Data presented as number (%) of patients in each group unless stated otherwise.

[b]This assay was performed only in 18 patients.

*Mann-Whitney U test

¥Chi-square test

Table 2. Peptides identified by LC/MS-MS in spots M1 and M2

Spot M 1: A0A251QUN8	Spot M2: A0A251QUN1
ARILGGTTIINAGVYAR	DTVASYWHYHGGAIVGK
FKVLILER	FNYYSDPVDLTHCVR
GDPNNLLVAVQASVEK	FVSEDGIDNVR
GTIATEYPNTLTADGFAYNLQQQDDGK	GDPDNLKVAVEAAVQK
GTIATEYPNTLTADGFAYNLQQQDDGKTPVER	GMKNVGVFLSTDALKPYK
HAADELLNK	HASDELLNK
HAADELLNKGDPNNLLVAVQASVEK	HASDELLNKGDPDNLK
KLGDLIR	HASDELLNKGDPDNLKVAVEAAVQK
ILGGTTIINAGVYAR	ILGGTTIINAGVYAR
TKALEPYK	NVGVFLSTDALKPYK
TKALEPYKAR	SRILGGTTIINAGVYAR
VLDDSFR	VAVEAAVQK
VLDDSFRVMGIK	VIDGNFRVMGINALR
VVDASTFPDEPNSHPQGFYLMLGR	VMGINALR
YVGLQILQER	VVDGSTFPSTPASHPQGFYLMLGR
	YVGTKIVQER

Table 3. Prevalence of sensitization to peach allergens by *in vitro* tests[a].

	Total (N=42)		RML-sensitized (N=22)		RML negative (N=20)		P value	
	Number[b] (%)	Median kU/L (range)	Number[b] (%)	Median kU/L (range)	Number[b] (%)	Median kU/L (range)	Number (Chi-square test)	sIgE (Mann-Whitney U test)
Peach	35 (83.3)	2.10 (0-29.3)	19 (86.4)	2.16 (0.09-23.3)	16 (80)	1.67 (0.0-29.3)	0.691	0.811
Pru p 1	2 (4.8)	0 (0-9.14)	2 (9.1)	0 (0-9.14)	0 (0)	0 (0-0)	0.489	0.208
Pru p 3	32 (76.2)	1.73 (0-35.3)	15 (85)	1.16 (0.01-23.9)	17 (68.2)	1.96 (0-35.3)	0.284	0.003
Pru p 4	4 (9.5)	0.01 (0-17.7)	4 (18.2)	0.01 (0-17.7)	0 (0)	0 (0-0.01)	0.109	0.002
Pru p 7	4 (9.5)	0.02 (0-15)	3 (13.6)	0.05 (0-15)	1 (5.0)	0.01 (0-6.72)	0.610	0.033

RML: R-mandelonitrile lyase

[a]Data presented as number (%) of patients in each group.

[b]The patient is considered positive if sIgE value >0.34 kU/L.

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FIGURE LEGENDS

Figure 1. Allergenic profile. A: Peach peel with individual serum (1 to 42), a no-serum negative control (C-) and a positive control with a pool of commercial plasma from patients reactive to peach (C+). All sera were diluted 1/2 the bands at 70 kDa are marked with a red asterisk (*). B: Immunoblot of peel and pulp of peach using a pool of sera made up of the patients who showed a 70-kDa reactive band in A.

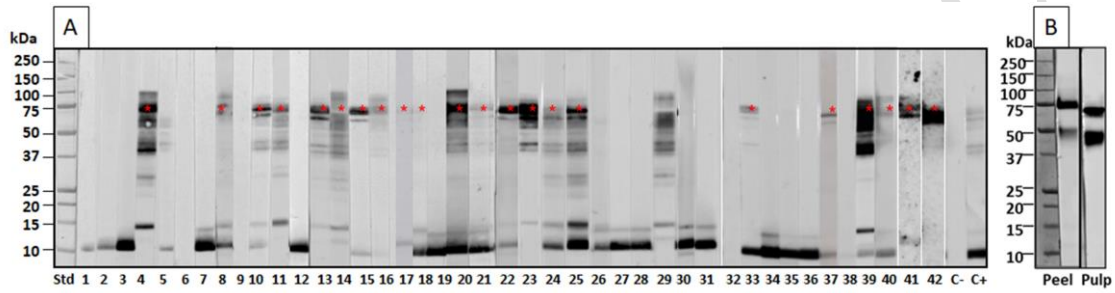

Figure 2. 2D protein and allergenic profile of the peach peel extract. A: 2D electrophoresis of peach peel extract; spots identified by LC/MS-MS are marked with a red circle and named M1 and M2. B: 2D immunoblot of peach peel using a pool of sera made up of patients with 70-kDa band in Fig 1A, diluted 1/2.

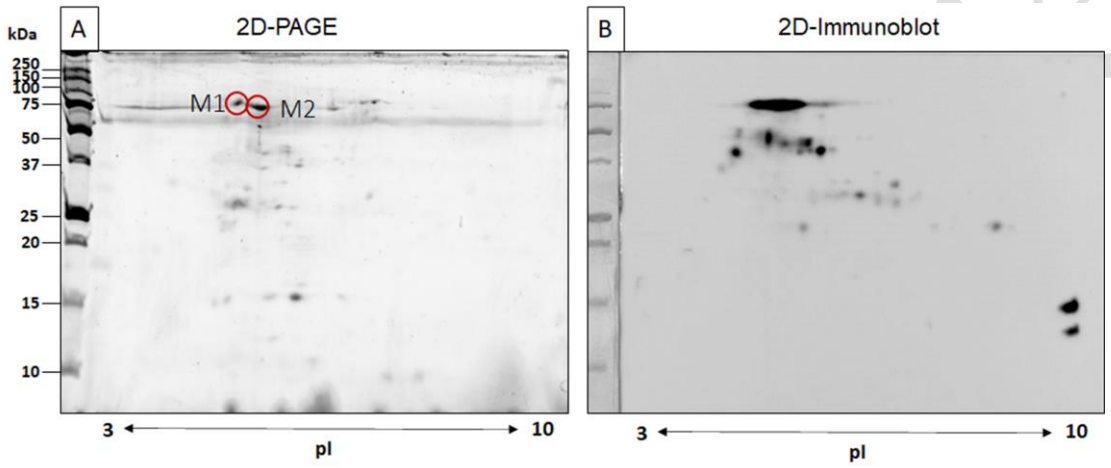

Figure 3. Protein identification by LC/MS-MS. The spot M1 corresponded to the protein A0A251QUN8, and the spot M2 was identified as A0A251QUN1. Both proteins are (R)-mandelonitrile lyases, homologues of Pru du 10. Peptides identified by LC/MS-MS are underlined. Identical amino acids in the three proteins are marked in red; amino acids of Pru du 10 that matched one of the identified peach proteins are marked in blue.

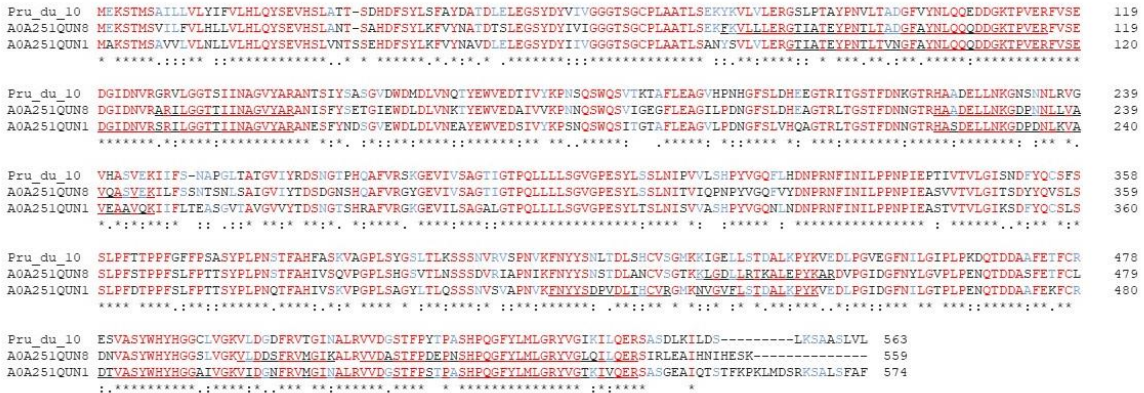

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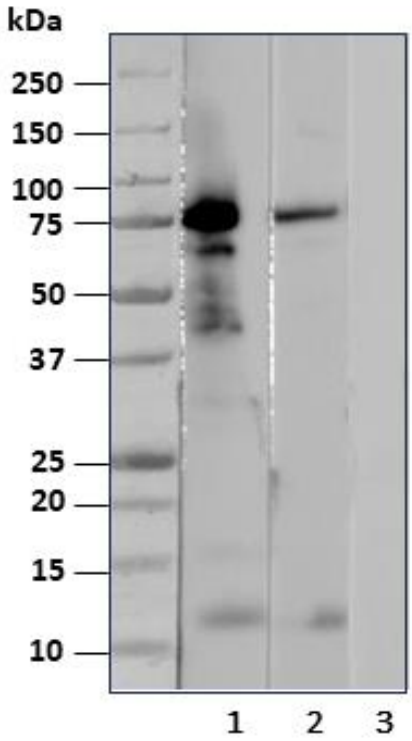
Figure 4. Immunoblot inhibition with almond. Lane 1: Immunoblot with peach peel in solid phase and the pool of RMA-sensitized sera diluted 1/2. Lane 2: Immunoblot with peach peel in solid phase and the pool of RMA-sensitized sera (diluted 1/2) inhibited with almond extract. Lane 3: Immunoblot with peel peach in solid phase and the pool of sera (diluted 1/2) inhibited with peel peach (positive control).



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