WILEY
Online Proofing System Instructions

The Wiley Online Proofing System allows authors and proof reviewers to review PDF proofs, mark corrections, respond to queries, upload replacement figures, and submit these changes directly from the PDF proof from the locally saved file or while viewing it in your web browser.

1. For the best experience reviewing your proof in the Wiley Online Proofing System please ensure you are connected to the internet. This will allow the PDF proof to connect to the central Wiley Online Proofing System server. If you are connected to the Wiley Online Proofing System server you should see the icon with a green check mark above in the yellow banner.


Connected


Disconnected

2. Please review the article proof on the following pages and mark any corrections, changes, and query responses using the Annotation Tools outlined on the next 2 pages.

3. To save your proof corrections, click the "Publish Comments" button appearing above in the yellow banner. Publishing your comments saves your corrections to the Wiley Online Proofing System server. Corrections don't have to be marked in one sitting, you can publish corrections and log back in at a later time to add more before you click the "Complete Proof Review" button below.

4. If you need to supply additional or replacement files bigger than 5 Megabytes (MB) do not attach them directly to the PDF Proof, please click the "Upload Files" button to upload files:

Click Here

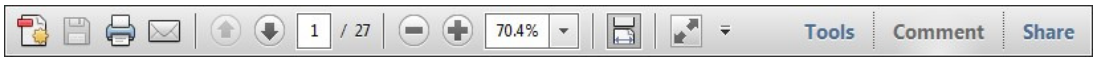
5. When your proof review is complete and you are ready to submit corrections to the publisher, please click the "Complete Proof Review" button below:

Click Here

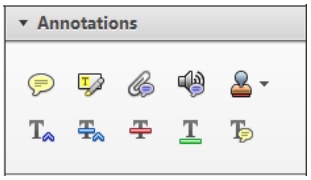
IMPORTANT: Do not click the "Complete Proof Review" button without replying to all author queries found on the last page of your proof. Incomplete proof reviews will cause a delay in publication.

IMPORTANT: Once you click "Complete Proof Review" you will not be able to publish further corrections.

Once you have Acrobat Reader open on your computer, click on the Comment tab at the right of the toolbar:



This will open up a panel down the right side of the document. The majority of tools you will use for annotating your proof will be in the Annotations section, pictured opposite. We've picked out some of these tools below:

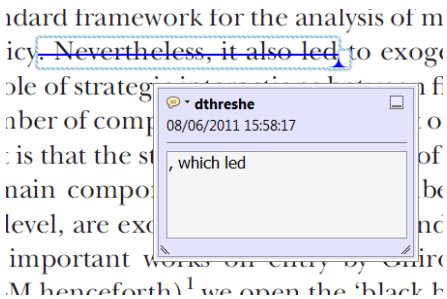


1. Replace (Ins) Tool – for replacing text.

Strikes a line through text and opens up a text box where replacement text can be entered.

How to use it

- Highlight a word or sentence.
- Click on the Replace (Ins) icon in the Annotations section.
- Type the replacement text into the blue box that appears.

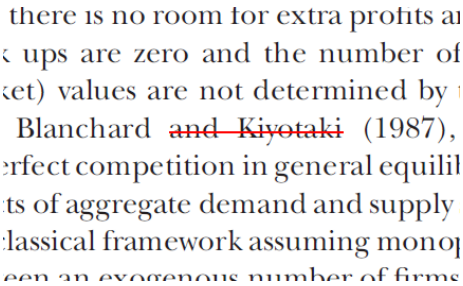


2. Strikethrough (Del) Tool – for deleting text.

Strikes a red line through text that is to be deleted.

How to use it

- Highlight a word or sentence.
- Click on the Strikethrough (Del) icon in the Annotations section.

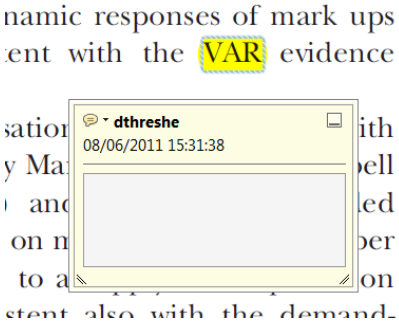


3. Add note to text Tool – for highlighting a section to be changed to bold or italic.

Highlights text in yellow and opens up a text box where comments can be entered.

How to use it

- Highlight the relevant section of text.
- Click on the Add note to text icon in the Annotations section.
- Type instruction on what should be changed regarding the text into the yellow box that appears.

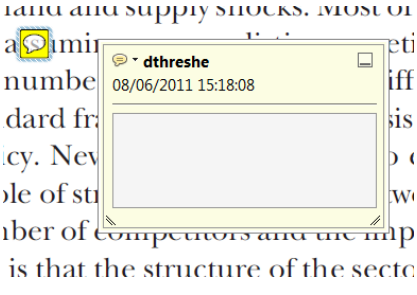


4. Add sticky note Tool – for making notes at specific points in the text.

Marks a point in the proof where a comment needs to be highlighted.

How to use it

- Click on the Add sticky note icon in the Annotations section.
- Click at the point in the proof where the comment should be inserted.
- Type the comment into the yellow box that appears.

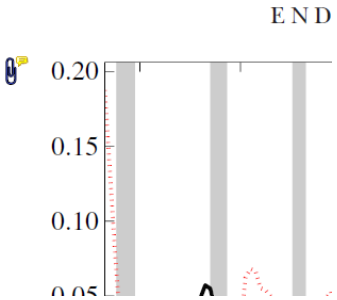


5. Attach File Tool – for inserting large amounts of text or replacement figures.

Inserts an icon linking to the attached file in the appropriate place in the text.

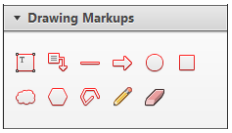
How to use it

- Click on the Attach File icon in the Annotations section.
- Click on the proof to where you'd like the attached file to be linked.
- Select the file to be attached from your computer or network.
- Select the colour and type of icon that will appear in the proof. Click OK.



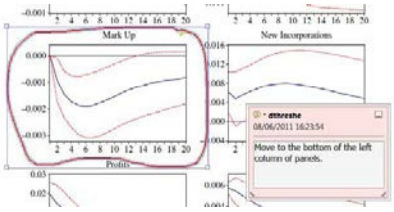
6. Drawing Markups Tools – for drawing shapes, lines and freeform annotations on proofs and commenting on these marks.

Allows shapes, lines and freeform annotations to be drawn on proofs and for comment to be made on these marks.



How to use it

- Click on one of the shapes in the Drawing Markups section.
- Click on the proof at the relevant point and draw the selected shape with the cursor.
- To add a comment to the drawn shape, move the cursor over the shape until an arrowhead appears.
- Double click on the shape and type any text in the red box that appears.



ORIGINAL ARTICLE

Molecular characterization of allergens in raw and processed kiwifruit

Francesca Uberti[1], Elena Peñas[1], Yuri Manzoni[1], Chiara di Lorenzo[1], Cinzia Ballabio[1], Alessandro Fiocchi[2], Luigi Terracciano[3] & Patrizia Restani[1]

[1]Dipartimento di Scienze Farmacologiche e Biomolecolari, Università degli Studi di Milano, Milan, Italy; [2]Ospedale Pediatrico Bambino Gesù, Rome, Italy; [3]Paediatric Division, Department of Child and Maternal Medicine, University of Milan Medical School at the Melloni Hospital, Milan, Italy

To cite this article: Uberti F, Peñas E, Manzoni Y, di Lorenzo C, Ballabio C, Fiocchi A, Terracciano L, Restani P. Molecular characterization of allergens in raw and processed kiwifruit. *Pediatr Allergy Immunol* 2015: **00**.

Keywords
allergy; immunoelectrophoretic techniques; kiwifruit; technological treatments

Correspondence
Prof. Patrizia Restani, Dipartimento di Scienze Farmacologiche e Biomolecolari, Università degli Studi di Milano, Via Balzaretti 9, 20133 Milan, Italy
Tel.: + 39 0250318350-8371
Fax: + 39 0250318284
E-mail: patrizia.restani@unimi.it

Accepted for publication xxxx

DOI:10.1111/pai.12345

Abstract

Background: The prevalence of allergy to kiwifruit is increasing in Europe since the last two decades. Different proteins have been identified as kiwifruit allergens; even though with geographic differences, Act d 1, a cysteine protease protein of 30 kDa, and Act d 2, a thaumatin-like protein of 24 kDa, are normally considered the most important. The aim of this study was (i) to identify at molecular level the sensitization pattern in a group of well-characterized patients allergic to kiwifruit and (ii) to assess the role of technological treatments on kiwifruit allergenic potential.

Methods: The differences in the pattern of antigenicity between fresh and processed kiwifruit were evaluated by both immunoelectrophoretic techniques and clinical tests.

Results: In the group of patients included in this study, three proteins were identified as major allergens in fresh kiwifruit, as the specific sensitization was present in ≥50% of the subjects. These proteins corresponded to actinidin (Act d 1), pectin methyl aldolase (Act d 6), and thaumatin-like protein (Act d 2). Kiwellin (Act d 5) and proteins of Bet v 1 family (Act d 8/act d 11) were also recognized as minor allergens. Immunoreactivity was totally eliminated by industrial treatments used for the production of kiwifruit strained derivative.

Conclusions: In this group of allergic children, the technological treatments used in the production of kiwifruit strained product reduced drastically the allergenic potential of kiwifruit.

Kiwifruit (kiwi) is considered a fruit with high nutritional value thanks to its vitamin C content and its strong antioxidant capacity associated with a large number of phytonutrients including carotenoids, lutein, phenolics, flavonoids, and chlorophyll (1). The most frequently consumed species are *Actinidia deliciosa* and *Actinidia chinensis*, which have green and yellow pulp, respectively, with some differences in flavor (2, 3).

With its increasing diffusion in European countries in the 1980s, reports of kiwi allergy started to become common, mainly among adults, with an increasing prevalence since then (4–6). Most allergic reactions occur within minutes of consuming a kiwifruit, either as such or in food preparations. The main symptoms are as follows: oral allergy syndrome (65–72% of kiwi-allergic subjects), urticaria, abdominal pain, moderate dyspnea, rhinitis, cyanosis, or anaphylaxis (6–10).

According to the International Union of Immunological Societies Allergen Nomenclature Subcommittee (IUIS-www.allergens.org), 13 kiwifruit allergenic proteins have currently been identified, with molecular weights ranging from 10 to 50 kDa. Among them, a 30-kDa protein, Act d 1, belonging to the cysteine protease protein family (11), is considered the most important, being identified in most kiwi-monosensitized patients (8, 12–14).

Act d 2, a 24 kDa thaumatin-like protein, is the second most important kiwifruit allergen; it cross-reacts with proteins of fruits from the same family, such as Mal d 2 in apple and Pru av 2 in cherry (15–17). Other proteins have been also described as kiwifruit allergens, but they showed lower sensitization rates than Act d 1 and Act d 2 (11, 18).

Heat treatment can sometimes reduce the allergenic potential of some food proteins by denaturating and aggregating

IgE-reactive epitopes (19), but some food allergens are described as heat stable, and others only partially labile (20, 21). Heat-processed kiwifruit is less allergenic than fresh fruit, as we showed previously using a double-blind placebo-controlled food challenge (5). None of the 20 children with a history of immediate allergic reaction to fresh kiwifruit showed clinical reactivity to heat-treated and strained kiwi in a commercial preparation. In that study, the pattern of sensitization to the different kiwifruit allergens was not investigated, and the effect of heating on the IgE-binding activity of such allergens remains unclear. Therefore, the aim of the present work was to assess, by immunoelectrophoretic techniques, the differences in the pattern of antigenicity between fresh and processed kiwi, in order to elucidate the impact of heating on its tolerance.

Materials and methods

Patients' enrollment

Table 1 shows the clinical data of the 10 subjects enrolled at the Department of Pediatrics of Macedonio Melloni Hospital (Milano, Italy), whose allergy to kiwifruit (and tolerance to banana, another ingredient of the strained product) was confirmed (5) by a double-blind placebo-controlled food challenge (DBPCFC): all the subjects showed immediate-onset symptoms after exposure to fresh fruit. The skin prick test was performed as described by Fiocchi et al. (22), using kiwifruit (i) fresh, (ii) heated at 100°C in water for 5 min, and (iii) 3 industrially processed (strained). Results were read after 15 min through a clear plastic caliper disk scaled in tenths of millimeters. Reactions were considered to be positive if the largest diameter of the wheal was 3 mm greater than the negative control (vehicle). Specific circulating IgE antibodies were measured by UniCAP® 100 (Pharmacia Diagnostics AB, Uppsala, Sweden); IgE titers ≥0.35 IU/ml were rated positive.

Sera used in immunoblotting consisted of the residual samples taken for diagnostic purposes; no extra blood was drawn for the study. Prior informed consent was obtained from the subjects or their parents.

Kiwifruit samples

Fresh kiwifruit (FK) and strained (SK) kiwifruit were purchased in a local supermarket. SK (Mellin, Milan, Italy) is a product for weaning containing kiwifruit (20%) and banana. To prepare it, fresh kiwifruit (*Actinidia chinensis* var. *deliciosa* cv. Hayward) was (i) heated at 90°C for 5 min; (ii) peeled and puréed; (iii) heated at 115°C for 15 s and stabilized at 110°C for 15 s; and (iv) strained and pasteurized at 65°C for 21 min.

Fresh and strained kiwifruit were suspended in 0.1 M phosphate buffer (pH 7.0) to obtain a final protein concentration of 30 mg/ml and maintained at 4°C overnight. After centrifugation at 10,000 rpm for 30 min at 4°C (Hermle 4 Labortechnik GmbH, Wehingen, Germany), the supernatant was diluted 1:1 (v/v) with sample buffer (0.25 M Tris-HCl buffer pH 6.8, containing 7.5% glycerol, 2% SDS, 5% β-mercaptoethanol).

Sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) and immunoblotting

Sodium dodecyl sulfate polyacrylamide gel electrophoresis was carried out in a gradient gel (9–19% acrylamide), having the following characteristics:

Gradient running gel: 9–19% acrylamide; 0.08–0.17% bis-acrylamide; 0.36 M TRIS-HCl buffer pH 8.8; 35% glycerol; 0.1% SDS; 0.02% ammonium persulfate; and 0.15% N,N,N',N'-tetramethylenediamine (TEMED).

Stacking gel: 3.5% acrylamide; 0.09% bis-acrylamide; 0.125 M TRIS-HCl buffer pH 6.8; 0.1% SDS; 0.02% ammonium persulfate; and 0.15% TEMED.

Running buffer: 25 mM TRIS, 0.19 M glycine and 0.1% SDS (w/v), pH 8.8.

After the electrophoretic run (90 V at room temperature, for approximately 6 h), gels were dyed with Coomassie Brilliant Blue G-250 by the method of Neuhoff et al. (23). All materials and instruments were purchased from Bio-Rad (Richmond CA, USA). Further details are reported by Ballabio et al. (24).

Table 1 Clinical characteristics of patients allergic to kiwifruit and included in the study

Subject	Age (yr)	Sex	STP-FK mm	STP-HK mm	STP-SK mm	cIgEs (IU/ml)	DBPCFC FK	DBPCFC HF	DBPCFC SF
1	2.0	M	4.0	–	–	<0.35	OAS	–	–
2	8.3	M	6.5	4.5	–	0.90	OAS	OAS	–
3	9.0	M	4.5	–	–	2.89	OAS/C	–	–
4	8.0	F	5.5	3.5	–	<0.35	OAS	–	–
5	6.3	M	10.0	–	–	<0.35	OAS	–	–
6	8.6	F	9.5	5.5	–	<0.35	OAS	–	–
7	11.9	M	4.0	–	–	<0.35	OAS/Rh	–	–
8	14.4	F	3.5	–	–		OAS	–	–
9	16.3	F	3.5	–	–	<0.35	OAS	–	–
10	9.2	F	6.5	4	–	1.40	OAS	–	–

STP, Skin Prick Test; FK, Fresh kiwifruit; HK, Heated kiwifruit; SK, Strained kiwifruit; cIgEs, circulating specific IgEs; OAS, Oral Allergy Syndrome; Rh, Rhinitis; C, Conjunctivitis; DBPCFC, Double-blind Placebo-Controlled Food Challenge.

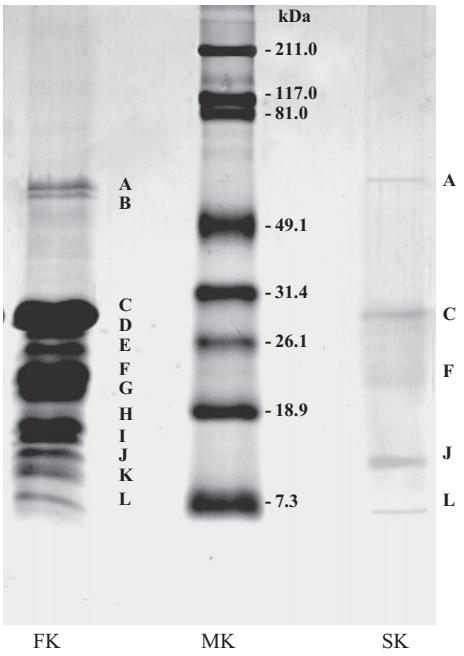


Figure 1 SDS-PAGE of fresh kiwifruit (FK) and strained (SK) kiwifruit. MK, molecular weight marker solution.

A prestained molecular weight marker solution (broad range, Bio-Rad) contained myosin (211.0 kDa), β-galactosidase (117 kDa), bovine serum albumin (81 kDa), ovalbumin (49.1 kDa), carbonic anhydrase (31.4 kDa), soybean trypsin inhibitor (26.1 kDa), lysozyme (18.9 kDa), and aprotinin (7.3 kDa) was run in parallel to the samples.

For immunoblotting analysis, after SDS-PAGE, kiwifruit proteins were transferred onto a PVDF membrane (Millipore, Billerica, MA, USA) by Western blotting in a Trans-blot Electrophoretic Transfer Cell (Bio-Rad). The membranes were blocked with 1% gelatin and washed three times with 0.25% gelatin solution (150 mM NaCl, 5 mM TRIS, 0.05% Triton-X) to prevent non-specific adsorption of the immunologic reagents. Afterward, the membranes were immersed in 30 ml of 0.25% gelatin solution containing 300 μl of allergic patient's serum. Antigen-IgE complexes were detected using 10 μl of goat anti-human IgE antibodies labeled with alkaline phosphatase (Sigma Aldrich, Milan, Italy). Finally, after incubation with the bromochloroindolyl phosphate–nitroblue tetrazolium (BCIP/NBT) solution, an intense black-purple precipitate was developed at the site of the enzyme binding. The developing solution contained 15% bromochloroindolyl phosphate and 30% nitroblue tetrazolium in alkaline phosphatase buffer (100 mM TRIS, 100 mM sodium chloride, and 5 mM magnesium chloride, pH 9.5).

The immunoreactive bands were quantified by a gel scanner (Sharp JX-330, Pharmacia Biotech) and the Image Master 1D software, calculating the average density of pixels across the band length and integrating over the bandwidth. Classes of positive reactions were defined on the basis of an arbitrary scale of densitometric values, and six classes of reactivity were identified (classes <3 were considered not significant for clinical reactions, being frequent also in non-allergic subjects).

Results

All subjects included in this study had positive responses in the skin prick test to FK (Table 1). However, only subjects 2, 4, 6, and 10 also presented a positive response to boiled kiwifruit (HK), and in all cases, wheal diameter was significantly smaller (from 31 to 42%) than for the fresh sample. There was no positive response to the commercial strained product (SK).

Fig. 1 illustrates the electrophoretic patterns of FK and SK samples; FK pattern includes 12 main proteins with molecular weight ranging from 8 to 73 kDa (Table 2). The protein bands with higher relative abundance were as

Table 2 Molecular characterization of kiwifruit allergens and their involvement in positive response in immunoblotting

Protein band	Published MW (kDa)*	Calculated MW (kDa)†	Allergen name	Presence in FK	Presence in SK	Immunoreactivity to FK proteins (% of total subjects)
A	–	72.8		+	+	10
B	–	68.0		+	–	10
C	30.0	29.6	Cysteine protease/actinidin (Act d 1)	+	+	70
D	28.0	26.3	Kiwellin (Act d 5)	+	–	40
E	24.0	23.7	Thaumatin-like protein (Act d 2)	+	–	50
F	18.0	~20.0	Pectin methylesterase inhibitor (Act d 6)	+	+	70
G	17.0	17.5	Proteins of Bet v 1 family (Act d 8/Act d 11)	+	–	40
H	14.0	13.8	Profilin (Act d 9)	+	–	20
I	–	12.4		+	–	20
J	11.4	11.4	2S Albumin (Act d 13)	+	+	20
K	10.0	10.0	nsLTP1 (Act d 10)	+	–	10
L	–	8.5		+	+	0

*Biblio allergenic.

†Calculated with the molecular weight standard solution.

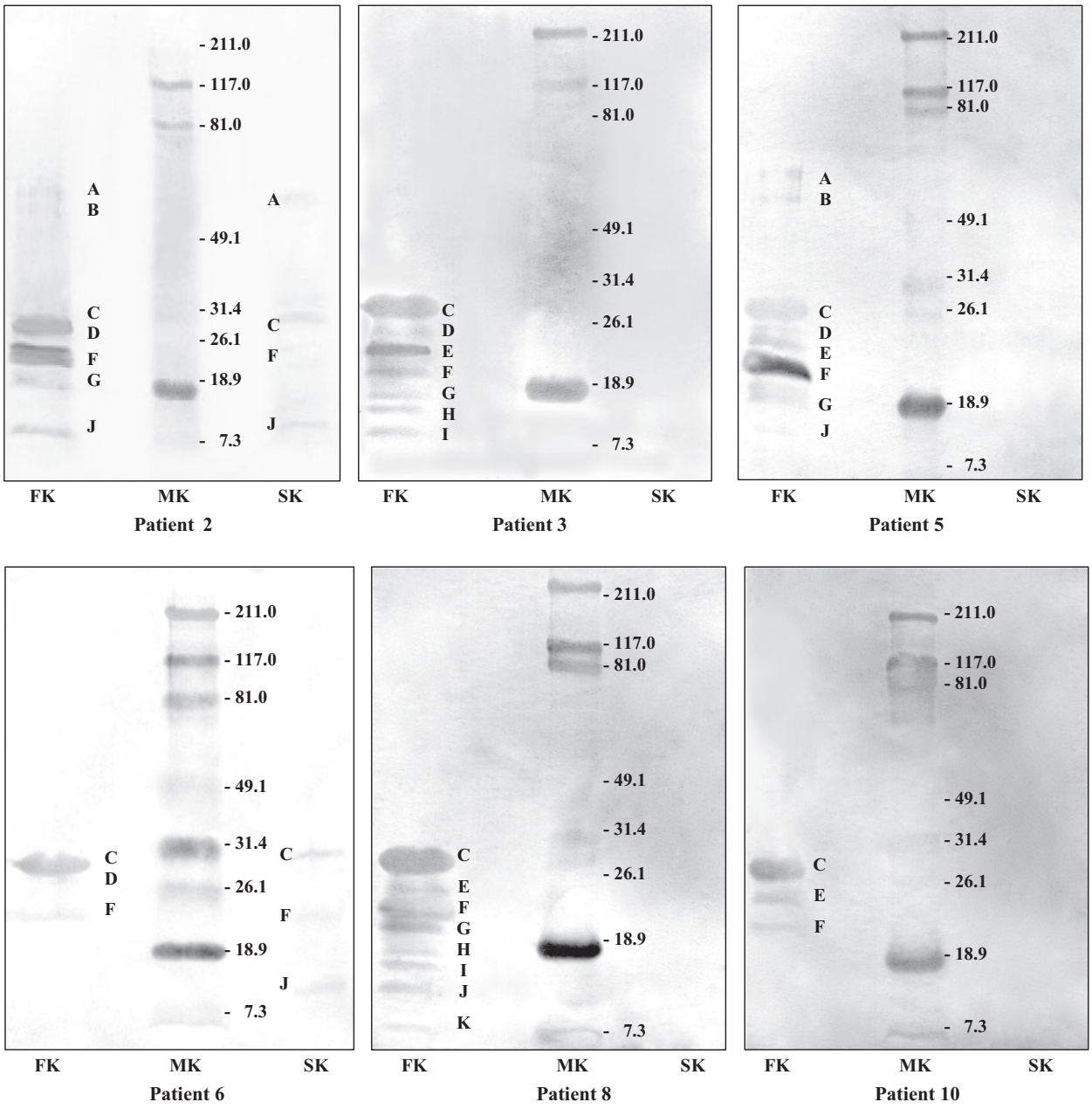


Figure 2 Immunoblotting obtained incubating the membranes with the sera of six subjects included in the study. Abbreviations as in Fig. 1.

follows: C (~29.6 kDa), D (~26.3 kDa), E (~23.7 kDa), F (~20.0 kDa), G (~17.5 kDa), and H (~13.8 kDa). SK presented a less complex profile, with proteins having an apparent general lower abundance distributed in the range from 8.5 to 73 kDa. Only bands A, C, F, J, and L were clearly identifiable in this sample.

Fig. 2 shows the membranes obtained after incubation with the most reactive sera (6/10) challenged in this study. Several proteins of fresh and strained kiwi were recognized by circulating IgEs from allergic patients (Tables 2 and 3). In FK, all proteins apart from band L were recognized by circulating IgEs with different frequencies (10–70% of subjects) and class of reactivity (from 2 to 6). In SK, however, only patients 2 and 6 showed a weak immunoreactivity (class 2) for Act d 1 (band C in Fig. 1 and Table 3).

As for the percentages of positive responses (Table 2), the major kiwifruit allergens (positive, with classes ranging between 3 and 6 in more than 50% of patients) for these patients were as follows: actinidin (Act d 1) recognized by 70% of patients; pectin methyl esterase (Act d 6) recognized by 70% of patients; and thaumatin-like protein (Act d 2) immunoreactive in 50% of patients.

All allergenic proteins lost their immunoreactivity after heating and straining, the only exception being in subjects 2

Table 3 Class of positivity measured in immunoblotting for Fresh (FK) and Strained (SK) kiwi

Allergen band	Allergen name	Patient 1		Patient 2		Patient 3		Patient 4		Patient 5		Patient 6		Patient 7		Patient 8		Patient 9		Patient 10		Total responses*	
		FK	SK	FK	SK	FK	SK	FK	SK	FK	SK	FK	SK	FK	SK	FK	SK	FK	SK	FK	SK	FK	SK
A	–			2	1					3												1	0
B	–			2						3												1	0
C	Act d 1			3	2	6				4		6	2	2		6		6		6		7	0
D	Act d 5	2		5		3		5		3		1										4	0
E	Act d 2					6		3		4						4				5		5	0
F	Act d 6	6		6	1	5		2		6		3	2			5				3		7	0
G	Act d 8/11			4		3				3						5						4	0
H	Act d 9					3										3						2	0
I	–					3										4						2	0
J	Act d 13			4	1					1			2			4						2	0
K	Act d 10															3						1	0
L	–																					0	0

*Including only reactivities ≥3.

and 6, who showed weak reactivity for Act d 1 (ranked in class 2) (Table 3).

Discussion

The results obtained by the skin prick test indicated that the reactivity to kiwi proteins was strongly influenced by boiling (HK) and technological treatments (SK). Although it has been reported that several kiwifruit allergens are heat-labile (16), the results of the present work indicate that boiling reduced the reactivity to kiwifruit, but it was not severe enough to eliminate completely kiwifruit antigenicity. Differently, technological treatments (heating followed by straining) removed drastically kiwifruit immunoreactivity.

The electrophoretic pattern of SK sample showed lower amount of proteins than that of FK. As the protein amount loaded onto the gel was the same for FK and SK, the differences in protein relative abundance must be due to a modified structure of the fruit matrix. In fact, kiwifruit, after heating and straining, appears partially jellified, indicating changes in kiwifruit matrix.

Immunoblotting identified three proteins (C, E, and F) as the major allergens in FK. These proteins corresponded to actinidin (Act d 1) and pectin methyl aldolase (Act d 6), both with 70% of subjects sensitized and thaumatin-like protein (Act d 2) with 50%. Kiwellin (Act d 5) and proteins of the Bet v 1 family (Act d 8/Act d 11) were recognized by 40% of the sera challenged with fresh kiwifruit.

Act d 1 represents more than 50% of the total soluble protein contained in green-fleshed kiwi (*A. deliciosa* cv. Hayward) (18), and its role in patients' sensitization here described confirms that it must be considered a major allergen in kiwi-allergic individuals (8, 19).

Act d 2, a thaumatin-like protein, and Act d 5 (kiwellin) have also been described among the major allergenic proteins in kiwifruit (13, 20, 21), showing sensitization rates ranging from 8 to 88% (7, 8, 19, 22); in the present study, Act d 2 and Act d 5 were involved in 50 and 40% of cases, respectively.

Act d 6, a pectin methylesterase inhibitor, has previously been described as a minor allergen (11), in contrast with this study, which found the protein to be among the major allergens (70%) with three subjects in class 6 and two in class 5 of reactivity.

Act d 8, a homolog of the major birch pollen allergen Bet v 1, has previously been identified as one of the most important allergens in kiwifruit, showing sensitization in 44 and 58% of patients from Western/Central and Eastern Europe (11); our study confirms its important role in sensitization (40% of patients).

Our results show Act 4, Act 9, and Act 10 to have the lowest sensitization rates (10–20%); this is only partially in agreement with a previously published paper (11) which showed a higher sensitizing role of profiline (Act d 9) in patients from southern European countries (31%) than in eastern, northern, and central-western European countries (7, 17 and 22%, respectively).

The absence of immunoreactivity in SK observed in immunoblotting shows that results at molecular level confirm the clinical tolerance of the commercial product previously described by Fiocchi et al. (5).

In conclusion, Act d 1, Act d 6, and Act d 2 were identified as the major allergens in fresh kiwifruit. Boiling treatment was not severe enough to eliminate the reactivity in the most sensitized subjects. On the other hand, technological treatments used during industrial production (*three* heating steps and straining) were enough to modify the antigenic potential of kiwi proteins or at least to decrease dramatically any clinical reaction. This phenomenon may be attributed to the stickiness of the kiwifruit matrix that we observed in preparing samples for immunoblotting.

Acknowledgments

Elena Peñas thanks the Spanish Ministry of Science and Innovation for the postdoctoral fellowship.

References

1. Cassano A, Figoli A, Tagarelli A, Sindona G, Drioli E. Integrated membrane process for the production of highly nutritional kiwi fruit juice. *Desalination* 2006: **189**: 21–30.
2. Bublin M, Mari A, Ebner C, et al. IgE sensitization profiles toward green and gold kiwis differ among patients allergic to kiwi from 3 European countries. *J Allergy Clin Immunol* 2004: **114**: 1169–75.
3. Lucas JSA, Lewis SA, Trewin JB, Grimshaw KEC, Warner JO, Hourihane JOB. Comparison of the allergenicity of *Actinidia deliciosa* (kiwi fruit) and *Actinidia chinensis* (gold kiwi). *Pediatr Allergy Immunol* 2005: **16**: 647–54.
4. Lucas JS, Lewin SA, Hourihane JOB. Kiwi fruit allergy: a review. *Pediatr Allergy Immunol* 2003: **14**: 420–8.
5. Fiocchi A, Restani P, Bernardo L, et al. Tolerance of heat-treated kiwi by children with kiwi allergy. *Pediatr Allergy Immunol* 2004: **15**: 454–8.
6. Lucas JSA, Grimshaw KEC, Collins K, Warner JO, Hourihane JOB. Kiwi is a significant allergen and is associated with different patterns of reactivity in children and adults. *Clin Exp Allergy* 2004: **34**: 1115–21.
7. Aleman A, Sastre J, Quirce S, et al. Allergy to kiwi: a double-blind placebo-controlled food challenge study in patients from a birch-free area. *J Allergy Clin Immunol* 2004: **113**: 543–50.
8. Bublin M, Pfister M, Radauer C, et al. Component-resolved diagnosis of kiwi allergy with purified natural and recombinant kiwi allergens. *J Allergy Clin Immunol* 2010: **125**: 687–94.
9. Möller M, Kayma M, Steinhart H, Paschke A. Isolation and characterization of a major allergen in kiwi. *Z Lebensm Unters Forsch A* 1997: **205**: 364–9.
10. Voitenko V, Poulsen LK, Nielsen L, Norgaard A, Bindslev-Jensen C, Skov PS. Allergenic properties of kiwi-fruit extract: cross-reactivity between kiwi-fruit and birch-pollen allergens. *Allergy* 1997: **52**: 136–43.
11. Le T-M, Bublin M, Breiteneder P, et al. Kiwi allergy across Europe: clinical manifestation and IgE recognition patterns to kiwi allergens. *J Allergy Clin Immunol* 2013: **131**: 164–71.
12. Pastorello EA, Pravettoni V, Ispano M, et al. Identification of the allergenic components of kiwi fruit and evaluation of their cross-reactivity with timothy and birch pollens. *J Allergy Clin Immunol* 1996: **98**: 601–10.
13. Popovic M, Grozdanovic M, Gavrovic M, Jankulovic C. Kiwi as a food allergen source. *J Serb Chem Soc* 2013: **78**: 333–52.
14. Bublin M, Dennstedt S, Buchegger M, et al. The performance of a component-based allergen microarray for the diagnosis of kiwi allergy. *Clin Exp Allergy* 2011: **41**: 129–36.
15. Gavrovic-Jankulovic M, Cirkovic T, Burazer L, Vuckovic O, Jankov RM. IgE cross-reactivity between meadow fescue pollen and kiwi fruit in patients' sera with sensitivity to both extracts. *J Investig Allergol Clin Immunol* 2002: **12**: 279–86.
16. Tamburrini M, Cerasuolo I, Carratore V, et al. Kiwellin, a novel protein from kiwi fruit. Purification, biochemical characterization and identification as an allergen. *Protein J* 2005: **24**: 423–9.
17. Tuppo L, Giangrieco I, Palazzo P, et al. Kiwellin, a modular protein from green and gold kiwi fruits: evidence of *in vivo* and *in vitro* processing and IgE binding. *J Agric Food Chem* 2008: **56**: 3812–7.
18. Palacin A, Rodriguez J, Blanco C, et al. Immunoglobulin E recognition patterns to purified kiwi (*Actinidia deliciosa*) allergens in patients sensitized to kiwi with different clinical symptoms. *Clin Exp Allergy* 2008: **38**: 1220–8.
19. Watanabe H, Toda M, Sekido H, et al. Heat treatment of egg white controls allergic symptoms and induces oral tolerance to ovalbumin in a murine model of food allergy. *Mol Nutr Food Res* 2014: **58**: 394–404.
20. Wal JM. Thermal processing and allergenicity of foods. *Allergy* 2003: **58**: 727–9.
21. Lodge N, Prera C. A review of kiwi processing. *N Z kiwifruit* 1992: **90**: 14–5.
22. Fiocchi A, Bouygue GR, Restani P, Bonvini G, Startari R, Terracciano L. Accuracy of skin prick tests in bovine protein allergy. *Ann Allergy Asthma Immunol* 2002: **89**: 26–32.
23. Neuhhoff V, Arold N, Taube D, Ehrhardt W. Improved staining of proteins in polyacrylamide gels including isoelectric focusing gels with clear background at nanogram sensitivity using Coomassie Brilliant Blue G-250 and R-250. *Electrophoresis* 1988: **9**: 255–62.
24. Ballabio C, Penas E, Uberti F, et al. Characterization profile to lupin in peanut-allergic children and assessment of cross-reactivity. *Pediatr Allergy Immunol* 2013: **24**: 270–5.

Author Query Form

Journal: PAI
Article: 12345

Dear Author,
During the copy-editing of your paper, the following queries arose. Please respond to these by marking up your proofs with the necessary changes/additions. Please write your answers on the query sheet if there is insufficient space on the page proofs. Please write clearly and follow the conventions shown on the attached corrections sheet. If returning the proof by fax do not write too close to the paper's edge. Please remember that illegible mark-ups may delay publication.
Many thanks for your assistance.

Query reference	Query	Remarks
1	**WILEY: Please supply date of acceptance.**	
2	**AUTHOR: Please confirm that given names (red) and surnames/family names (green) have been identified correctly.**	
3	**AUTHOR: Please suggest whether the term "plastric" can be changed to "plastic" in the sentence "Results were read. . .of millimeters".**	
4	**AUTHOR: 10,000 rpm: please replace this with the correct *g* value.**	
5	**AUTHOR: Please give address information for "Pharmacia Biotech": town, state (if applicable), and country.**	
6	**AUTHOR: Please check journal title for reference [9].**	
7	**AUTHOR: Please check journal title for reference [21].**	