demonstrate the utility of this method and up to 80% recovery with a purity of 98% was observed. Gel electrophoresis of the purified samples reflects yield and purity of precipitation procedure. The strategy outlined here make metal affinity precipitation a rapid, simple and easy to scale up technique for the purification of His-tagged recombinant proteins and introduce a high-throughput alternative for metal affinity chromatography.

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Animal model for melon allergy

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Background: More than 25% of populations are suffering from type I Allergy. Food allergens such as Melon (*Cucumis melo*) represent a major cause of type I hypersensitivity. Profilin was identified and characterized as the melon major allergen. Profilin is an actin binding protein and has been identified as one of the common allergens in pollens and fruits. According to previews studies.
Objectives: The main aim of this study was to development an animal model for characterization of IgE mediated response against melon major allergen (Cuc m 2).
Methods: Recombinant melon profilin was generated by cDNA cloning and expressed in *Escherichia coli* and purified with metal affinity chromatography. Balb/C mice were sensitized by subcutaneousinjection of rCuc m 2 using hydrated $Al(OH)_3$ as the adjuvant. In all cases subcutaneous injection was repeated every 7 days and sera was collected. In order to visualize skin reaction, animals were injected i.v. with Evans blue/PBS solution. Recombinant melon profilin and some cross reactive allergenic sources were injected intradermally and blueing reactions were read after 15 min. Histamine and 0.9% NaCl were considered as the positive and negative control respectively.
Results: The primary data indicated that subcutaneous injection of rCuc m 2 could induce allergenic reaction in Balb/C mice. Mice were sensitized through subcutaneous injection of allergen in combination with alum adjuvant. Sensitized mice were indicated positive skin reaction to recombinant melon profilin and some cross reactive allergenic natural extraxts in compare with control group.
Conclusion: Our data support the value of Balb/C mouse model to study hypersensitivity to melon profiln and could be suitable for the evaluation of therapeutic strategies.

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Purification of natural and recombinant melon profilin using affinity chromatography column

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Background: Profilin is one of actin binding proteins and has been identified as one of the common allergens in pollens and fruits. Different methods have been developed in purifying specific proteins, such as affinity chromatography.
Objectives: Affinity chromatography was chosen to purify profilin. Separation of profilin could be done by the high affinity of the allergen to IgG.
Methods: Recombinant profilin, generated by cDNA cloning, was expressed in *Escherichia coli* and purified with metal affinity chromatography. Purified rCuc m 2 was used to generate specific polyclonal antibody in rabbit. Rabbit IgG anti Cuc m 2 was affinity purified with protein A sepharose column. Purified IgG from previews step was used to develop (set up) an immunoaffinity column for purification of natural melon extract. nCuc m 2 was purified from melon extract, using glycin-HCl 0.2 M pH 2.8 as dilution buffer.
Result: Natural melon profilin was purified to homogenous band from total melon extract by one step immunoaffinity chromatography. Recombinant melon profilin was purified with metal affinity chromatography. Purified proteins were characterized by SDS-PAGE and immunoblot.
Conclusion: The aim of this study was development of purification method for separation and characterization of natural and recombinant melon profilin for *in vivo* and *in vitro* diagnostic tests.

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Coconut allergy and latex sensitisation in pediatric age

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Background: Coconut (*Cocos nucifera*) is an *Arecaceae* family fruit. Allergic reactions to coconut are very rare, being scarcely reported in childhood.
Clinical case: An 8-years-old female child with atopic eczema since her 6 months and asthma and allergic rhinitis since 2 years of age; with no malformation or previous surgery personal history. At 6 years old, the child presented to the emergency department with an episode of generalized urticaria and lips and tongue angioedema 5 min after eating a small portion of fresh coconut. Previously the child ingested food containing coconut vestiges without complaints. One year later, 5 min after eating fresh kiwi she suffered oropharynx pruritus and lips and tongue angioedema which resolved after anti-histaminic therapy. There were previous ingestions of this fruit without symptoms. The child refused to eat banana, pineapple, mango and melon. Skin prick tests were strongly positive to coconut, kiwi and banana commercial extracts and to fresh pineapple, melon and mango. Skin prick tests to inhalant allergens were positive to house dust mites, grass pollen and latex commercial extracts. Latex challenges were negative. Oral challenges with banana, pineapple, mango and melon were refused. Serum total IgE was 1508 IU/mL; serum specific IgE to coconut was 15.10 kU/L, to kiwi – 2.35 kU/L and to latex – 0.94 kU/L. Coconut IgE-immunoblotting was strongly positive, with identification of several bands with molecular weight varied from 15 to 75 kDa. Latex IgE-immunoblotting identified three bands with 34, 36 and 42 kDa. Kiwi IgE-immunoblotting showed an IgE binding protein of about 27 kDa. On the inhibition assays (AlaBLOT® Inhibition Assay Procedure, DPC), IgE binding to latex allergens was completely inhibited by coconut and kiwi extracts. Latex extract completely inhibited kiwi immunoblot, but it inhibited in only 14% the IgE binding to coconut allergens.
Conclusions: We present a rare clinical case of IgE-mediated coconut allergy in paediatric age, associated with other fruit IgE-mediated allergies. The presence of cross-reactive allergens between coconut and latex was demonstrated. The child presents asymptomatic sensitisation to latex, being the primary sensitizing agents the fruits. To the best of our knowledge, there is no previous report of cross-reactivity between coconut and latex.

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Fruits, vegetables and latex sensitisation in a pollen allergic patient

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Background: Fruit and vegetable sensitivity in patients allergic to pollens can be partially explained by the existence of ubiquitous panallergens throughout higher order plants, with subsequent cross reactivity.
Patient and methods: We present the case of a male patient, 21 years old, with a history of severe allergic rhinitis and asthma during the pollen season since he was 9 years old. Five years later, he started to suffer from

Table 1: for abstract 954.

Allergen	SPT (mm)	IgE (kU/L)	Allergen	SPT (mm)	IgE (kU/L)
Histamine	5	–	Kiwi	5	10 (cl 3)
Dactylis glomerata	6	>100 (cl 6)	Strawberry	2	17.7 (cl 4)
Poa pratensis	16	>100 (cl 6)	Peach	8	16.5 (cl 3)
Phleum pratensis	8	68.2 (cl 5)	Banana	6	17.5 (cl 3)
Parietaria officinalis	7	68.2 (cl 5)	Melon	3	–
Artemisia vulgaris	8	4.7 (cl 3)	Apple	6	–
Triticum sativum	14	–	Peanut	5	28 (cl 4)
Corylus anellana	6	–	Hazelnut	5	17.5 (cl 4)
Plantago major	8	–	Carrot	4	25 (cl 4)
Latex	3	17.9 (cl 4)	Celery	6	22.4 (cl 4)
			Onion	5	17.8 (cl 4)

oral allergy syndrome after the ingestion of peach, melon, tomato, onion, strawberry, apple, banana, kiwi and nuts and contact eczema to natural rubber latex material. He always lived in the eastern region of Portugal, where high airborne pollen concentrations predominantly to grass, artemisia, parietaria and *Olea europea* can be found from March to July. Skin prick tests (SPT), serum specific IgE and immunoblotting assays were carried out in order to confirm the sensitisation and identify the molecular weight range of the proteins involved.

Results: He presented a high level of total IgE 2270 kU/L and the results of SPT and specific IgE are shown in the table.

Immunoblotting assay results were as follows: Apple – IgE binding at 13.43 kDa band; Peanut – IgE binding at 29.59 kDa, three bands between 40.73 and 56.97 kDa; Peach: IgE binding at 82.49 kDa; Banana – IgE binding at 13.93 kDa; *Artemisia vulgaris* – IgE binding at 14.70 kDa; Latex – IgE binding at 12.41 kDa; The patient was medicated with inhaled budesonide 400 μg, nasal budesonide, cetirizine and terbutaline as needed. Avoidance of *Rosaceae* and *Cucurbitaceae* fruits, banana, kiwi, tomato and nuts was recommended. Specific immunotherapy to pollens (*Dactylis*, *Phleum* and *Parietaria*) was started in 2001 with clinical improvement. Immunoblotting inhibition assays between banana and latex revealed total inhibition of latex by banana extract and partial inhibition of banana by latex.

Conclusions: The results are compatible with a primary sensitisation to pollen grass, *Parietaria* and *Artemisia*, with clinical cross-reactivity with *Rosaceae* and *Cucurbitaceae*

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Banana allergens and protein distribution in different banana cultivars

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Background: Banana fruit belongs to the *Musaceae* family and is extremely nutritious food, but also well known as an allergenic source, eliciting IgE-mediated reaction in sensitized individuals. Some banana proteins are proven to be allergens (profilin and class I chitinase) while the allergenic potential of other abundant proteins like thaumatin-like protein (TLP), β-1.3-glucanase and banana lectin has not yet been well studied. Evaluation of allergenic potential of individual proteins should indicate relevant allergens for the component-resolved allergy diagnosis.

Methods: Banana extracts from five different bananas were made using the standard procedure and analyzed for protein content by SDS-PAGE, IEF and 2D electrophoresis. After electrophoresis proteins were electrotransfered or printed to the nitrocellulose membrane. They were then analyzed with polyclonal antiserum obtained by immunization of the rabbit with common banana extract, pooled sera from patients with positive skin-prick test to banana extract and polyclonal anti-chitinase antibodies. ELISA inhibition studies for five banana extracts were also performed using rabbit polyclonal antiserum and pooled patient's sera.

Results: Using polyclonal rabbit antiserum it was possible to detect all antigens from the common banana extract and also to compare the pattern of antigenicity between common banana and four banana cultivars analyzed in this study. Banana cultivars contain more or less the same protein profile, but there is a difference in the amount of proteins and isoforms thereof in the extracts. IgE antibodies from pooled patients sera were able to detect not only different isoforms of class I chitinase, but also β-1.3-glucanase. The difference in the amount of TLP was detected by patients' IgE and rabbit polyclonal sera in the banana protein extracts.

Discussion: Rabbit polyclonal antiserum enabled us to analyze the presence and distribution of proteins and their isoforms in different banana cultivars. Results show that apart from the class I chitinase, which is known to be the major banana allergen, both β-1.3-glucanase and TLP can bind IgE antibodies from patients allergic to banana. This result suggests that fruit extracts could be replaced by well defined panel of relevant allergenic isoforms to achieve more reliable diagnostic reagents for food allergy.

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Methods for improving natural fruit extracts

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Background: Allergy to fruit and vegetables is often associated with pollinosis. However, cross-reactivity patterns differ between geographical areas and climates depending on the differences in exposure to inhaled and ingested allergens. In central and northern Europe food allergy to fruits of the Rosaceae family is strongly associated with birch pollinosis because of the existence of allergens with Bet v 1 homology. In contrast, in the Mediterranean population allergic reactions to these fruits are more often related to lipid transfer proteins (LTPs). Bet v 1 analogues are labile and might be reduced by conventional extraction procedures of natural raw material. The ambition with this study was to explore new extraction buffer trying to find the optimal formula for conserving labile Bet v1 analogues during extraction.

Methods: Six fruits, where the ImmunoCAP™ reagent showed less than optimal uptake for Bet v 1 sensitized patient samples, were selected. We produced extracts with a panel of extraction procedures, and coupled extracts from the optimal extract to an Improved ImmunoCAP™. Patient samples from different European regions were analysed with this new reagent

