



Novel low-abundance allergens from mango via combinatorial peptide libraries treatment: A proteomics study

Erik Elvin Gomez Cardona^{a,b}, Karen Heathcote^e, Luis M. Teran^{a,b,*}, Pier Giorgio Righetti^c, Egisto Boschetti^d, Alfonsina D'Amato^{e,f,*}

^a Instituto Nacional de Enfermedades Respiratorias, Universidad Autónoma de México, Calz. Tlalpan 4502, c.P. 14080 Mexico D.F., Mexico

^b Biomedicine in The Post-Genomic Era, Huitzilac, Morelos, Mexico

^c Department of Chemistry, Materials and Chemical Engineering “Giulio Natta”, Politecnico di Milano, Via Mancinelli 7, Milano 20131, Italy

^d Scientific Consultant, Paris, France

^e Proteomics Unit, Quadram Institute Bioscience, Norwich Research Park, NR4 7UA England, United Kingdom

^f Department of Pharmaceutical Sciences, Università degli Studi di Milano, Via L. Mangiagalli 25, 20133 Milano, Italy

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ABSTRACT

Mango allergy is a rare condition, which may cause severe hypersensitivity reactions, such as anaphylaxis, angioedema, asthma and contact dermatitis. By exploiting the combinatorial peptide ligand library (CPLL) technology, mango proteomes have been extracted and the presence of traces of allergens assessed via Western blot analysis two-dimensional maps. Upon reactive spot elution and mass spectrometry analyses, four major mango allergens could be identified for the first time and shown to be in common with three of the five known banana species. These allergens include: Mus a 1, Mus a 2 and Mus a 5. Additional mango allergens detected do not seem to be in common with the banana species. In particular, a pectinesterase and a superoxide dismutase, both widely described as allergens, could be identified in mango extracts. Conversely, plain mango extracts not treated with CPLLs did not exhibit any reactive spots in Western blot analysis.

1. Introduction

Food allergy and anaphylaxis are increasing in the world, and affect 8% of the adult population and 6% of children. Allergen-specific immunotherapy has been developed in recent years and it utilizes increasing doses of allergens to induce tolerance. Several clinical trials, using allergen (e.g. peanut or hazelnut)-specific immunotherapy, related to placebo, have reported that > 50% of subjects responded to the therapy (Feuille & Nowak-Węgrzyn, 2018). However, these therapies have efficacy limitations and adverse effects are associated with them. There is an increasing need to discover new food protein allergens, and more efficient target immunotherapies.

Mango (*Mangifera Indica*) belongs to the family “Anacardiaceae”, such as cashew, pistachio and hog plum, with which it could exhibit allergenic cross reactivity (Noorbakhsh et al., 2011).

Mango allergy is a rare condition but can cause allergic diseases that can be manifested as an immediate hypersensitivity reaction presenting as anaphylaxis, angioedema, erythema, urticaria, wheezing dyspnoea and a late reaction presenting as contact dermatitis and periorbital

edema (Sareen & Shah, 2011).

In this study we have investigated the unknown allergen map of Mango, and its potential cross reactivity with banana pulp (*Musa balbisiana*), using an immune-proteomics approach. These fruits represent “recalcitrant tissues” in that minute amounts of proteins (of the order of 1%) are embedded on a very large matrix of plant-specific material (e.g., polysaccharides for banana, and up to 32% of oil in avocado). Mango contains 20% oil, constituted by more than 9% of triglycerides, high content of carotenoids, ascorbic acid and phytochemicals (Nadeem, Imran & Khaliq 2016; Singh et al., 2004; Gouado, Schweigert, Ejoh, Tchouanguep & Camp, 2007). In order to tackle this problem, we resorted to the use of combinatorial peptide ligand libraries (CPLL), a methodology now well ingrained in the field of proteomics (Righetti, Candiano, Citterio, & Boschetti, 2015).

In two of our previous studies we showed the protein profiling of banana and mango and the discovery of known and potentially unknown allergens of these fruits. For instance, from banana protein extracts the extensive use of CPLL extraction, under certain conditions, allowed the identification of 1131 proteins for the first time, a far cry

* Corresponding authors at: Instituto Nacional de Enfermedades Respiratorias, Universidad Nacional Autónoma de México, Calz. Tlalpan 4502, c.P. 14080 Mexico D.F., Mexico (L.M. Teran). Department of Pharmaceutical Sciences, Università degli Studi di Milano, Via L. Mangiagalli 25, 20133 Milano, Italy (A. D'Amato).

E-mail addresses: lmteran@iner.gob.mx (L.M. Teran), alfonsina.damato@unimi.it (A. D'Amato).

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from the handful of species assessed in previous work (Esteve, D'Amato, Marina, Garcia & Righetti, 2013). Among the large amount of species found, several already known allergens, such as Mus a 1, pectinesterase and superoxide dismutase, as well as potentially new allergens, were detected. The proteome of mango was also explored (Fasoli & Righetti, 2013); this evidenced a proteome composed of 2855 unique gene products, 334 of them being specifically located in the peel.

Mango fruit is a particularly interesting and pertinent material because of its strong allergic threats that are relatively poorly known in terms of protein allergens and identification. Only two major mango allergens are partially known (Paschke, Kinder, Zunker, Weißbecher & Vieluf, 2001; Renner, et al., 2008). Man i 1 and Man i 2. The sequence of the first one has been recently elucidated and the protein expressed for better biochemical knowledge in *E. coli* (Tsai, Wu, Chiang & Wen, 2017). In this study Man i 1, the major allergen, was identified as glyceraldehyde 3-phosphate dehydrogenase, a relatively ubiquitous, known abundant allergen found in other species, which shares 86.2% sequence homology with GAPDH from wheat. Recent investigation confirms that CPLL is effective for the discovery of novel allergens from banana and has added another interesting immuno-reactive protein identified as catalase. According to the authors this deserves to be added to the panoply of official allergens (Nikolic, et al., 2018). This probably simply means that these proteins are pan-allergens likely to be found in many vegetable species.

It is within this context, and also by the fact that mango exhibits powerful allergic reactions, that we describe the results of our investigations on mango pulp protein extract with the aim of finding the presence of new allergenic low-abundance species never described before and comparing the allergens to what is known for banana proteome.

2. Materials and methods

2.1. The global technical approach

Experimentally the discovery of protein allergens from mango pulp was based on a simple principle involving first the enrichment of a protein extract by means of CPLL, followed by a proteomics analysis using two-dimensional electrophoresis (2-DE). The resulting two-dimensional image was submitted to an immunoblot detection, using blood serum of allergic patients and positive spots analysed by conventional mass spectrometry (LC-MS/MS). The first important step was the proper protein enrichment that is possible when the amount of biological sample is large enough to “compress” the dynamic range, allowing the detection of low-abundance allergens. Another important step is the harvesting of enriched proteins, optimized over years of CPLL technology improvement, where a number of precautions are necessary to achieve success, such as the use of performant elution processes. 2-DE and LC-MS/MS are also important, as is the accurate mode of making immunoblots; however, they are more art-dependent approaches than technologically-driven operations. This approach has been inspired by previous detections of novel allergens from other biological extracts using CPLL, such as in cow milk, where a polymorphic alkaline protein, identified as an immunoglobulin, was observed for the first time from the serum of allergic patients (D'Amato et al., 2009). Several other minor protein allergen signals were also observed but not identified. In this work the enriched protein sample was separated by isoelectric focussing. With reference to plant proteins a similar approach has been undertaken for the analysis of *Hevea brasiliensis* lattice (D'Amato et al., 2010) and cypress pollen extract (Shahali et al., 2012) where the enriched protein extract was separated by SDS-polyacrylamide gel electrophoresis and 2-DE, respectively. Supplementary Fig. 1 gives the experimental set-up adopted in the present investigation for allergen discovery from both SDS-PAGE (1D blots) and two-dimensional maps (2-DE blots).

Supplementary data associated with this article can be found, in the

online version, at <https://doi.org/10.1016/j.foodchem.2018.06.113>.

2.2. Chemicals and biologicals

ProteoMiner™ (combinatorial hexapeptide ligand library beads, PM), Laemmli buffer, 40% acrylamide/Bis solution, N,N,N',N'-tetramethylethylenediamine (TEMED), molecular mass standards and electrophoresis apparatus for one-dimensional electrophoresis were from Bio-Rad Laboratories, Hercules CA. β -Mercaptoethanol, dithiothreitol (DTT), ammonium persulphate, 3-[3-cholamidopropyl dimethylammonio]-1-propanosulfonate (CHAPS), acetonitrile (ACN), trifluoroacetic acid (TFA), sodium dodecyl sulphate (SDS), iodoacetamide (IAA), formic acid (FA) and all other chemicals used during the experimental work were current pure analytical grade products and purchased from Sigma-Aldrich, St Louis, MO. C₁₈ resins were purchased from Pierce Chemical Company, Rockford, IL, USA. Complete protease inhibitor cocktail tablets and sequencing grade trypsin were from Roche Diagnostics, (Basel, CH).

2.3. Cplls treatment of mango protein extract

Mango samples (from market) were peeled; the pulp was cut into pieces and homogenised by a beater with zirconium beads. The proteins were extracted from the homogenised tissue, incubated with CPLL beads and the proteins eluted (Fasoli & Righetti, 2013). The different protein samples were separated by either SDS-PAGE or 2-DE.

2.4. SDS-polyacrylamide gel electrophoresis

SDS-PAGE was performed as previously described (Shahali et al., 2012). Briefly, the PBS extract was mixed with 0.04 M Tris-acetate buffer pH 6.8 containing 4% SDS and submitted to an SDS-PAGE separation. Mr markers from 14.4 to 94 kDa were used as references. A part of the gel was silver-stained (Blum, Beier & Gross, 1987) and another part was transferred onto a nitrocellulose sheet for immunoblots.

2.5. 2-DE analysis

The desired volume of non-treated sample and the mixture of all eluates was solubilized in the “2-D sample buffer” (TUC, 40 mM Tris) to a final concentration of 2 mg/ml protein and the disulphide bridge reduction allowed to proceed at room temperature for 60 min by addition of Tris-(2-carboxyethyl)-phosphine hydrochloride at a final concentration of 5 mM. For alkylating the reduced -SH groups, 150 mM De-Streak (bis-(2-hydroxyethyl)disulphide), diluted directly from the stock 8.175 M, was added to the solution, followed by 0.5% carrier ampholytes (diluted directly from the stock, 40% solution) and a trace amount of bromophenol blue. All samples were analysed in duplicate: seven-cm long IPG strips, pH 3–10 I, were rehydrated with 150 μ l of protein solution, for 4 h. Isoelectric focusing (IEF) was carried out with a Protean IEF Cell in a linear voltage gradient from 100 to 1000 V for 5 h, 1000 V for 4 h, followed by an exponential gradient up to 5000 V, for a total of 25 kV/h. For the second dimension, the IPG strips were equilibrated for 25 min in a solution containing 6 M urea, 2% SDS, 20% glycerol, 375 mM Tris-HCl (pH 8.8) under gentle shaking. The IPG strips were then laid on an 8–18% acrylamide gradient SDS-PAGE gel slab with 0.5% agarose in the cathodic buffer (192 mM glycine, 0.1% SDS and Tris-HCl to pH 8.3) (Hamdan & Righetti, 2005). The electrophoretic run was at 5 mA/gel for 1 h, followed by 10 mA/gel for 1 h and 15 mA/gel until the dye front reached the gel bottom. One half of the gels were incubated in a colloidal Coomassie Blue solution and destaining was performed in 7% acetic acid until a clear background was reached, followed by a rinse in pure water. The 2-DE gels were scanned with a Versa-Doc image system, by fixing the acquisition time at 10 s; the relative gel images were captured via PDQuest software. After filtering the gel images for removing the background, spots were automatically

detected, manually edited and then counted. The other half of gels was blotted on a CNBr-activated nitrocellulose (NCa) sheet, performed with nitrocellulose according to Leduc et al. (Leduc, et al., 1999). Nitrocellulose sheets were rehydrated first in PBS and, second, in anodic transfer Tris buffer (20 mM), glycine (150 mM), ethanol 20%, pH 10.5. The transfer of proteins was made by a semi-dry blotting apparatus for 1¼ h at 1 mA/cm² of gel.

2.6. Immunoblot

After SDS-PAGE and 2-DE separations, the proteins were transferred as described above. For 1D screening, each NC was then cut into 2.5 mm-wide strips. Each strip (SDS-PAGE) and sheet (2-DE) was blocked with PBS containing 0.3% Tween 20. One tenth (or one fifth) diluted patient's sera were individually incubated overnight at 20 °C with each strip or the whole blotted NC sheet. The blots were incubated, in sealed bags, with 300 µl (or 60 µl) for 3 ml of final allergic patient serum in PBS and 0.1% Tween. After washing, the NC sheets were incubated with alkaline phosphatase (AP)-conjugated goat anti-human IgE for 2 h at 20 °C, followed by AP substrate 5-bromo-4-chloro-3-indolyl phosphate + nitro blue tetrazolium in 0.1 M Tris-HCl buffer, pH 9.5.

2.7. Protein identification of immunoreactive 2-DE spots by nano LC-MS/MS

2-DE spots were cut and proteins reduced by 10 mM DTT and alkylated by 55 mM iodoacetamide. The gel pieces were shrunk in acetonitrile and dried under vacuum; proteins were digested overnight with bovine trypsin as previously described. The tryptic mixtures were acidified with formic acid up to a final concentration of 1%. The tryptic digests were examined with an Orbitrap Fusion trihybrid mass spectrometer coupled with a nano flow UHPLC system (Thermo Fischer Scientific, USA). The peptides were separated, after being trapped on a C₁₈ pre-column, using a gradient of 3–40% acetonitrile in 0.1% formic acid, over 50 min at flow rate of 300 nl/min, at 40 °C. The peptides were fragmented in the linear ion trap by a data-dependent acquisition method, selecting the 40 most intense ions. All analyses were performed in triplicate. The raw data were analysed by Mascot (version 2.4.1), consulting the Uniprot *Anacardiaceae* (2849 sequences) protein database; the tolerance on parents was 10 ppm and on fragments was 0.60 Da. The variable modifications allowed were oxidation on methionine and carboxyamidomethylation on cysteine as fixed modification. The false discovery rate was below 1%, using a decoy database, and the identified proteins contained at least 2 peptides. The mass spectrometry proteomics data were deposited on the ProteomeXchange Consortium via the PRIDE (Vizcaino et al., 2016) partner repository with the dataset identifier PXD008768 (Username: reviewer63625@ebi.ac.uk, Password: Yd0mZdas).

3. Results

A quite large harvest of data has been extracted from the analysis of enriched protein extracts. Fig. 1 shows an SDS-PAGE profile of protein extracts from mango pulp. Tracks 1 and 3 show the patterns of extracts under reducing and non-reducing buffer conditions, respectively. Tracks 2 and 4 represent the eluates after CPLL capture at pH 7.0 of the same mango pulp here too under reducing and non-reducing buffer conditions, respectively. Finally tracks 5 and 6 show the same eluates from CPLLs but at pH 2.2, again performed under reducing and non-reducing buffer conditions, respectively. It can be appreciated that the eluates from CPLLs show many more zones distributed between 6 and 180 kDa than the control extractions. Moreover, it appears that the capture at pH 2.2 gives eluates with more intensive staining but globally of lower molecular mass, suggesting a better capturing ability than at pH 7.0.

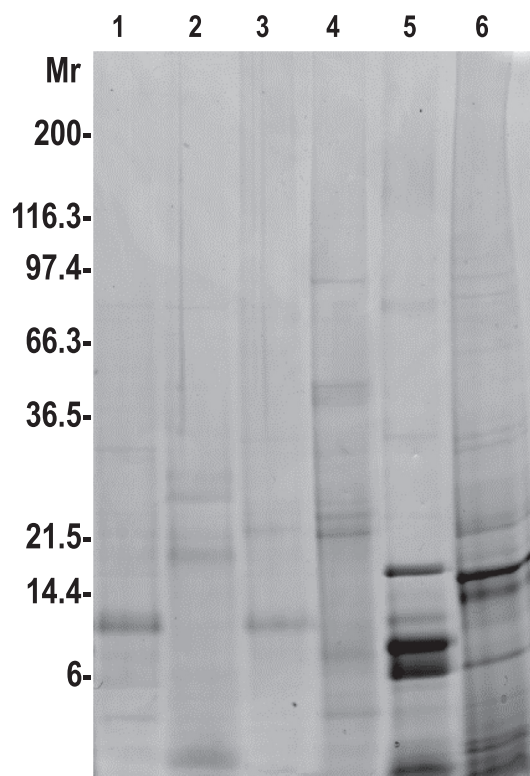


Fig. 1. Coomassie stained SDS-PAGE of mango protein extracts: 1. protein extract in reducing buffer (input), 2. elution after CPLLs incubation at pH 7 with proteins in reducing buffer, 3. protein extract in non-reducing buffer (input), 4. elution after CPLLs incubation at pH 7 with proteins in non-reducing buffer, 5. elution after CPLLs incubation at pH 2.2 with proteins in reducing buffer, 6. elution after CPLLs incubation at pH 2.2 with proteins in non-reducing buffer. Non-reducing buffer: 50 mM Tris-HCl pH7.2, 50 mM NaCl, 2% CHAPS, Protease inhibitors. Reducing buffer: 50 mM Tris-HCl pH7.2, 50 mM NaCl, 2% CHAPS, 1% SDS, 25 mM DTT, Protease inhibitors. Elution buffer: 4% SDS, 20 mM DTT.

Fig. 2 shows the SDS-PAGE profiling of controls (left panel, A) vs. the immune-reactivity profiles obtained from healthy persons and patients sensitive to mango proteins (right panel, B). In panel A, tracks 1 and 3, show the mango patterns after capture with CPLLs at pH 7.0 and 2.2, respectively, whereas track 2 is a control, non-treated mango extract. Panel B shows the respective immune-reactivity profiles of the previous three samples confronted with the blood serum from healthy individuals (C) as compared to patients' sensitive to mango proteins (P). It can be appreciated that, whereas the controls show no immune-reactivity, tracks P1 and P3 produce one (P1) and a few (P3) immune-reactive zones, among which is a very intense one at an apparent Mr value around 20 kDa. The control extract shows a blurred zone at around 2 kDa, a much too low Mr to represent a true protein allergen. Thus, it would appear that, in a control extract, the potential allergenic proteins are present at much too low levels to elicit an immuno-response, which is on the contrary very visible in the two CPLL extracts (it is known that CPLL treatment can enhance the concentration of LAP species up to three orders of magnitude and even higher) (Righetti, et al., 2015). The immuno-detection patterns were obtained by utilizing a total of seven serum samples to create a pool of allergic patients.

In order to obtain a clearer pattern, allowing excision of bands for mass spectrometry analysis and identification of allergens, the experiments were repeated in a 2-DE (two dimensional gel electrophoresis) format, utilizing a pH 3–10 immobilized pH gradient in the first dimension, followed by an SDS-PAGE gel slab in an 8–16% porosity gradient in the second dimension. Fig. 4 shows the immuno-reactivity profile obtained from the CPLL eluate after capture at pH 7.0. Nine spots (circled in red) were excised from control, SDS-PAGE gels (in

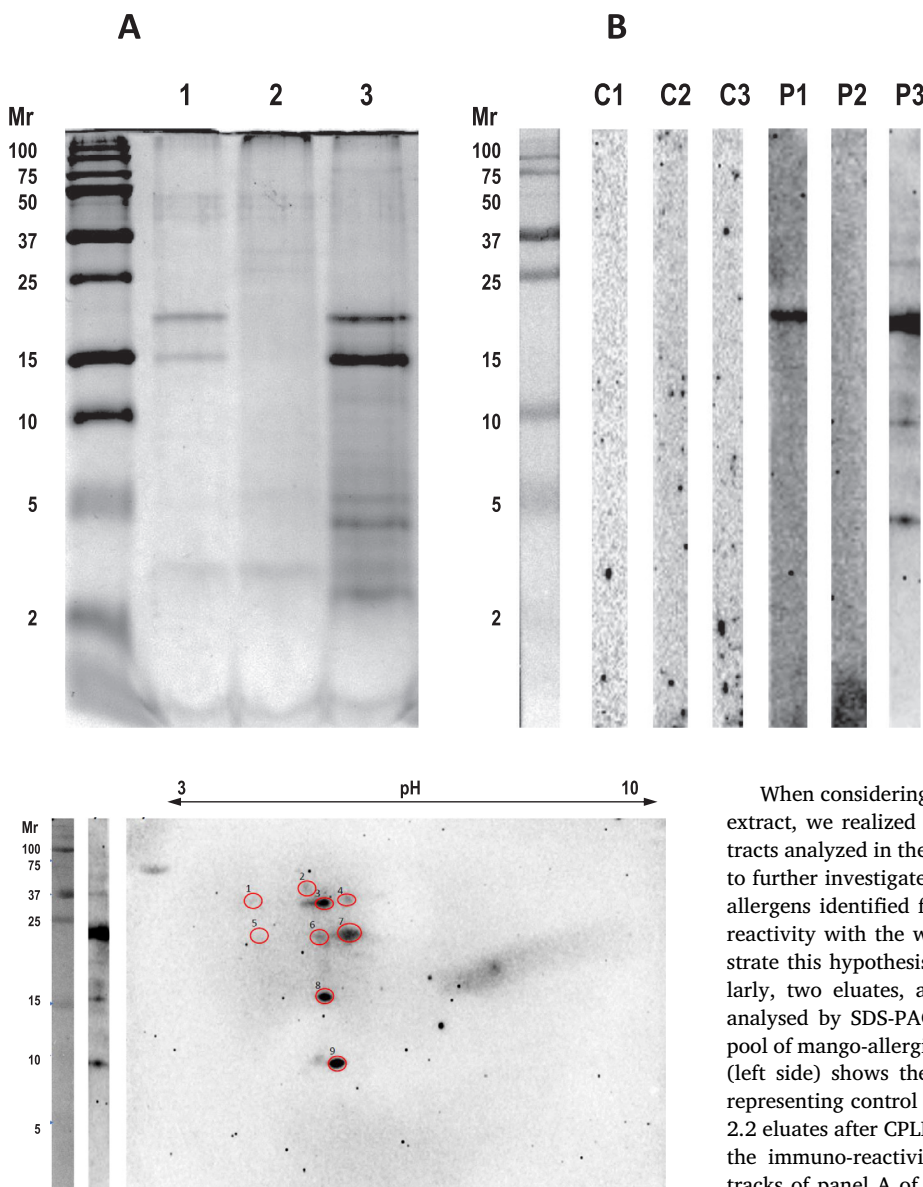


Fig. 2. SDS-PAGE and immuno-reactivity 1D of patients sensitive to mango proteins. A) Protein profile of 15 μ g of mango protein extracts stained with CoomassieG-250. B) Reactivity to mango protein extracts of healthy controls (C) and the serum pool of mango allergic patients (P). In total, 7 serum samples were used to create the mango allergic patient pool and 30 μ g of protein were used in WB experiments. 1: Reducing lysate (mango protein samples 1 and 3); 2: CPLLs lysate (mango protein samples 2 and 4). 3: pH 2.2 lysate (mango protein samples 5 and 6).

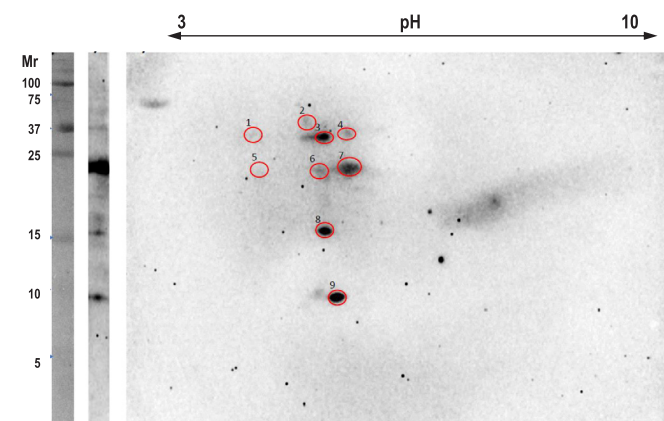


Fig. 3. Immuno-blot from a 2-DE map of mango protein extracts. Spots 1–9, circled in red, were excised from three replica gels, digested with trypsin and analysed by mass spectrometry. This gel refers to the pH 7.0 capture and eluates after CPLLs sample treatment. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

triplicate) stained with Coomassie blue, digested and analysed via MS. Supplementary Table 1 gives the experimental pI and Mr values of the nine reactive spots in the mango extract. It can be appreciated that all these spots represent acidic proteins with pI values ranging from apparent pH 4.06 up to pH 5.48 and Mr values from 9.5 up to 32.1 kDa. Supplementary Fig. 2 shows a similar 2-DE gel slab, stained with Coomassie blue, of the CPLL eluate after capture at pH 2.2. By comparing these spots with the immuno-reactive zones of Fig. 3, we have here excised nine spots (circled in red, in triplicate gel slabs) and submitted them to MS analysis.

Table 2 reports the identification of spot-extracted proteins by mass spectrometry. A fragment of Glyceraldehyde-3-phosphate dehydrogenase (Man i 1) was found, together with eleven other new allergens. It should be noted that many of the allergens detected are present as several spots in the gel slab, as is common in most 2-DE map analyses: such spots likely represent post-translational modifications, including proteolytic cleavages.

When considering this list of immune-reactive proteins from mango extract, we realized that a certain homology existed with banana extracts analyzed in the past few years (Gouado, et al., 2007) and decided to further investigate to see any possible correlation. It was found that allergens identified from the CPLL mango extracts would show cross-reactivity with the well-known banana allergens. To formally demonstrate this hypothesis banana pulp was also treated with CPLLs. Similarly, two eluates, after CPLL capture at pH 7.0 and pH 2.2, were analysed by SDS-PAGE, followed by an immunoblot, using the same pool of mango-allergic patients. The results are shown in Fig. 4. Panel A (left side) shows the profiles of three different extracts (tracks 1–3, representing control or the non-treated protein extract, pH 7.0 and pH 2.2 eluates after CPLL capture, respectively). Panel B (right side) shows the immuno-reactivity profiles obtained from the same 1–3 sample tracks of panel A of three healthy individuals (tracks C1–C3) as compared to three patients allergic to banana proteins (tracks P1–P3). Here too, whereas the healthy persons do not show any reactivity, the three allergic individuals show a pattern of reactive zones essentially identical to those of mango (see Fig. 1). It is concluded, as also shown in Table 1, that the allergens exhibited in mango extracts are the very same as reported for bananas. Table 2 lists all the reactive spots (a total of 42) analysed by mass spectrometry. Three of the five described banana allergens were identified: Mus a 1 (profilin), Mus a 2 (class I chitinase), and Mus a 5 (β -1,3-glucanase). The other potential allergens do not seem to be in common with the banana ones. In particular, we could identify a pectinesterase and a superoxide dismutase, both widely described as allergens too.

4. Discussion

In this study, we have investigated mango proteins using CPLLs in combination with proteomics technology with the aim of discovering low-abundance allergens that cannot be detected using a regular crude sample. Several allergens were identified, including profilin, class I chitinase, β -1,3-glucanase and lipoxygenase. Interestingly, plant allergens can be similar from one source to another as was also found in the present work. In spots number 1 and 3 (Fig. 3), we found an IgE reactivity which, on mass spectrometric analysis, was identified as a β -

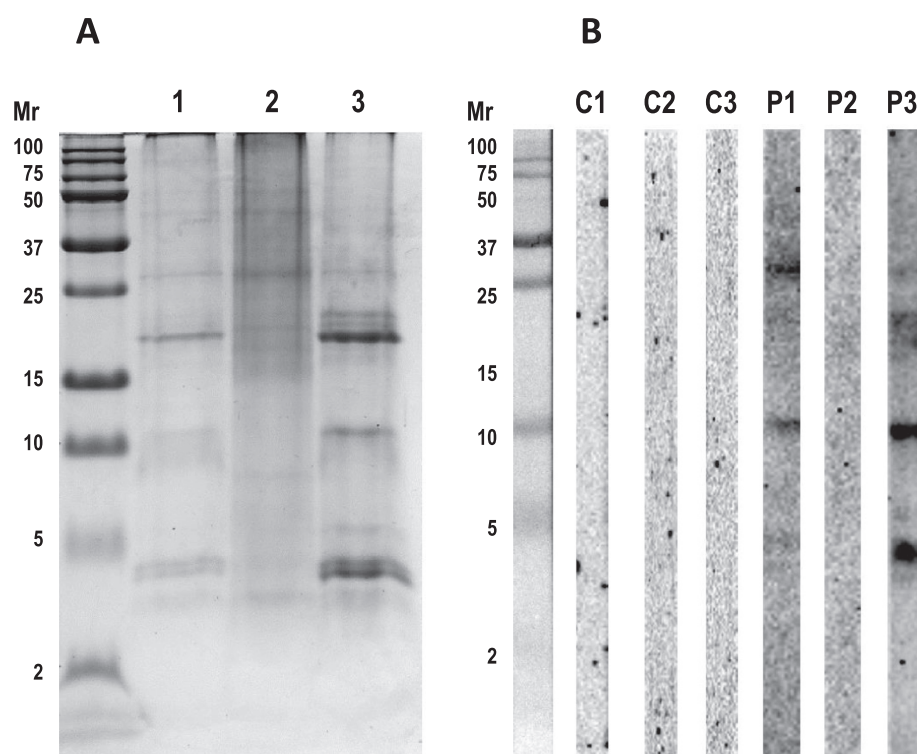


Fig. 4. SDS-PAGE and immuno-reactivity of mango-sensitive patients to banana proteins. A) Protein profile of banana protein extracts (10 µg) stained with Coomassie G-250. B) Reactivity to banana eluates of healthy controls (C1–C3) and the serum pool of mango allergic patients (P1–P3). 30 µg of protein were used in the immunoblot experiments. 1: Reducing lysate (banana protein samples 1 and 3); 2: CPLLs lysate (banana protein samples 2 and 4). 3: pH 2.2 lysate (banana protein samples 5 and 6).

Table 1
Common allergens between mango and banana.

Banana	Mr	Mango
Mus a1 Profilin	15 kDa	Yes
Mus a2 Class 1 chitinase	33 kDa	Yes
Mus a3 Non-specific lipid transfer protein (nsLTP1)	9 kDa	No
Mus a4 Thaumatin-like protein	30 kDa	No
Mus a5 Beta-1-3-glucanase	27 kDa	Yes

1,3-glucanase; this protein sequence exhibits 100% homology to other β -1,3-glucanases: *Mangifera indica* (100%), *Eucalyptus* (72%) and *Populus* (70%). Beta-1,3-glucanase, belongs to the pathogenesis-related (PR-2) which exhibits protective responses against pathogen attack. Their antifungal activity in plants has made these enzymes important tools for strategies to develop resistance in crop plants against fungal pathogens. Beta-1,3-glucanases are also constitutively expressed in several tissues of plants and have been implicated in physiological and developmental processes in organs such as bud, style of flower, anther and seed from uninfected plants, e.g. cell division or pollen development. The crucial roles of these molecules, acting as allergens and triggering the symptoms among allergic patients (Torres et al., 2015), make their characterization relevant for the diagnosis of such hypersensitivity disorder.

Lipoxygenase from peanut seeds is also induced upon fungus infection and seems to be associated with the two well-known major allergen genes Ara h 1 and Ara h 2 (Guo, Abernathy, Zeng & Ozias-Akins, 2015).

Profilins constitute a family of highly conserved proteins that have been identified as allergens in many plants and fruits. They exhibit an extremely conserved structure and are responsible for the cross-reactive nature of IgE antibodies against different allergen sources (Hauser, Roulias, Ferreira & Egger, 2010; Mani et al., 2015). The profilin identified in this study, exhibits homology to hevea profilin 1 (85%), *Arabidopsis thaliana* profilin 1 and soja bean profilin (83%). Profilins are found in all eukaryotic cells and are involved in cell motility via regulation microfilament polymerization upon binding to actin (Hauser,

et al., 2010). In plant cells, profilins play a role in cytokinesis, cytoplasmic streaming, cell elongation as well as growth of pollen tubes and root hairs. In the allergic reaction, IgE cross-reactivity results from the common three-dimensional profilin fold composed of two α -helices and a five stranded anti-parallel β -sheet. Moreover, profilin-specific IgE may cross-react with homologues from virtually every plant source. Thus the identification of a profilin from mango suggests a risk factor for allergic reactions to multiple pollen and food allergen sources as demonstrated by the homology found to other profilins in the BLAST analysis.

Class I chitinases have also been identified as the major pan-allergens in fruits, such as avocado, banana, turnip and chestnut (Volpicella, et al., 2014). The allergenicity of plant chitinases derives from the presence of the additional chitin-binding protein (CtB) which was originally identified in avocado (Posch, et al.1999; O'Riordain et al., 2002). The identification of chitinase 1 in the present study is a novel finding and should be considered as an important mediator of mango allergy.

It is important to highlight that CPLLs were essential for the isolation of allergenic proteins. Indeed, in the absence of CPLL capture, it was not possible to identify any single allergen by proteomic studies, as was the case in many other situations.

Another point worth discussion is the cross-reactivity that has been found between mango and banana allergens. This was quite unexpected, considering that these two fruits belong to completely different species: *Mangifera indica* is a member of the family Anacardiaceae, whereas banana belongs to the genus *Musa*, comprising two wild species—*Musa acuminata* and *Musa balbisiana*. Indeed, profilin (Mus a 1), Class 1 chitinase (Mus a 2) and Beta 1, 3-glucanase (Mus a 5) which were identified in the present study, are also banana proteins). A recent investigation confirms that CPLL is effective for the discovery of novel allergens from banana and has added another interesting immuno-reactive protein identified as catalase that according to the authors deserves to be added to the panoply of official allergens (Nikolic, et al., 2018). This probably means that these proteins are pan-allergens likely to be found in many vegetable species.

In summary, we have identified several low-abundance mango

Table 2
Protein identified in 2DE spots by using Uniprot_Anacardiaceae database.

Spot number	Accession Numbers	Protein Name	Taxonomy	Mascot Score	Mr	N unique peptides	Peptide Sequence
1	Q2ERX5	Beta-1,3-glucanase	<i>Mangifera indica</i>	4882	19,553	13	AGDWVQTNVLAYSADVK DKLQDLTDSAK DNNIGQMR GSNIELILDVVK GSNIELILDVVKDK GSNIELILDVVKDKLQDLTDSAK IYDPDQATLQALR LQDLTDSAK NGNNLPPQAEVVTLYK NVYNAIAAANLQGQIK PGDAEAQYVLPAMR YIAVGNEIR YIAVGNEIRPGDAEAQYVLPAMR
2	Q2ERX5	Beta-1,3-glucanase	<i>Mangifera indica</i>	1190	19,553	13	AGDWVQTNVLAYSADVK DKLQDLTDSAK DNNIGQMR GSNIELILDVVK GSNIELILDVVKDK GSNIELILDVVKDKLQDLTDSAK IYDPDQATLQALR LQDLTDSAK NGNNLPPQAEVVTLYK NVYNAIAAANLQGQIK PGDAEAQYVLPAMR YIAVGNEIR YIAVGNEIRPGDAEAQYVLPAMR
	D3TIC1	Chitinase (Fragment)	<i>Mangifera indica</i>	692	26,367	8	INGALECGGK SNAYCDQSNQYPCVPGK AALWFWMTNVHSMNQGFQATIQK DGFLNAANSFPQFGSGSADESK INGALECGGKQPDKNAR IGYYTDYQK DGFLNAANSFPQFGSGSADESKR DSTLMQLLR EAAESTLTAYK IISIEQKEER NLLSVAYK APLGNPDVVLQGMGK KPNQAENEPSSLK VNTQTSVIVK EIFPTNEDNLFEPFPTQVIQGR LGDIEVR MSDFLGALK NNIPLELLK SKLDPELYGDQDSK
	D3TIB7	14-3-3 (Fragment)	<i>Mangifera indica</i>	143	29,427	4	AGDWVQTNVLAYSADVK YIAVGNEIRPGDAEAQYVLPAMR IYDPDQATLQALR NVYNAIAAANLQGQIK
	Q4QYX3	Beta-galactosidase (Fragment)	<i>Mangifera indica</i>	118	93,465	3	SNAYCDQSNQYPCVPGK INGALECGGKQPDK SNAYCDQSNQYPCVPGK AALWFWMTNVHSMNQGFQATIQK EIAAFFAHVTHETGHLCTEEDK INGALECGGKQPDKNAR DGFLNAANSFPQFGSGSADESKR IGYYTDYQK DGFLNAANSFPQFGSGSADESK
	A0A1U9ZCC3	Lipoxygenase	<i>Mangifera indica</i>	102	96,421	5	YIAVGNEIR AGDWVQTNVLAYSADVK NVYNAIAAANLQGQIK NGNNLPPQAEVVTLYK DNNIGQMR GSNIELILDVVK PGDAEAQYVLPAMR YIAVGNEIRPGDAEAQYVLPAMR DKLQDLTDSAK LQDLTDSAK GSNIELILDVVKDK IYDPDQATLQALR
3	Q2ERX5	Beta-1,3-glucanase	<i>Mangifera indica</i>	206	19,553	4	SNAYCDQSNQYPCVPGK INGALECGGKQPDK SNAYCDQSNQYPCVPGK AALWFWMTNVHSMNQGFQATIQK EIAAFFAHVTHETGHLCTEEDK INGALECGGKQPDKNAR DGFLNAANSFPQFGSGSADESKR IGYYTDYQK DGFLNAANSFPQFGSGSADESK
4	D3TIC1	Chitinase (Fragment)	<i>Mangifera indica</i>	1767	26,367	9	YIAVGNEIR AGDWVQTNVLAYSADVK NVYNAIAAANLQGQIK NGNNLPPQAEVVTLYK DNNIGQMR GSNIELILDVVK PGDAEAQYVLPAMR YIAVGNEIRPGDAEAQYVLPAMR DKLQDLTDSAK LQDLTDSAK GSNIELILDVVKDK IYDPDQATLQALR
	Q2ERX5	Beta-1,3-glucanase	<i>Mangifera indica</i>	1652	19,553	12	

(continued on next page)

Table 2 (continued)

Spot number	Accession Numbers	Protein Name	Taxonomy	Mascot Score	Mr	N unique peptides	Peptide Sequence
5	F1CWB0	Actin 9	<i>Mangifera indica</i>	130	41,929	5	DAYVGDEAQS EITALAPSSMK VAPEEHPVLLTEAPLNPK DLTDALMK IWHHTFYNELR
	Q2ERX5	Beta-1,3-glucanase	<i>Mangifera indica</i>	144	19,553	2	NVYNAIAAANLQGQIK IYDPDQATLQALR
	D3TIC1	Chitinase (Fragment)	<i>Mangifera indica</i>	111	26,367	7	SNAYCDQSNTPQYPCVPGK AALWFWMTNVHSMNQGFQATIQK INGALECGGKQPDKNAR DGFLNAANSFPQFGSGSADESK IGYYTDYCQK DGFLNAANSFPQFGSGSADESK INGALECGGK
6	Q2ERX5	Beta-1,3-glucanase	<i>Mangifera indica</i>	621	19,553	11	YIAVGNEIR GSNIELLDVVK PGDAEAQYVLPAMR DKLQDLTDSAK AGDWVQTNVLAYSADV DNNIGQMR YIAVGNEIRPGDAEAQYVLPAMR LQDLTDSAK GSNIELLDVVKDK NVYNAIAAANLQGQIK IYDPDQATLQALR AALWFWMTNVHSMNQGFQATIQK INGALECGGKQPDKNAR DGFLNAANSFPQFGSGSADESK IGYYTDYCQK DGFLNAANSFPQFGSGSADESK INGALECGGK
	D3TIC1	Chitinase (Fragment)	<i>Mangifera indica</i>	400	26,367	6	EITALAPSSMK VAPEEHPVLLTEAPLNPK DLTDALMK AGFAGDDAPR TTGIVLDSGDGVSHTVPIYEGYALPHA-ILR GYMFTTTAER NYELPDGQVITIGAER DSTLIMQLLR IISIEQKEESR NLLSVAYK IISIEQK SAQDIANAELAPTHPIR VPTVDVSVVDLTVR AASFNIIPSTGA SSIFDAK
	F1CWB0	Actin 9	<i>Mangifera indica</i>	240	41,929	7	DAYVGDEAQS EITALAPSSMK VAPEEHPVLLTEAPLNPK DLTDALMK IWHHTFYNELR
7	D3TIB7	14–3–3 (Fragment)	<i>Mangifera indica</i>	130	29,427	5	DAYVGDEAQS EITALAPSSMK VAPEEHPVLLTEAPLNPK DLTDALMK IWHHTFYNELR
	I3RXT2	Glyceraldehyde-3-phosphate dehydrogenase (Fragment)	<i>Anacardium occidentale</i>	55	21,221	3	DAYVGDEAQS EITALAPSSMK VAPEEHPVLLTEAPLNPK DLTDALMK IWHHTFYNELR
	Q2ERX5	Beta-1,3-glucanase	<i>Mangifera indica</i>	722	19,553	12	YIAVGNEIR GSNIELLDVVK DKLQDLTDSAK LQDLTDSAK GSNIELLDVVKDK NGNNLPPQAEVVTLYK AGDWVQTNVLAYSADV DNNIGQMR NVYNAIAAANLQGQIK IYDPDQATLQALR PGDAEAQYVLPAMR YIAVGNEIRPGDAEAQYVLPAMR SNAYCDQSNTPQYPCVPGK EIAAFFAHVTHETGHLCTEEDK IGYYTDYCQK SNAYCDQSNTPQYPCVPGK INGALECGGKQPDK DGFLNAANSFPQFGSGSADESK INGALECGGKQPDKNAR DGFLNAANSFPQFGSGSADESK EITALAPSSMK VAPEEHPVLLTEAPLNPK DLTDALMK AGFAGDDAPR TTGIVLDSGDGVSHTVPIYEGYALPHA-ILR GYMFTTTAER NYELPDGQVITIGAER
	D3TIC1	Chitinase (Fragment)	<i>Mangifera indica</i>	388	26,367	8	SNAYCDQSNTPQYPCVPGK EIAAFFAHVTHETGHLCTEEDK IGYYTDYCQK SNAYCDQSNTPQYPCVPGK INGALECGGKQPDK DGFLNAANSFPQFGSGSADESK INGALECGGKQPDKNAR DGFLNAANSFPQFGSGSADESK EITALAPSSMK VAPEEHPVLLTEAPLNPK DLTDALMK AGFAGDDAPR TTGIVLDSGDGVSHTVPIYEGYALPHA-ILR GYMFTTTAER NYELPDGQVITIGAER
	F1CWB0	Actin 9	<i>Mangifera indica</i>	164	41,929	7	DAYVGDEAQS EITALAPSSMK VAPEEHPVLLTEAPLNPK DLTDALMK IWHHTFYNELR

(continued on next page)

Table 2 (continued)

Spot number	Accession Numbers	Protein Name	Taxonomy	Mascot Score	Mr	N unique peptides	Peptide Sequence
	M4PZ23	Chalcone-flavonone isomerase family protein	<i>Rhus chinensis</i>	118	26,568	3	DIVTGPFEK LLAESVLESIIIGK SLGLYTDAESK
	S4S7Y5	Enone oxidoreductase	<i>Mangifera indica</i>	117	40,616	4	ATDSPLPTVPGYDVAGVVVK EFKEGDEVYGDINEK QFGSLAEYTAVEEK VVAAALNPVDAK
	O04976	Beta-galactosidase (Fragment)	<i>Mangifera indica</i>	108	74,518	3	LGAQSSLK LTFSQNVK TAVFNTAR
8	Q2ERX5	Beta-1,3-glucanase	<i>Mangifera indica</i>	326	19,553	7	GSNIELILDVVK LQDLTDSAK PGDAEAQYVLPAMR YIAVGNEIRPGDAEAQYVLPAMR AGDWVQTNVLAYSADVK NVYNAIAAANLQGQIK IYDPDQATLQALR
	D3TIC1	Chitinase (Fragment)	<i>Mangifera indica</i>	217	26,367	4	EIAAFFAHVTHETGHLCTEEIDK IGYYTDYCQK DGFLNAANSFPQFGSGSADESKR DGFLNAANSFPQFGSGSADESK
	S4S7Y5	Enone oxidoreductase	<i>Mangifera indica</i>	217	40,616	6	EFKEGDEVYGDINEK QFGSLAEYTAVEEK VVAAALNPVDAK EGDEVYGDINEK SLGTDLAIDYTK ATDSPLPTVPGYDVAGVVVK
9	Q2ERX5	Beta-1,3-glucanase	<i>Mangifera indica</i>	575	19,553	12	GSNIELILDVVK PGDAEAQYVLPAMR DKLQDLTDSAK GSNIELILDVVKDK NGNNLPPQAEVVTLYK LQDLTDSAK YIAVGNEIRPGDAEAQYVLPAMR AGDWVQTNVLAYSADVK NVYNAIAAANLQGQIK IYDPDQATLQALR DNNIGQMR GSNIELILDVVKDKLQDLTDSAK QFGSLAEYTAVEEK VVAAALNPVDAK SLGTDLAIDYTK ATDSPLPTVPGYDVAGVVVK
	S4S7Y5	Enone oxidoreductase	<i>Mangifera indica</i>	236	40,616	4	DFDEPGSLAPTGLHLGGTK LNPEETAINK LNPEETAINKDFDEPGSLAPTGLHLG- GTK KGPGGVTVK YMVIQGEPAVIR
	Q2XPB2	Profilin	<i>Mangifera indica</i>	164	14,177	5	IGYYTDYCQK DGFLNAANSFPQFGSGSADESKR DGFLNAANSFPQFGSGSADESK IISIEQKEESR LAEQAERYEEMVEFMEK YEEMVEFMEK
	D3TIC1	Chitinase (Fragment)	<i>Mangifera indica</i>	103	26,367	3	
	D3TIB7	14–3-3 (Fragment)	<i>Mangifera indica</i>	161	29,427	3	

allergens, including profilin, class I chitinase, and β -1,3-glucanase. Interestingly three of the proteins identified in mango have previously been identified in banana. These findings are of great importance as Mango can be the cause of allergic diseases that can be of dramatic issue.

5. Conclusions

Although mango is a fruit capable of inducing drastic allergic reactions, its allergen map had not been fully investigated. Obstacles to the detection of allergens from fruits in general are the very low concentration of protein allergens, necessitating specific manipulations except for high-abundance protein allergens that do not necessitate preliminary concentrations operations. To simultaneously concentrate

on all these low-abundance proteins CPLL is here used a well-ingrained tool for the reduction of high abundance proteins that concomitantly act for the concentration of low and very-low-abundance species. With the resulting protein solution, of narrower dynamic range, it has been possible to focus on allergens that are generally undetectable by current means. The fact that they are undetectable might provoke strong allergy reactions. For the first time they have been identified. Further studies demonstrated that they were common to other allergic proteins and among them are the ones responsible for banana allergic reactions. This unexpected finding opens an avenue for investigating the reasons why these proteins are common for allergic reactions. Allergy, being considered as a defence reaction, it could be of interest to see if there is a cause-effect involvement associated with the responses against pathogen attack, such as antifungal activity.

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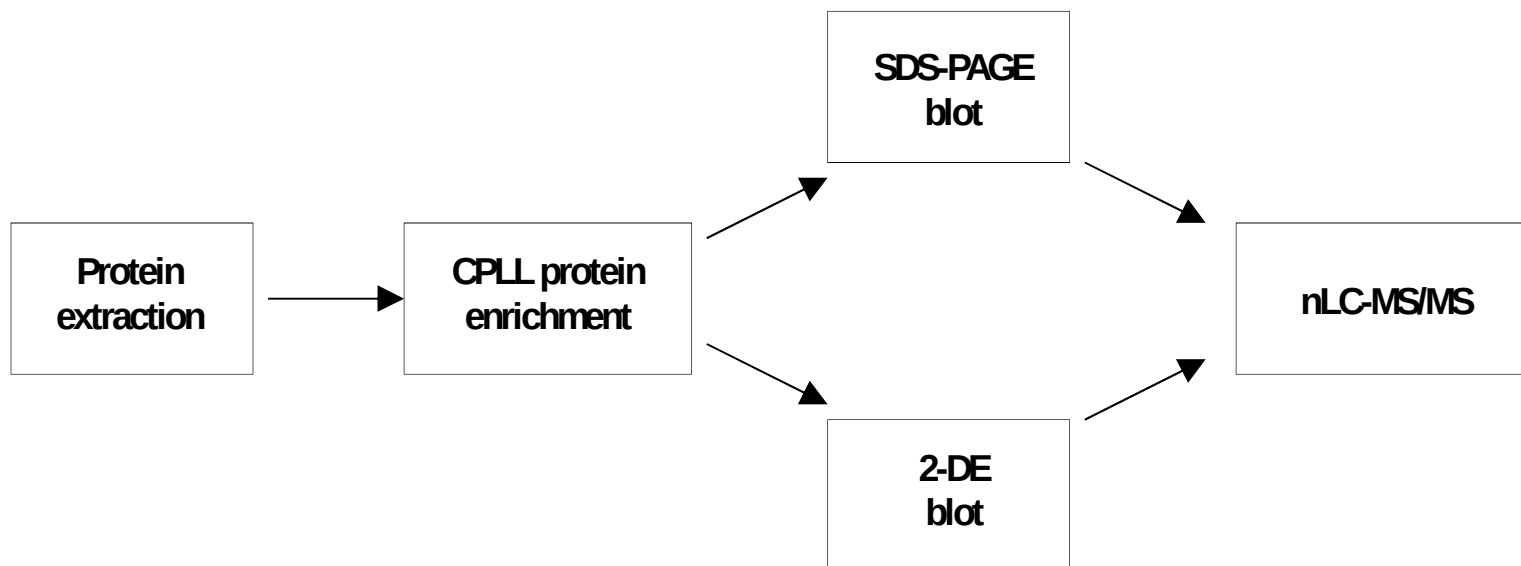


Fig Suppl. 1: Mango allergens, Gomez Cardona et al. 2018

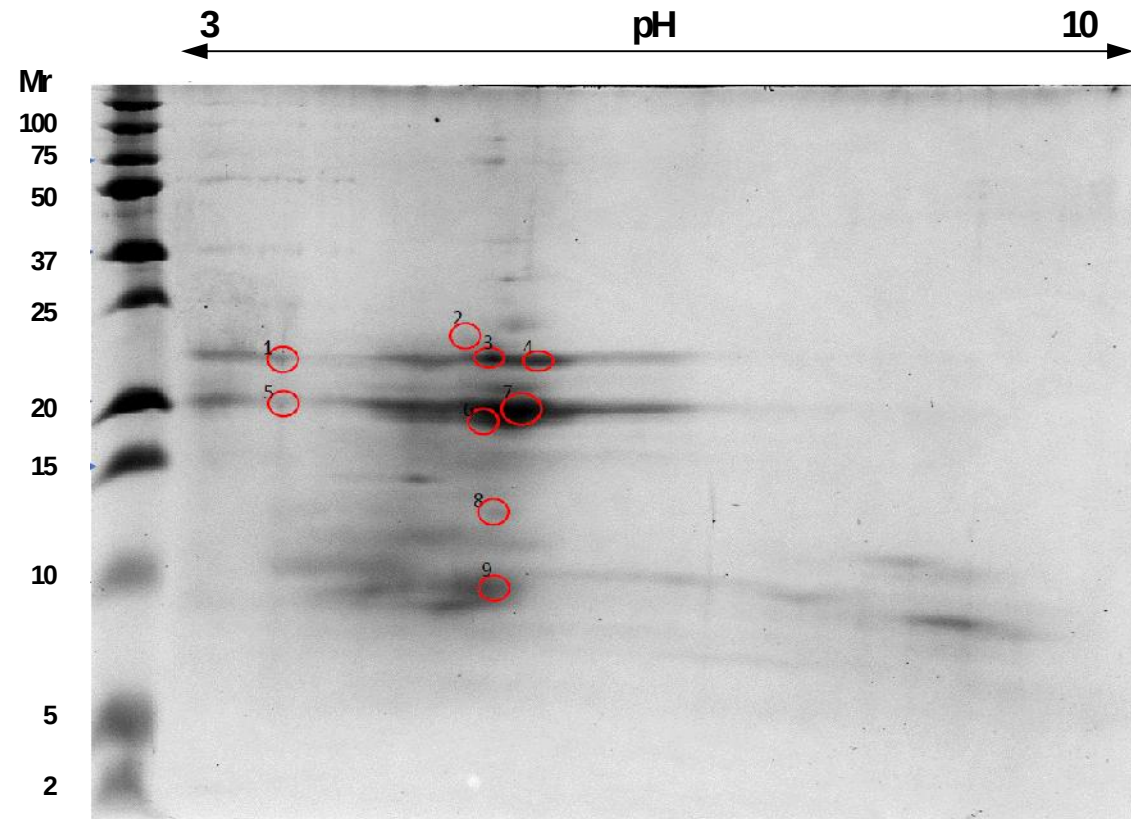


Fig Suppl.2: Mango allergens, Gomez Cardona et al. 2018