SUPPLEMENTARY MATERIAL

METHODS

Study Population

The exclusion criteria of the study were: patients under 6 years of age, patients in treatment with antihistamine that could not be interrupted, patients in treatment with topical corticosteroids on their arm, patients in treatment with omalizumab in the previous 3 months, patients receiving immunotherapy against peach, patients allergic to placebo components, pregnant women or patients with a physical or mental disability to participate.

Open Oral Food Challenge with Kiwifruit

An open oral food challenge (OFC) with kiwifruit (var. *Actidinia deliciosa*, Zespri®, New Zealand) was performed in 17/36 patients. Oral food challenge was not performed in 15 patients with previous history of anaphylaxis and four patients who had suffered a clear kiwifruit ingestion-related systemic symptom within the previous two years and had positive tests (SPT or specific immunoglobulin E [sIgE] against kiwifruit as determined by an ImmunoCAP assay). The OFC was performed progressively administering the following doses: 2.7 gr, 5.7, 11 gr, 23 gr, and 46 gr, every 15-30 minutes (1). The challenge was positive when objective symptoms of an IgE-mediated reaction or subjective symptoms developed following at least two consecutive doses as assessed by the visual analog scale (VAS ≥2).

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Skin Prick Test

Cow milk (Leti) and banana (Leti) used for the SBOFC recipe were evaluated with a SPT in the 36 cases and fresh lemon, chia seeds, and green food coloring (Dr. Oetker, Bielefeld, Germany) were assessed with a p-p test.

Preparation of Homemade Kiwifruit Extracts (Pulp and Seed)

Briefly, kiwifruit seeds were manually separated from the kiwifruit *(A. deliciosa)* pulp and lyophilized. Powder of the kiwifruit seeds was then suspended in sodium borate buffer, pH 8.0, at a 10% (w/v) ratio and gently stirred for 1 h. After centrifugation at 4 °C, the supernatant was collected, and the pellet was re-extracted twice using the same buffer and under the same conditions. The collected supernatants were lyophilized, and the resulting material was then extracted 3 times with 10% (w/v) acetone to remove the lipid components. The resulting pellets containing the extracted proteins were air desiccated and dissolved in ammonium bicarbonate 0.15 mol/L, pH 8. After centrifugation at 8500 rpm and 4 °C, the supernatants were lyophilized, quantified using the method described by Lowry *et al*. (2), and stored at -20 °C until their use. Pulp kiwifruit extract was prepared as starting material using seedless kiwifruit pulp that was homogenized in 10% (w/v) ammonium bicarbonate (50 mmol/L) and phenylmethylsulphonyl fluoride (1 mmol/L) in a blender. After 1 h of mixing at 4 °C, the homogenate was centrifuged for 30 minutes at 8500 rpm and 4 °C. The pellet was extracted once again using the same solvent and the supernatants were collected, lyophilized, and stored at -20 °C until their use. The lyophilized protein extracts were resuspended in ammonium bicarbonate 0.15 mol/L, pH 8.0, and quantified using the method described by Lowry *et al*. (2)

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Simple Blind Oral Food Challenge with Kiwifruit Seeds

Ingredients used to prepare the active smoothie included natural yogurt, banana, lemon, green food coloring, and the isolated seeds of one whole kiwifruit (approximately 1.2-1.5 g). In the case of the inactive smoothie, chia seeds (0.9 g) were used instead of kiwifruit seeds.

Immunoblotting

Sodium dodecyl sulfate-polyacrylamide gel electrophoresis was performed with kiwifruit seed extract (20 µg/strip) in 17% polyacrylamide gels. Proteins were visualized by means of Coomassie blue staining or, alternatively, transferred to nitrocellulose membranes (Amersham, Piscataway, NJ). The protein concentration was determined using the bicinchoninic acid method (Pierce Chemical, Rockford, Ill). Immunodetection of allergenic proteins was performed with patient sera (n = 36) diluted at a ratio of 1:5. The binding of human IgE was detected using anti-human mouse IgE antibodies diluted at a ratio of 1:5000 (ALK-Abello, Hørsholm, Denmark), followed by horseradish peroxidase-labeled goat anti-mouse IgG antibodies diluted at a ratio of 1:5000 (Pierce). The signal was developed with the ECL-Western blotting reagent (Amersham). Sensitization to the kiwifruit storage proteins Act d 12 and Act d 13 corresponded to bands immunodetected at 51 and/or 12 kDa, respectively, in the kiwifruit extract immunoblotting.

Enzyme-Linked Immunosorbent Assay (ELISA)

Allergens Act d 1, Act d 2, and Act d 5 were purified from *Actinidia deliciosa* fruits using the method published by Palacin *et al*..(3) Briefly, polystyrene 96-well microtiter plates (Costar 3590, Corning) were coated with 50 µl of purified Act d 1, Act d 2, or Act d 5 at

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a concentration of 5 µg/ml as a solid phase and each individual serum sample at a dilution of 1:6. After washing them with 0.1% phosphate-buffered saline (PBS) with Tween-20, the wells were incubated with a peroxidase-labeled anti-human IgE (FisherScientific; 1:3000 dilution) for 1 hour at 25 °C. The plates were washed again and developed with 50 µl of peroxidase substrate buffer (ThermoScientific). After 30 minutes, the reaction was stopped with 50 µl of 2N HCl, and the optical density was measured at 492 nm. PBS with 1% bovine serum albumin (BSA) was used as a negative control. All assays were performed in triplicate (3,4).

ImmunoCAP Inhibition with Purified Act d 1

Specific IgE inhibition studies with ImmunoCAP were performed using ImmunoCAP FEIA 250 (Thermofisher Scientific, Uppsala, Sweden). Sera from patients showing high levels of sIgE against Act d 1 as detected by ImmunoCAP ISAC or ELISA assays were inhibited with Act d 1 (purified as previously described)(3) at room temperature, for 2 h, at 100 µg/ml and with 5 sequential concentrations diluted at a ratio of 1:10. The inhibitor mixtures (including sera with no inhibitor used as a control) were analyzed to detect sIgE against kiwifruit (f84) in the ImmunoCAP assays. The assays were performed following the manufacturer´s protocol. The percentage of inhibitory IgE binding was calculated using the following formula: ([kUA/L non-inhibited control - kUA/L inhibited sample]/ non-inhibited control kUA/L) x 100.

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RESULTS

Results of the Kiwifruit Extract Immunoblotting with 40 µg/Strip

Immunoblotting of kiwifruit extract at 40 µg/strip (following the same method described above) was performed in patients with a positive SPT against kiwifruit seed extract (n = 17) (Supplement Figure 2). Eleven out of 17 patients (64.7%) had a protein of 51 kDa (expected for Act d 12; patients 6, 10, 13, 14, 15, 24, 25, 26, 28, 34, and 36), 3 out of 17 (17.6%) had a protein of 12 kDa (expected for Act d 13; patients 11, 15, and 24), and 10 out of 17 (58.8%) had a protein of 25 kDa (patients 6, 11, 13, 15, 16, 26, 28, 30, 33, and 34), which, identified by mass spectrometry, corresponded to a thaumatin-like protein of the kiwifruit seed (Supplement Figure 3). This seed thaumatin-like protein was different from the pulp Act d 2, as one patient (patient 34) had it in the seed extract IB but not in the pulp extract IB. Sequence coverage between both proteins was 44%. This seed thaumatin-like protein was not previously described in the literature; nevertheless, further studies are needed to determine its clinical importance. In the literature, sensitization against a 7S-globulin protein of kiwifruit seeds was found in sera from kiwifruit-allergic pediatric patients (5) but, again, we are unaware of its clinical relevance.

Comparison of the Diagnostic Performance of the Different Available *In Vitro* Techniques

In order to increase the sample size for the comparative analysis of the *in vitro* techniques, sera from the 36 prospectively recruited subjects and from 33 kiwifruit-allergic patients of a previous multicenter study (FIS PI 11/01634) were used together. The 35 controls of

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the retrospective study were also used in this analysis. The 33 patients who had been retrospectively selected experienced allergy symptoms after ingesting kiwifruit on at least two occasions (14 developed local or systemic symptoms, a positive SPT against kiwifruit [ALK-Abelló], and positivity for sIgE to kiwifruit [ImmunoCAP, Uppsala, Sweden]; 10 developed local or systemic symptoms, had a positive SPT against kiwifruit, and negativity for sIgE against kiwifruit; and 9 developed systemic symptoms or anaphylaxis and had negative tests). In sera of these 33 patients, the presence of sIgE was determined by the ISAC and FABER assays, ImmunoCAP (kiwifruit extract and Act d 8), and an ELISA (Act d 1 and Act d 2). The diagnostic yield of the CAP and FABER assays did not differ significantly in the detection of kiwifruit extract ($p = 0.118$). The ISAC microarray had a better diagnostic performance than the FABER assay in the detection of Act d 1 ($p < 0.001$) and Act d 2 ($p = 0.031$). The ISAC and CAP assays did not differ in terms of the detection of Act d 8 ($p = 0.5$) (Supplement Table II).

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Supplement Table 1. Prick test with purified Act d 1 extract in ten patients with positivity for Act d 1 in the ELISA

	ISAC Act d 1 (ISU)	ELISA Act d 1 (positive cut point >0.235 units)	Prick test with purified Act d 1 extract (mm)
Patient 5	0.00	0.406	0
Patient 6	12.62	0.434	5.5
Patient 7	6.07	0.472	5.5
Patient 9	0.00	0.401	0
Patient 17	0.00	0.366	0
Patient 21	0.00	0.443	0
Patient 22	0.00	0.38	0
Patient 23	0.00	0.37	0
Patient 24	0.3	0.459	3.5
Patient 25	0.00	0.449	0

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Supplement Table 2. Concordance between the ImmunoCAP, ISAC, and FABER assays in the detection of kiwifruit components using McNemar's comparative test

	Kiwifruit extract	**Act d 1**	**Act d 2**	**Act d 5**	**Act d 8**
Patients: [Positive rate, n (%)]	ImmunoCAP: 34/62 (54.8) FABER: 41/62 (66.1) McNemar $p = 0.118$	ISAC: 24/62 (38.7) FABER: 5/62 (8.1) McNemar $p = 0.000$	ISAC: 6/62 (9.7) FABER: 0/62 McNemar $p = 0.031$	ISAC: 2/62 (3.2) FABER: 2/62 (3.2) McNemar $p = 1$	ISAC: 3/69 (43.5) ImmunoCAP: 5/69 (72.5) McNemar $p = 0.5$
Controls: [falls positive rate, n (%)]	ImmunoCAP: 2/16 (12.5) FABER: 1/35 (2.9) McNemar $p = 0.5$	ISAC: 1/35 (2.9) FABER: 0/35 McNemar $p = 1$	ISAC: 0/35 FABER: 0/35 McNemar $p = 1$	ISAC: 0/35 FABER: 0/35 McNemar $p = 1$	

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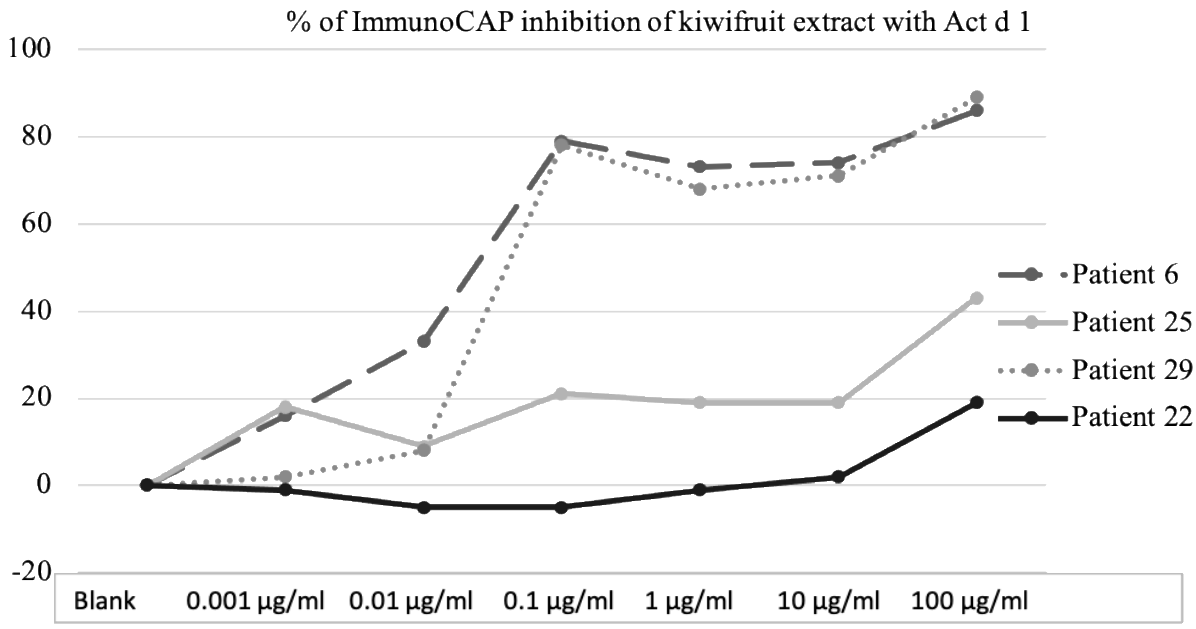
Supplement Figure 1. ImmunoCAP inhibition of kiwifruit extract with Act d 1. Patients 6 and 29, who were positive for Act d 1 in the ISAC assay, showed a high inhibition with purified Act d 1, and patients 25 and 22, who were negative for Act d 1 in the ISAC assay, showed poor inhibition with purified Act d 1.



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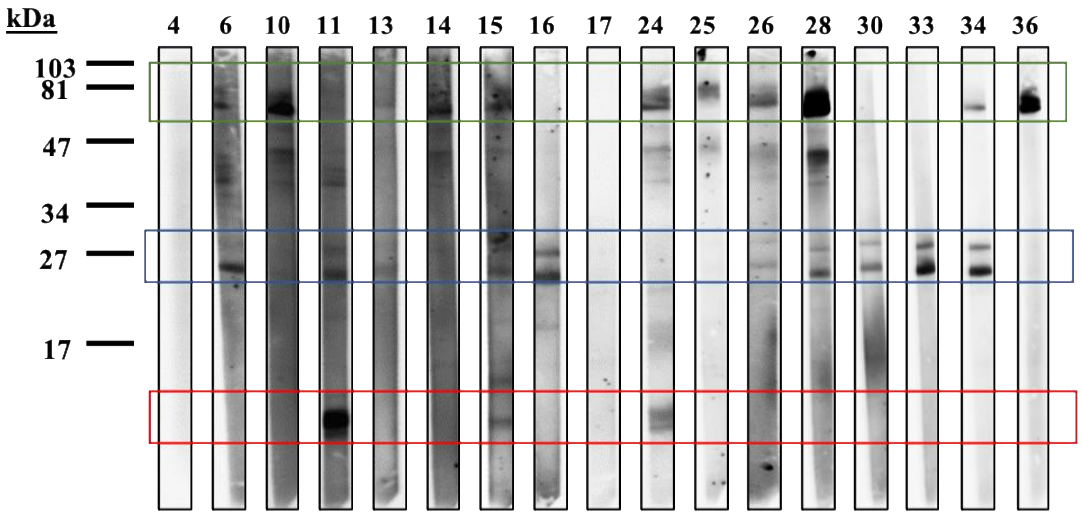
Supplement Figure 2. Immunoblot of kiwifruit seed extract (40 µg/strip) in sera of the 17 patients with a positive skin prick test against kiwifruit seed extract. A band of 25 kDa is identified as a seed-specific thaumatin-like protein.



Supplement Figure 3. Identification of the 25-kDa kiwifruit seed protein by MALDI-TOF mass spectrometry

Thaumatin-like protein OS = *Actinidia deliciosa* OX = 3627 GN = TLP1 PE = 2 SV =1

Database: UP-Viridiplantae_191001 ***Score***: 107 ***Expect***: 0.00017

Monoisotopic mass (Mr): 25175 Da ***Calculated pI***: 8.29

Protein sequence coverage: 44% Matched peptides shown in **bold.**

1 MSTFKSLSLS ALLFIAFLFT CARGATFNI NNCPFTVWAA AVPGGGKR**LD**

51 **RGQNWIINPG** **AGTK**GARVWP R**TGCNFDGAG** **RGK**CQTGDCN GLLQCQAFGQ

101 PPNTLAEYAL NQFNNLDFFD ISLVDGFNVA MEFSPTSGGC TRGIK**CTADI**

151 **NGQCPNELRA** **PGGCNNPCTV** **FKTDQYCCNS** **GNCGLTNFSK** FFK**DRCPDAY**

201 **SYPKDDQTST** **FTCPAGTNYK** VVFCP

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