
Identification and quantification of tomato allergens: in vitro characterization of six different varieties

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Background: The prevalence of sensitization to tomato has been estimated in 6.5% in a Mediterranean population.

Objectives: To characterize the allergenic profile of 6 commonly ingested varieties of tomato and to identify and quantify the most representative allergens.

Methods: Rama, Rambo, Canario, Kumato, Pera and Raf tomato varieties were carefully peeled and extracts from peel and pulp prepared. The extracts were characterized by SDS-PAGE and immunoblot. Lyc e 3 and osmotin-like protein (OLP) were identified by LC MS/MS and PG2A by immunoblot with a specific polyclonal antibody. Protein content and allergen concentration (Lyc e 3 and PG2A) were measured by scanning densitometry after identification. One hundred and ninety-one patients were enrolled in the study. Specific IgE to all tomato extracts was evaluated by direct ELISA.

Results: Eighty (41.7%) individuals reported symptoms after contact/ingestion with tomato, most of them oral symptoms. Protein profile and allergen quantification of the 6 varieties showed differences in the allergen composition. Lyc e 3 was visualized in all extracts, being the most abundant in all cases. PG2A and OLP were identified in all peel extracts and in some pulp extracts. Higher specific IgE values were obtained for the Kumato variety. Immunoblot showed the existence of several bands with IgE-binding capacity, especially at 12 and 45-50 kDa.

Conclusions: The study demonstrates differences in the antigenic and allergenic profiles of the analyzed varieties and variations in their allergenic composition, affecting the diagnostic capacity. LTP and PG2A are important tomato allergens in our population but other IgE-binding capacity proteins are also involved.

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INTRODUCTION

Tomato (*Lycopersicon esculentum*) is one of the most widely consumed foods for its edible fruits. The easy adaptation of the tomato plant to different climatic conditions has allowed their cultivation worldwide. Hundreds of available tomato varieties have been described for human consumption and the cultivars are classified into several categories, based mainly on shape and size.¹

Although the real prevalence of tomato allergy remains still unknown, the prevalence of sensitization can be consid-

ered as important, especially in areas where this fruit is frequently consumed. Larramendi *et al*, estimated that the prevalence of tomato sensitization among subjects attending Allergy Clinics in the Mediterranean Coast of Spain, was approximately 6.5%, though more than 80% of the individuals tolerated the consumption.² In a survey of the general population, about 1.3% of subjects from England reported symptoms upon tomato ingestion,³ whereas worldwide the self-reported estimation of allergy to tomato is about 0.3%.⁴ In allergic patients, the most frequent symptoms after ingestion or contact with tomato are local symptoms, mainly oral allergy syndrome (OAS), characterized by IgE mediated symptoms that occur mainly at the mucosa of the lips, tongue, and pharynx after ingestion, or cutaneous symptoms such as pruritus, erythema or hives after contact or ingestion. Systemic reactions, including anaphylaxis, are not very frequent.^{5,6}

Different studies have demonstrated that the allergenicity of fruits may be affected by biological and physicochemical parameters. The selected variety,^{7,8,9} the protein/allergen content,¹⁰ specially differences between peel and pulp¹¹ or the ripening stage¹² of the fruits are critical points in their allergenicity. Beside, other factors, such as the extraction process (temperature, pH, time, etc) or the solvent,¹³ are also crucial in the allergenicity. Furthermore, differences in allergenicity

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between different varieties could have clinical relevance, if “natural hypoallergenic” varieties are found.¹⁴

Several tomato allergens have been characterized to date, including Lyc e 1 (profilin of 14 kDa)^{15,16} a minor tomato allergen, Lyc e 2 (β -fructofuranosidase) of 50 kDa¹⁷ and Lyc e 3 (Lipid transfer protein –LTP–) of 6 kDa¹⁸ (Molecular weights (MW) obtained from allergen.org). Other IgE-binding capacity proteins have been described such as polygalacturonase2A (PG2A) with a MW of 46 kDa,¹⁹ glucanase,²⁰ peroxidase,²¹ pectinesterases,¹⁸ superoxidedismutase,¹⁸ chitinase,²² patatin²³ and a heat-labile and-pepsin resistant protein (9 kDa).²⁴

The aims of this study were to analyze the antigenic and allergenic profiles of 6 different tomato varieties, to determine the allergen content and to study the eventual allergenic differences among these varieties.

METHODS

Extract Manufacturing

Six different tomato varieties, including, Rama, Rambo, Canario, Kumato, Pera, and Raf were purchased the same day at a local market, in a similar ripening stage and optimal conditions for human consumption.

Tomatoes were washed and carefully peeled; peels and pulps from each variety were individually homogenized, extracted, dialyzed, sterile-filtered, frozen and freeze-dried.²

Patient Population

Patients residing in the Mediterranean coast of Spain were enrolled for the study following one or both of the following criteria: Self-reporting symptoms with tomato (ingestion or contact) and/or positive skin tests with peel or pulp Canario tomato extract in the current or previous visits. Asymptomatic patients were identified in the course of an epidemiological study of tomato sensitization.² Canario tomato peel and pulp were routinely tested for nearly two years in all participating centers. All individuals fulfilling the criteria were skin prick tested with peel and pulp extracts of the six tomato varieties. Subjects with negative skin tests to all the extracts were discarded for further studies. Patients were recruited in the following centers, after the approval of the Ethic Committee: Hospital Clinic (Barcelona), Hospital General Básico de la Defensa (Valencia), Hospital Marina Baixa (Villajoyosa), Hospital Vega Baja (Orihuela), Hospital Santa María del Rosell (Cartagena) and Hospital de la Arrixaca (Murcia). Serum samples were collected from 142 patients (mean age 27.8 ± 10.0 , range 9-57; males 46.2%, females 53.8%) after oral consent, frozen and stored at -20°C (Figure 1).

Skin Prick Tests

All patients were skin prick tested with different tomato extracts (peel and pulp -10 mg/ml-) and with a battery of the most common aeroallergens present in our area, including

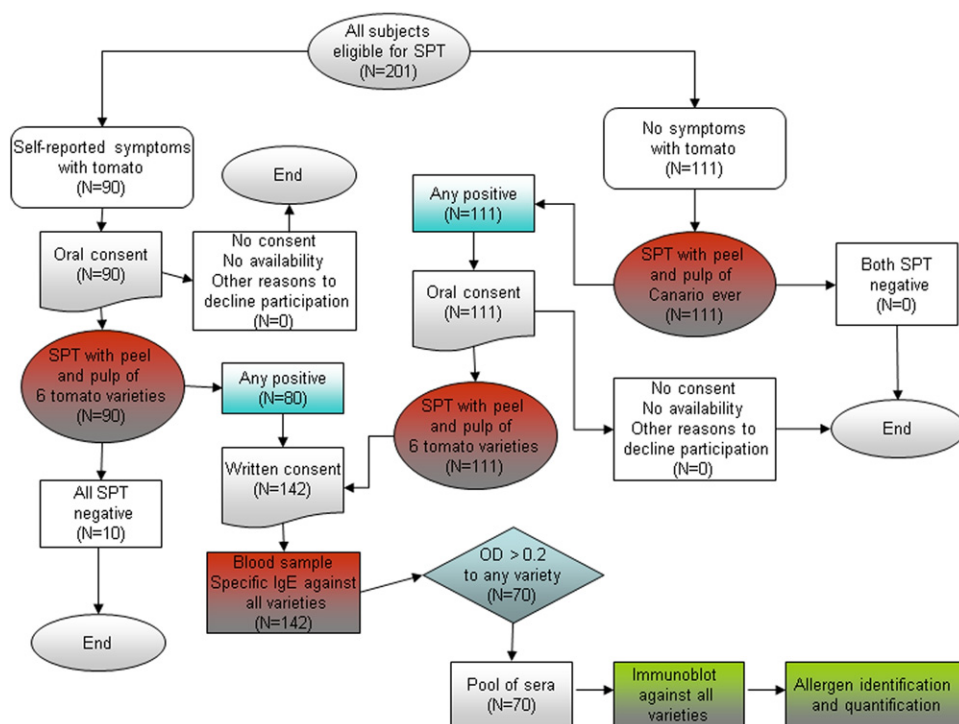


Figure 1. Flow-chart of the study.

standardized extracts of mites, pollens, moulds and animal dander's. Latex and some vegetable foods were also tested.

Specific IgE

Specific IgE to peel and pulp tomato of each variety was determined by direct ELISA in the available sera. Briefly, microtiter plates (Immulon IV; Dynex Technologies, Chantilly, VA) were coated (20 µg freeze-dried extract/ml). Each serum sample was diluted 1:2 vol/vol in PBS (0.01M) and incubated (100 µL) for 2 hours in the coated wells. The plates were then washed and incubated for 2 hours with α-human IgE (Ingenasa, Madrid, Spain) conjugated with peroxidase. After 5 washes, the reaction was developed for 30 minutes and stopped with sulfuric acid (1N). Three individual serum samples from non-allergic patients were used as negative controls. Optical densities (OD) higher than 0.2 were considered positive. A serum pool was prepared with serum aliquots from 70 individual sera with a specific IgE levels higher than 0.2 O.D. in any of the analyzed varieties.

Protein Profile

The protein profile of the extracts was evaluated using sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE). A total of 150 µg of freeze-dried from each extract was loaded. After electrophoresis, gels were fixed and silver stained (BioRad, Hercules, CA). The protein content was measured using the Lowry-Biuret method (Sigma-Aldrich, St. Louis, MO). Glycoprotein content was detected using a glycoprotein detection kit (Sigma-Aldrich).

Allergen Identification

Two bands at 12 kDa and 27 kDa, ran in a SDS-PAGE gel, were cut from the gel (coomassie stained), digested with trypsin and individually identified by LC-MS/MS (Liquid Chromatography-Mass spectrometry/mass spectrometry). The allergen PG2A was identified in extracts by western blot using a polyclonal anti-mouse anti-PG2A (internally produced). Reaction was developed using anti-mouse IgG (Nordic, Tilburg, The Netherlands) 1:2,000 as a secondary antibody.

Allergen Measurement

Bands previously identified were quantified in each extract using scanning densitometry (Scanner Sharp JX-330) and analyzed using the Image Master 1-D Elite v 4.00 (Pharmacia Biotech, Uppsala, Sweden) respect to the total protein content of the extract.²⁵

Allergenic Profile

Allergenic profile of the pool of sera was determined by Immunoblot using peel and pulp tomato extracts in solid phase.

After electrophoresis, gels were electrotransferred onto Immulon-P membrane (Millipore, Bedford, MA). Membranes were incubated overnight with the pool of sera (diluted 1:2; PBS Tween 0.1%). After washing, the membrane was incubated with monoclonal α-human IgE-PO (Ingenasa).

RESULTS

Patient Population

Two hundred and one individuals were skin prick tested with peel and pulp extracts of the six tomato varieties. Ten individuals (all reporting symptoms with tomato) were skin prick test negative to all extracts and were not further studied.

A total of 191 patients (mean age 27.8 ± 10.4 , range 4-57; males 49.5%, females 50.5%), were skin prick test positive to at least one of the extracts. Eighty (41.7%) individuals reported symptoms after ingestion or contact with tomato. Fifty three of these individuals (66.3%) experienced OAS after tomato consumption, 33 (41.3%) digestive symptoms and 15 (18.8%) contact symptoms. No patient reported anaphylaxis. One hundred and eighty-two (95.3%) individuals were sensitized to aeroallergens: 174 (91.1%) to pollens, 101 (52.9%) to mites and 9 patients (4.7%) were not sensitized to any of the inhalant allergens tested. Regarding food sensitization, 148 individuals (77.5%) were sensitized to any vegetable food. Eight of the 9 non-sensitized aeroallergens individuals showed food sensitization, and only 1 individual was exclusively sensitized to tomato. Serum was available from 142 (74.3%) of these subjects. No statistically significant differences in symptoms and wheal sizes were observed between serum sample donors ($n=142$) and the rest of the population ($n=49$). Wheal sizes were larger ($P < .05$) in symptomatic individuals, for all the varieties except Rama peel, and Kumato, Rambo and Raf pulp extracts.

Skin Prick Test

Canario peel extract detected the highest percentage of sensitized patients (94.8%). Only 10 patients had negative skin prick test with this extract.

Kumato, Rambo and Canario peel extracts induced the largest wheal sizes and Raf, Rama and Rambo pulp extract induced the lowest. Wheal sizes induced by all extracts are shown in Figure 2A. Significant differences were obtained (Kruskall-Wallis One Way Analysis of Variance on Ranks) after comparing all peel and pulp extracts ($P < .05$), except peel Pera with pulp Kumato. The comparison between peel extracts showed significant differences between peel of Rambo, Kumato and Canario with Pera (Mann-Whitney Rank Sum Test) ($P < .05$). When pulp extracts were compared, only Kumato pulp extracts showed significant differences ($P < .05$) with Rambo, Rama, Canario and Raf. No significant differences were obtained between other pulp varieties (Table 1).

Specific IgE

Seventy individuals (49.3%) had positive specific IgE (≥ 0.2) to at least one of the tomato varieties. The highest OD values were obtained with the Kumato peel variety extract (Figure 2B). Kumato peel extract recognized the highest percentage of individuals ($n=62$; 88.6%) and Rambo pulp extract the lowest, only recognizing 13 individuals (18.6%). Differences

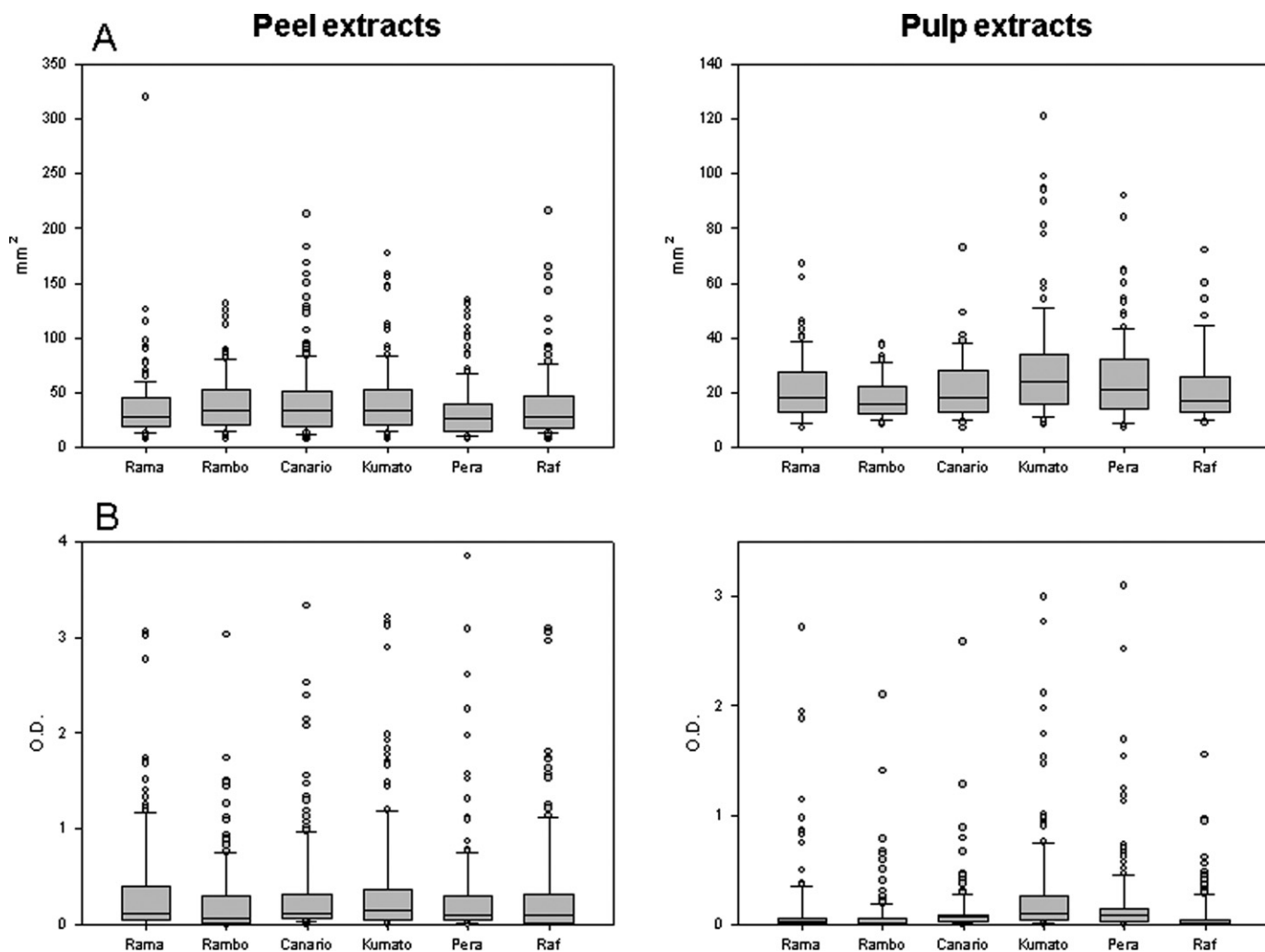


Figure 2. A, Wheal sizes and B, specific IgE obtained with different extracts in the patient population.

were obtained among most peel extracts compared with other peels and pulps and among several pulp extracts (Table 1).

Allergen Identification

The 12 kDa band was sequenced by mass spectrometry and corresponded to an LTP (Lyc e 3) (MW calculated by MS sequencing) according to the homology in the aminoacid composition.²⁶ Six different peptide sequences were identified in the 27 kDa band, corresponding to an osmotin-like protein (OLP) (Table 2). Bands corresponding to Lyc e 3, OLP and PG2A (identified by specific anti-PG2A) are marked in Figure 3.

Protein Profile

SDS-PAGE of the peel and pulp tomato varieties are represented in Figure 3. Several bands were visualized in a MW range between 6 and 90 kDa. Bands at 12 and 45 kDa were identified as Lyc e 3 and PG2A respectively. Lyc e 3 was the

most prominent band in all extracts though was more represented in peel than in pulp extracts. OLP was detected in all extracts. PG2A was not detected in pulp of Rama, Rambo, Canario and Raf varieties. Glycoproteins were detected in peel and pulp extracts with a MW of 16, 25, 30 and 45 kDa approximately (data not shown).

Allergen Quantification

Total protein content and Lyc e 3 and PG2A content are represented in Figure 4. The mean value of protein content was $94.36 \pm 14.04 \mu\text{g}$ of protein/mg freeze-dried material in peel extracts and varied from $114 \mu\text{g}$ of proteins/mg of freeze-dried material in Pera variety to $75 \mu\text{g}$ in Rambo variety. For pulp extracts the mean value was $51.5 \pm 14.8 \mu\text{g}$ of protein/mg freeze-dried material, varying between $69 \mu\text{g/mg}$ of freeze-dried material in Pera and $36 \mu\text{g/mg}$ of freeze-dried material in Rambo. Total protein content was 2 times higher in peel than in pulp extracts.

Table 1. Statistical Results Obtained After Comparing Individually Each Extract With All the Others (Mann-Whitney Rank Sum Test)

A: Skin prick test													
	Peel Rama	Pulp Rama	Peel Rambo	Pulp Rambo	Peel Canario	Pulp Canario	Peel Kumato	Pulp Kumato	Peel Pera	Pulp Pera	Peel Raft	Pulp Raft	
		*	N.S.	*	N.S.	*	N.S.	*	N.S.	*	N.S.	*	Peel Rama
			*	N.S.	*	N.S.	*	*	*	N.S.	*	N.S.	Pulp Rama
				*	N.S.	*	N.S.	*	*	*	N.S.	*	Peel Rambo
Peel Rama					*	N.S.	*	*	*	N.S.	*	N.S.	Pulp Rambo
Pulp Rama	*					*	N.S.	*	*	*	N.S.	*	Peel Canario
Peel Rambo	*	*					*	*	*	N.S.	*	N.S.	Pulp Canario
Pulp Rambo	*	N.S.	*					*	*	*	N.S.	*	Peel Kumato
Peel Canario	N.S.	*	*	*					N.S.	N.S.	*	*	Pulp Kumato
Pulp Canario		*	N.S.	*	*					*	N.S.	*	Peel Pera
Peel Kumato	N.S.	*	*	*	N.S.	*					*	N.S.	Pulp Pera
Pulp Kumato	N.S.	*	*	*	N.S.	*	N.S.					*	Peel Raft
Peel Pera	N.S.	*	*	*	N.S.	*	N.S.	N.S.					Pulp Raft
Pulp Pera	*	*	N.S.	*	*	*	*	*	*				
Peel Raft	N.S.	*	N.S.	*	*	N.S.	*	N.S.	N.S.	N.S.			
Pulp Raft	*	*	*	*	*	*	*	*	*	*	*		
	Peel Rama	Pulp Rama	Peel Rambo	Pulp Rambo	Peel Canario	Pulp Canario	Peel Kumato	Pulp Kumato	Peel Pera	Pulp Pera	Peel Raft	Pulp Raft	
B: IgE													

A, wheal sizes; B, specific IgE; N.S, Non-statistically significant differences.

* Statistically significant differences ($P < .05$) between extracts. Statistical results of each pair of extracts can be observed in the intersected grids.

Table 2. Peptide Sequences Obtained After Trypsin Digestion and Identification by LC-MS/MS: Peptide Sequences, Position, and Accession Number to Genbank

Peptide sequence	Position	Match to	
		Name	Genbank
SAASSFTGLNLGK	76–88	Lipid transfer protein	CAJ19705
AAALPNTCSVNIPYK	89–103	Lipid transfer protein	CAJ19705
GPLGGCCGVK	45–55	Lipid transfer protein 2	P93224
AAGLPGVCGVNIPYK	88–103	Lipid transfer protein 2	P93224
ISPSTDCSTVQ	104–114	Lipid transfer protein 2	P93224
NNCPYTVWAASPIGGGR	28–45	Osmotin-like protein	P50701
GQTWWINAPR	50–59	Osmotin-like protein	P50701
TNCNFDGAGR	70–79	Osmotin-like protein	P50701
CHAIHCTANINGECPSLR	141–159	Osmotin-like protein	P50701
VPGGCENNPTTFGGQQYCCTQGPGPTDLSR	160–190	Osmotin-like protein	P50701
CPDAYSYQDDPTSTFTCPSGSTNYR	196–221	Osmotin-like protein	P50701

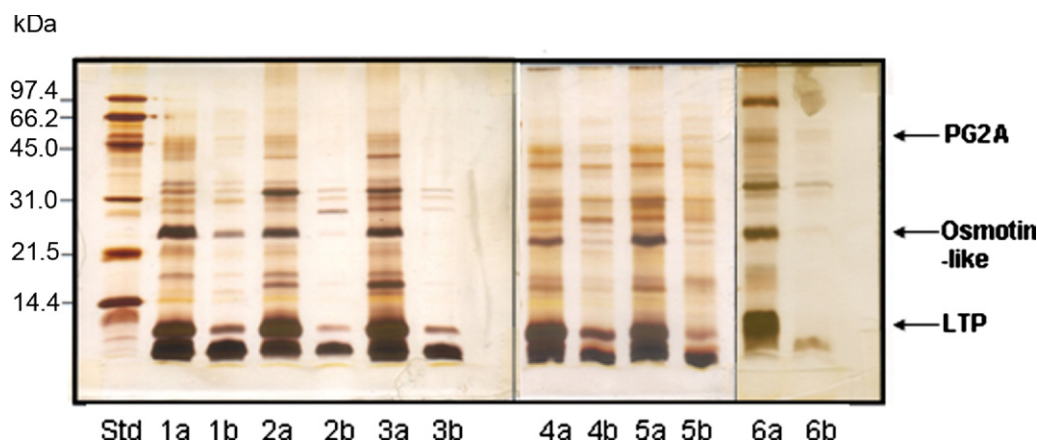


Figure 3. Antigenic profile of the different extracts. Standard Low Molecular Weight Standard 1a, Rama peel; 1b, Rama pulp; 2a, Rambo peel; 2b, Rambo pulp; 3a, Canario peel; 3b, Canario pulp; 4a, Kumato peel; 4b, Kumato pulp; 5a, Pera peel; 5b, Pera pulp; 6a, Raf peel; 6b, Raf pulp.

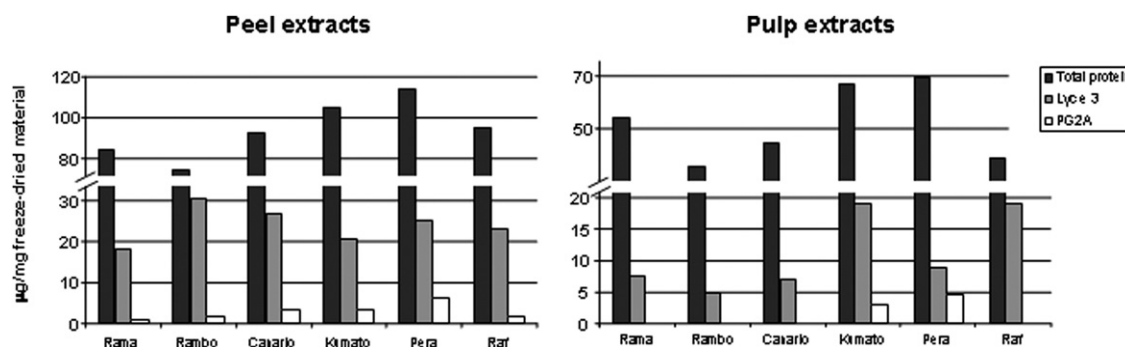


Figure 4. Total protein content and allergen content (Lyc e 3 and PG2A) of peel and pulp extracts.

Lyc e 3 was the most abundant allergen in all cases. Rambo (30.4 $\mu\text{g}/\text{mg}$) and Canario (26.6 $\mu\text{g}/\text{mg}$) were the varieties with the highest concentrations. PG2A was detected in all peel extracts. Peel Pera (6.2 $\mu\text{g}/\text{mg}$) was the variety with the highest concentration. In general, PG2A was not detected in pulp extracts, however, Kumato (3 $\mu\text{g}/\text{mg}$) and Pera (4.5 $\mu\text{g}/\text{mg}$) pulp extracts had a concentration greater than peel extracts such as Rama (1 $\mu\text{g}/\text{mg}$), Rambo (1.4 $\mu\text{g}/\text{mg}$) or Raf (1.7 $\mu\text{g}/\text{mg}$).

Allergenic Profile

The pool of sera recognized bands of the different varieties in a MW range of 12 and 95 kDa. Most clearly visualized bands in peel and pulp extracts were Lyc e 3 (12 KDa) and three bands with a MW of 45–50 kDa, one of them corresponding to PG2A. A band of 16 kDa was recognized only in Rambo and Kumato peel extracts, and a band of 32 kDa was also recognized in

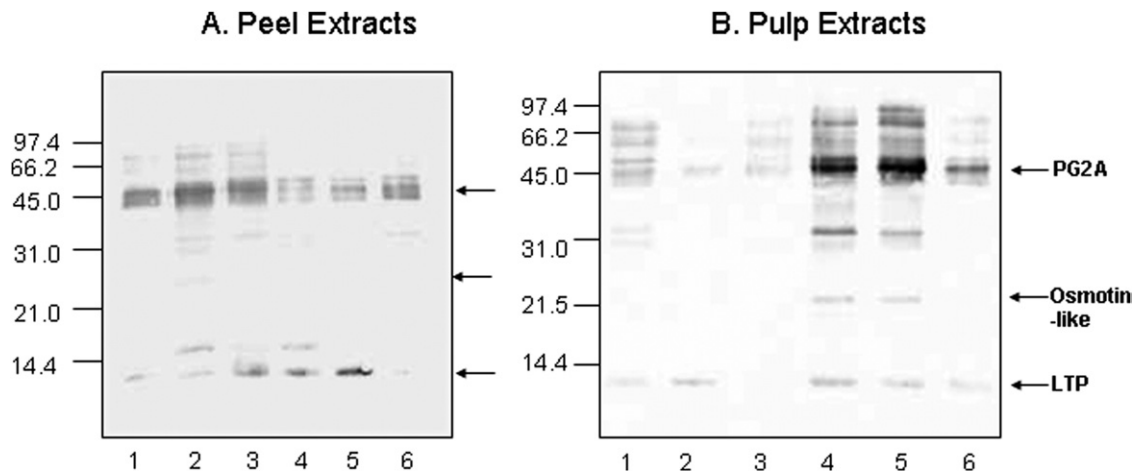


Figure 5. Immunoblot of different tomato varieties with the serum pool. A, Peel extracts. B, Pulp extracts. Lane 1, Rama; Lane 2, Rambo; Lane 3, Canario; Lane 4, Kumato; Lane 5, Pera; Lane 6, Raf.

Rambo, Canario and Raf peel varieties and in Rama, Kumato and Pera pulp extracts. The OLP was recognized in Kumato and Pera pulp extracts and in Rambo peel extract (Figure 5).

DISCUSSION

Tomato sensitization can be explained as a primary sensitization or as a consequence of cross-reactivity with pollens and other fruits.²⁷ However, although tomatoes are frequently consumed worldwide, not many studies have analyzed their allergen composition and the allergenicity of different edible varieties. Previous studies have demonstrated the variability in the allergen concentration and in the allergenic activity of fruit,⁸ vegetables,²⁸ and pollen extracts,²⁹ depending on the varieties, climatic conditions,^{30,31} ripening stage,^{32,33} or post-harvesting (manufacturing and packaging) processes.^{34,35}

Tomatoes of six common varieties were purchased at the same market in a similar ripening stage, reproducing usual tomato consumption. Peel and pulp extracts of each variety were prepared using previously described methods to prevent the loss of protein during the manufacturing process. In all cases, peel extracts contained approximately double protein content than pulp extracts.

In peel extracts, a band of 12 kDa approximately was the most abundant protein in the 6 extracts though other proteins were also visible. This protein was identified by LC MS/MS as Lyc e 3, a common and characteristic marker of sensitization in Mediterranean countries.^{36,37} Rambo and Canario varieties showed the highest concentration of Lyc e 3 but no statistically significant differences were detected in the allergen content compared with other varieties. Lyc e 3 was also the most abundant allergen in pulp extracts. PG2A (45 kDa) was identified by immunoblot with a polyclonal antibody. It was also prominent in all extracts, especially in peel extracts, suggesting that this allergen should be borne in mind. This is a protein present in the outer pericarp and during ripening is also synthesised in the inner pericarp. This

location explains its presence in peel extracts and its poor detection in pulp extracts.³⁸

Another protein described as a tomato allergen, OLP, was identified as a band of 27 kDa approximately. This band was recognized by the serum pool suggesting a relative importance in our population. This protein belongs to the thaumatin allergen family (PR-5) and has a great homology with other allergens such as Cap a 1, Jun a 3, Pru av 2 or Mal d 2.³⁹ This protein has been previously named as Lyc e NP24⁴⁰ (pathogenesis related protein).

A band of 16 kDa, probably corresponding to profilin (not confirmed by LC MS/MS) was only recognized in Rambo, Canario and Kumato peel extracts and seems to have a secondary role in this population.

With the exception of LTP, the studied population recognized allergens of medium and high MW, suggesting that these proteins have an important IgE binding capacity and in consequence, an important role in sensitization to tomato in the included population. LTPs (Lyc e 3) were recognized in all extracts and the importance of this allergen cannot be neglected.

Nearly two hundreds of individuals, residing in the Mediterranean coast of Spain were included in the study. Less than half experienced symptoms (OAS, contact urticaria, or other symptoms, mainly digestive) after tomato ingestion or contact. Most patients were sensitized to pollens (91%) and to other food extracts (fruits and nuts) (77%), which could be explained by cross-reactivity between fruits or co-sensitization. Although Pera variety showed the higher protein content, it induced the smaller wheals in the patients, being the only peel extract with non-significant differences respect to pulp extract (Kumato pulp variety). Rambo peel extract, which had the lower protein content, produced similar wheals than those induced by Kumato and Canario and with significant differences with Pera peel extract. Kumato pulp extract produced wheals significantly higher than all other pulp extracts. Canario variety detected the highest rate

of sensitization as expected, because patients were selected with this extract. Specific IgE binding to the 6 tomato varieties demonstrated significant differences in the specific IgE binding capacity between peel and pulp extracts, showing that peel extracts, at the same protein concentration, are more recognized than pulp extracts, probably due to the dilution of protein content in the matrix composition.^{9,10} Kumato variety showed the highest capacity for detection of tomato sensitization. This extract detected 43.7% of positive sera, suggesting that it could be used for diagnosis. This percentage is close to the Canario variety (40.8%) used as a standard in this study. The highest OD values in peel and pulp extracts were also obtained for the Kumato variety.

The present study represents a cross-sectional survey of the allergenicity of different tomatoes purchased at a local market. The Kumato variety seems to be the most allergenic and the Pera the least. Unless experiments are conducted with tomatoes at different ripening stages, there is no answer to the question of whether the differences among varieties could represent variability within each cultivar. The lack of studies measuring protein content and allergenicity of the same tomato variety in sequential years makes it difficult to draw a definitive conclusion on recommending consumption or avoidance of a certain tomato variety to a tomato allergic individual. It seems, therefore, that Kumato variety could be used to diagnose tomato allergy in this population. Kumato variety is not a commonly ingested variety. It belongs to a group of tomatoes known as black tomatoes, with an intense taste, higher sweetness and juiciness than usual tomatoes. These characteristics could be related with its high allergenicity, especially considering that it contains other components in the pulp fraction, such as carbohydrates, in higher proportion. Due to its high consumption and its good performance in our study, we think that Canario tomato variety is also a good option for use in the diagnosis of tomato sensitization. We have found a differential expression in protein/allergen content between varieties, but several factors, besides the variety itself, may contribute to these differences and should be further studied.

In summary, differences in protein, allergen composition, and allergen concentration have been demonstrated between 6 different tomato varieties suggesting that some tomato varieties could be less allergenic. In our population, although only 41% had clinical symptoms and there were no cases of anaphylaxis, tomato sensitization seems to be involved, mainly, with oral and mild symptoms. The results demonstrated that the use of selected varieties could improve the diagnosis of sensitization to tomato. However, further studies should be conducted to confirm this hypothesis, studying more varieties, controlling more variables and performing in vivo experiments before safe recommendations about the use of peeled or hypoallergenic varieties can be reached.

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