

Peels of Rosaceae fruits have a higher allergenicity than pulps

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Summary

Background It is not uncommon that patients allergic to fruits such as apple, pear, and peach, refer adverse reactions after the ingestion of the whole fruit, but subsequently tolerate the pulp.

Objective This study aimed to compare the allergenicity of peels and pulps of apple, peach, and pear in 33 patients allergic to these fruits.

Methods Clinical reactivity to the ingestion of whole fruit (peel + pulp) and pulp was established by medical history. Peels and pulps were tested separately in skin prick tests (SPTs), histamine release tests (HRTs) and RASTs. Cross-allergenicity between peel and pulp of apple and peach was studied by RAST inhibition.

Results Adverse reactions appeared more frequently and were more severe when the whole fruit was eaten. More than 40% of patients allergic to apple and pear tolerated the ingestion of the pulp of these fruits, and reactions were only elicited by the intake of the whole fruit. Peels induced higher SPTs, HRTs and RASTs than pulps. An important cross-allergenicity was found between the peel and pulp of apple and peach, although the amount of the shared allergenic epitopes seemed to be higher in peels.

Conclusion Our results suggest that peels of Rosaceae fruits such as apple, peach, and pear, have a higher allergenicity than pulps, which is clinically relevant. This aspect should be considered in the evaluation of patients allergic to Rosaceae fruits, and in the production of diagnostic materials.

Keywords: apple, cross-allergenicity, fruit allergy, oral allergy syndrome, peach, Rosaceae fruits

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Introduction

Oral allergy syndrome (OAS) to vegetable foodstuffs is the most frequent food allergy observed in adult patients in our area (Central Spain). Fruits of the Rosaceae family are the foods most often involved, specially peach and apple (unpublished data). Similar observations have already been reported in other geographical areas [1–3].

In our daily practice of allergy it is not uncommon that subjects allergic to fruits such as apple, peach and pear refer symptoms exclusively after the ingestion of the whole fruit, with a good tolerance to the pulp. Cuesta-Herranz *et al.* [4] have recently reported a series of 70 subjects allergic to peach: all of them had symptoms to unpeeled peach, but 39% tolerated the ingestion of peach pulp. Additionally, the

composition of peels and pulps of apple, pear, and peach is different: peels are richer in proteins, and pulps have a higher carbohydrate content [5]. Do the peel and the pulp of the same fruit have a different allergenicity? If this is the case, this aspect should be taken into account in the clinical evaluation of these patients and in the production of diagnostic materials.

This study aimed to compare the allergenicity of peels and pulps of apple, peach, and pear in a group of 33 patients allergic to these fruits.

Patients, materials and methods

Patients

The population was selected among adult outpatients (> 14 years old) referred to the Allergy Departments of Ramón y Cajal Hospital in Madrid and Virgen del Valle Hospital in

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Toledo (Spain). To be admitted to the study patients had to describe immediate clinical symptoms suggestive of a type I immunological reaction at least on two occasions after the ingestion of apples, pears and/or peaches (at least one of them). Patients also had to have a SPT response with the implicated fresh fruit/s (the peel, the pulp or both) resulting in a weal size similar or larger than that of the histamine control (3+/4+) [6].

The medical history was taken carefully to find out whether there was an influence of the peel of the fruits in the clinical reactivity. When a reaction was induced by the whole fruit (peel + pulp), the patient was asked whether s/he had subsequently eaten the pulp. Viceversa, if the reaction was induced by the pulp, the patient was asked whether s/he had subsequently eaten the whole fruit. Additionally, when a patient reported adverse reactions to one or two of the study fruits, the subsequent tolerance to the remaining fruit(s) (peel and pulp separately) was investigated.

In vivo methods

Skin prick tests

SPTs were performed with fresh apple (Golden delicious), pear (Blanquilla), and peach (Babygold 9) testing peel and pulp separately, in duplicate, by the prick-prick method described by Dreborg and Foucard [7]. All patients were tested with a battery of inhalants (Dome/Hollister-Stier, Química Farmacéutica Bayer S.A., Barcelona, and Ifidesa-Arístegui, Bilbao, Spain) which included perennial allergens (mites, molds, cockroach, cat, dog) and the most relevant pollens in our area (grasses, trees and weeds) [8]. SPTs were performed with the 'Allergy pricker' lancet (Dome/Hollister-Stier, Miles Ltd, Basingstoke, UK) according to the EAACI recommendations [6]. Histamine dihydrochloride 10 mg/mL (Ifidesa-Arístegui, Bilbao, Spain), and phosphate-buffered saline (PBS) solution served as positive and negative controls, respectively. The study was performed out of the pollen season. A SPT was considered positive if the weal area was greater than 7 mm² (diameter > 3 mm) [9].

In vitro methods

Fruit extracts

Fresh apples (Golden Delicious), and peaches (Babygold 9) were purchased at a local food store. Peels were removed very carefully with a sharp knife. Peels and pulps from apple, and peach were extracted following the method described by Björkstén *et al.* [10], and freeze dried. Average yields (grams of lyophilized material obtained from 100 g of

fresh fruit) were 3.76% for apple peel, 4.85% for apple pulp, 4.82% for peach peel, and 5.41% for peach pulp.

Histamine release

A histamine release test (HRT) was performed by using an automated fluorimetric method as described previously [11]. Peels and pulps of apple and peach were tested in duplicate at protein concentrations (Bradford method) [12] of 10, 50 and 250 µg/mL, which did not demonstrate fluorescence. Results were expressed as a percentage of the total histamine content after subtracting the spontaneous release. A release greater than 10% was considered as positive. A group of 10 nonatopic patients who tolerated the ingestion of these fruits was used as control.

Total IgE

Total IgE was determined by Pharmacia CAP System IgE FEIA following the standard procedure (Pharmacia Diagnostics AB).

RAST

Fruit extracts were coupled with cyanogen bromide activated filter paper disks following the method of Yman *et al.* [13] with a protein concentration in the coupling solution of 3 mg/mL for apple peel, 2 mg/mL for peach peel, and 1 mg/mL for pulps of apple and peach. These were the optimal allergen concentrations obtained by a direct titration method [13]. Each fruit disk was incubated with 50 µL of serum for 3 h at room temperature. Afterwards a standard RAST procedure was performed (Phadezym RAST, Pharmacia). Results are expressed in PRU/mL.

RAST inhibition

Cross-reactivity between peel and pulp of apple and peach was investigated by RAST inhibition. Each fruit peel RAST was inhibited by itself and by its pulp, and viceversa. All the inhibitors were tested in duplicate in four tenfold dilutions (1 : 1 to 1 : 1000). Pooled serum (50 µL, undiluted) was pre-incubated with 20 µL of inhibitor (or PBS as a control) for 1 h at room temperature. Then the fruit disk was added and the standard procedure was followed (Phadezym RAST, Pharmacia). Protein concentrations of inhibitors in the 1 : 1 dilutions were the following: 3 mg/mL for apple peel, 2 mg/mL for peach peel, and 1 mg/mL for pulps of apple and peach.

Statistical analysis

Statistical analysis was performed on computer using Epi

Table 1. Clinical background and total IgE

Patient				Apple		Pear		Peach	
No.	Sex	Age	IgEt	Peel + pulp	Only pulp	Peel + pulp	Only pulp	Peel + pulp	Only pulp
1	F	21	465	A	–	U	–	A	?
2	F	25	591	–	–	–	–	CU	OAS
3	F	25	480	OAS	–	–	–	OAS	OAS
4	F	16	1262	G	G	OAS	–	CU	G
5	F	24	364	OAS, U	–	OAS	OAS	CU	OAS
6	M	14	927	OAS, U	–	–	–	CU	?
7	M	23	385	U	U	G	?	CU	OAS, U
8	F	25	753	G	–	–	–	CU, OAS	G
9	M	24	851	–	–	OAS	?	CU, U	?
10	F	20	428	–	–	–	–	CU, OAS	OAS, CU
11	M	39	230	OAS	OAS	–	–	OAS	–
12	M	21	337	–	–	–	–	CU, OAS	?
13	M	22	153	OAS	OAS	OAS	OAS	CU, OAS	OAS
14	M	19	255	OAS	OAS	OAS	OAS	OAS	OAS
15	M	22	1180	–	–	–	–	CU, OAS, U	?
16	F	27	451	OAS	OAS	OAS	OAS	OAS	OAS
17	M	18	145	OAS	–	OAS, U	–	U	?
18	F	23	265	OAS	OAS	OAS	OAS	OAS, CU	OAS
19	F	22	68	–	–	–	–	OAS, CU	?
20	F	23	533	OAS	OAS	OAS	–	A, S	?
21	M	19	243	A	–	–	–	OAS	OAS
22	M	21	225	?	?	A	?	A, S	?
23	F	14	174	U, A	?	?	?	U, A	?
24	F	25	8	OAS	?	–	–	OAS	OAS
25	F	23	13	A, S	?	?	?	CU, OAS, U	?
26	M	24	204	–	–	–	–	CU, A, S	?
27	M	21	459	OAS	?	?	?	OAS, G	?
28	F	22	127	A	?	A, S	?	CU, A	?
29	F	24	71	U	–	OAS, U	–	CU, OAS	OAS
30	M	58	123	A	?	?	?	OAS	OAS
31	M	20	459	OAS, A, S	OAS	OAS	OAS	CU	CU, OAS
32	M	31	26	A	?	–	–	OAS	OAS
33	M	24	14	A	A	A	A	?	?

F, female. M, male. IgEt, total IgE (kU/L). –, good tolerance. OAS, oral allergy syndrome. G, gastric complaints. U, generalized urticaria. A, anaphylaxis. S, anaphylactic shock. CU, contact urticaria. Anaphylaxis includes systemic reactions with skin involvement (urticaria, angioedema), together with respiratory and/or gastrointestinal symptoms, but without hypotension. Age is given in years.

Info 6 and SPSS 6.1 softwares (Universidad Autónoma de Madrid). Comparisons of frequencies were done by a chi-square test. Associations between qualitative variables were analysed by a chi-square test or a Fisher's exact test. Difference in total IgE between the two groups was compared by a Mann–Whitney *U*-test. The differences between peel and pulp of the same fruit in SPTs, HRTs and RASTs were studied by a Wilcoxon test for paired data. In the RAST inhibition assays the self inhibition straight-line and the line of the inhibitor were compared

with a *t*-test for parallelism. Values were considered significant at a *P*-value less than 0.05.

Results

Thirty-three patients who fulfilled the inclusion criteria and gave their consent were included in the study. They comprised 17 males and 16 females, with a mean age of 23.57 years. Adverse reactions were reported by 97% of patients to peach, 76% to apple, and 48% to pear. Patients

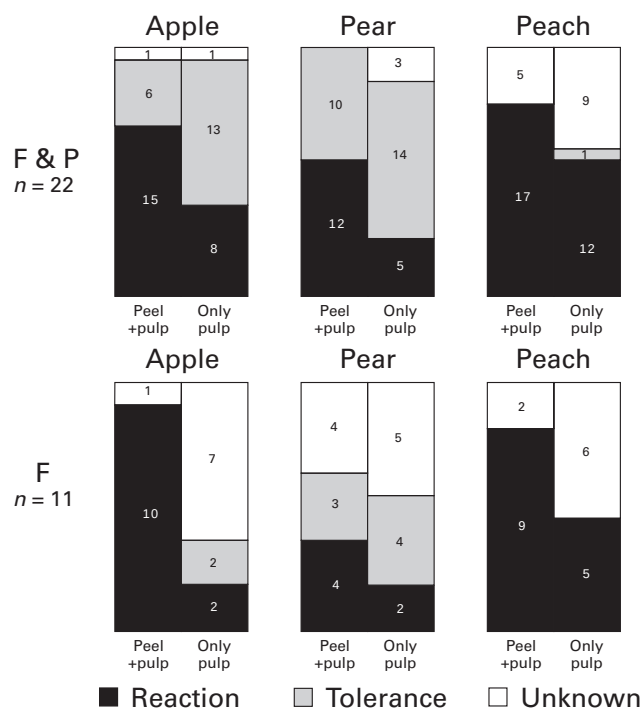


Fig. 1. Frequency of responses to the ingestion of apple, pear and peach with peel ('peel + pulp') and without it ('only pulp'). Responses are classified into three items: reaction, tolerance, and unknown. Frequency is given in absolute value (number of patients). F & P group includes patients 1–22 of Table 1. F group includes patients 23–33 of Table 1.

1–22 had an associated grass pollinosis (fruit and pollinosis group, F & P), and patients 23–33 did not present any type of inhalant allergy (fruit group, F) (Table 1). The characteristics and differences of these two groups of patients have been previously reported [8,11,14].

The responses to the ingestion of 'peel + pulp' and 'only pulp' are shown in Fig. 1. Adverse reactions were more frequently observed after the ingestion of 'peel + pulp'. In the F & P group the ingestion of whole apples and pears induced reactions more frequently than the ingestion of apple and pear pulps (apple peel + pulp 15/21 vs apple pulp 8/21, $P = 0.02$; pear peel + pulp 12/22 vs pear pulp 5/19, $P = 0.06$). The frequency of reactions to whole peach (17/17) and peach pulp (12/13) was similar. However, 9/22 patients did not eat peach pulp after having experienced reactions with whole peach. In the F group all the patients were first seen with serious immediate adverse reactions after ingestion of whole apples and/or whole peaches. For this reason the subsequent tolerance of apple and peach pulps, and pear is frequently unknown: fear to a new reaction made them avoid these fruits. Comparisons of frequencies between 'peel + pulp' and 'only pulp' are not done in the F group because of the important number of patients with unknown tolerance to pulps.

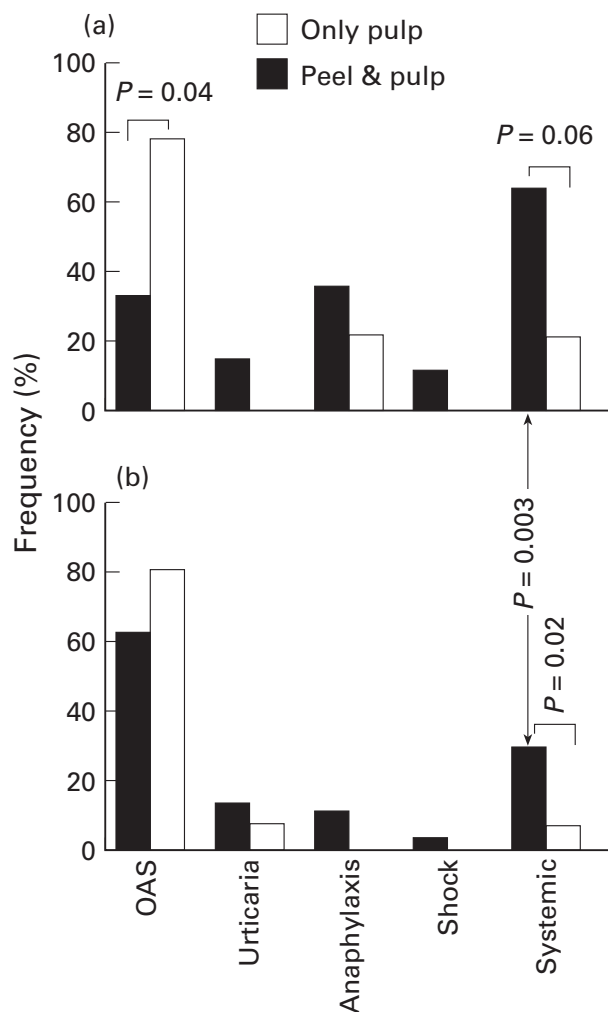
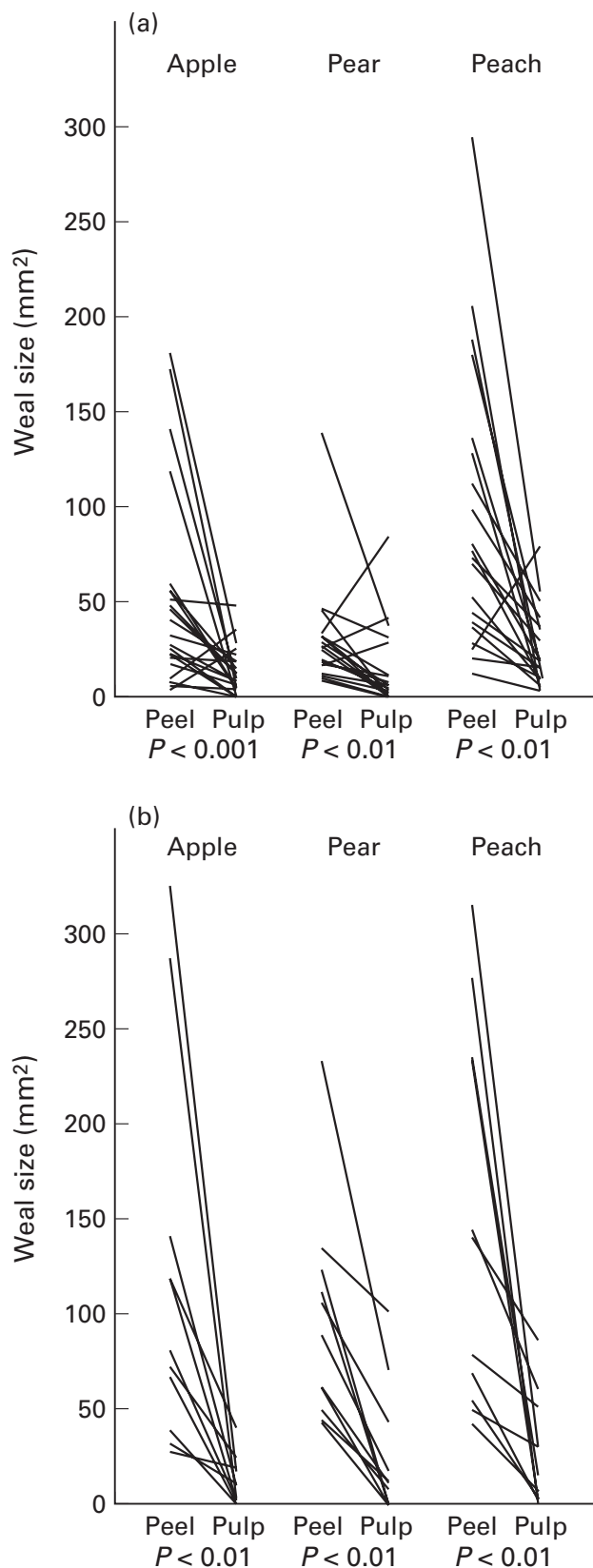


Fig. 2. Frequency of clinical manifestations induced by the ingestion of apple, pear and peach with and without peel. Frequencies are given as percentage of the total number of different adverse reactions reported. Apple, pear and peach reactions are combined and presented under the items 'peel + pulp' and 'only pulp'. 'Systemic' includes all the systemic reactions (generalized urticaria, anaphylaxis and anaphylactic shock). Total number of reactions reported in (b) F & P patients: 49 for 'peel + pulp', 26 for 'only pulp'. Total number of reactions reported in (a) F patients: 33 for 'peel + pulp', nine for 'only pulp'. Statistics: comparison of frequencies by a chi-square test. Only $P \leq 0.10$ values are presented in the figure.

Figure 2 shows the frequency of the clinical manifestations related to the total number of different reported reactions. OAS was the most frequent presentation after ingestion of pulps for the three fruits (independently and together) in the two groups of patients. OAS was observed in 80% of the reactions to pulps. Systemic involvement was reported only four times (11% of pulp reactions) after pulp ingestion: two generalized urticarias (apple pulp and peach



pulp) in F & P patients, and two anaphylaxis (apple pulp and pear pulp) in F subjects. Anaphylactic shock was never reported after pulp ingestion. In contrast, the ingestion of unpeeled fruits induced a significantly higher frequency of systemic reactions, and a lower frequency of OAS in both groups of patients. Urticaria and anaphylaxis were observed in F & P patients after the ingestion of unpeeled apples, pears and peaches, but only the latter induced anaphylactic shock (two patients). Systemic involvement in the intake of whole fruits was significantly higher in the F group: 64% of all the reported reactions (vs 31% in F & P, $P = 0.003$). Nine out of 11 F patients had presented systemic reactions, 5/11 to two or three fruits, and 4/11 anaphylactic shock. Unpeeled apple elicited 52% of the systemic reactions in the F patients ($P = 0.06$, chi-square test).

To evaluate more accurately the influence of the peel of a certain fruit in the induction of reactions, it was essential to know the clinical reactivity to the ingestion of the pulp and the whole fruit. Patients who fulfilled this condition were further evaluated: 18 patients for apple (15 F & P, 3 F), 12 subjects for pear (9 F & P, 3 F), and 13 individuals for peach (9 F & P, 4 F) (patients with '?' – unknown tolerance – in Table 1 were excluded for this analysis). Eight patients (numbers 1, 3, 5, 6, 8, 17, 21, 29) tolerated the ingestion of apple pulp but presented reactions when eating apples with the peel (8/18 = 44.44%; 95% confidence interval [CI]: 21.53–69.24%). Five patients reported adverse reactions after eating unpeeled pears (numbers 1, 4, 17, 20, 29), but tolerated subsequently the intake of pear pulp (5/12 = 41.66%; 95% CI: 15.16–72.33%). A significant association was observed between the ingestion of apples with peel and the appearance of a reaction ($P = 0.02$, chi-square test). For pear the analysis did not reach the level of statistical significance ($P = 0.07$, chi-square test).

The contact with peach peel induced urticaria in 13 F & P patients (13/22 = 59.09%, 95% CI: 36.35–79.29%), and in five F subjects (5/11 = 45.45%, 95% CI: 16.74–76.62%), but only two of them (numbers 10 and 31) also presented contact urticaria (CU) with peach pulp. This type of reaction was never elicited by apple or pear. A significant association was found between the contact with peach peel and CU ($P < 0.001$, Fisher's exact test).

Total IgE (Table 1) was significantly higher in the F & P group (mean 481.42 kU/L, SE 69.70) than in F patients (mean 152.73 kU/L, SE 49.92; $P = 0.001$, Mann–Whitney *U*-test).

Results of SPTs, RASTs, and HRTs for both groups are shown in Figs 3, 4 and 5, respectively. SPTs and RASTs were performed in all the patients, and HR in 25 subjects (18

Fig. 3. SPT with peels and pulps of apple, pear and peach. (a) F&P patients, (b) F patients. Statistics: Wilcoxon test for paired data.

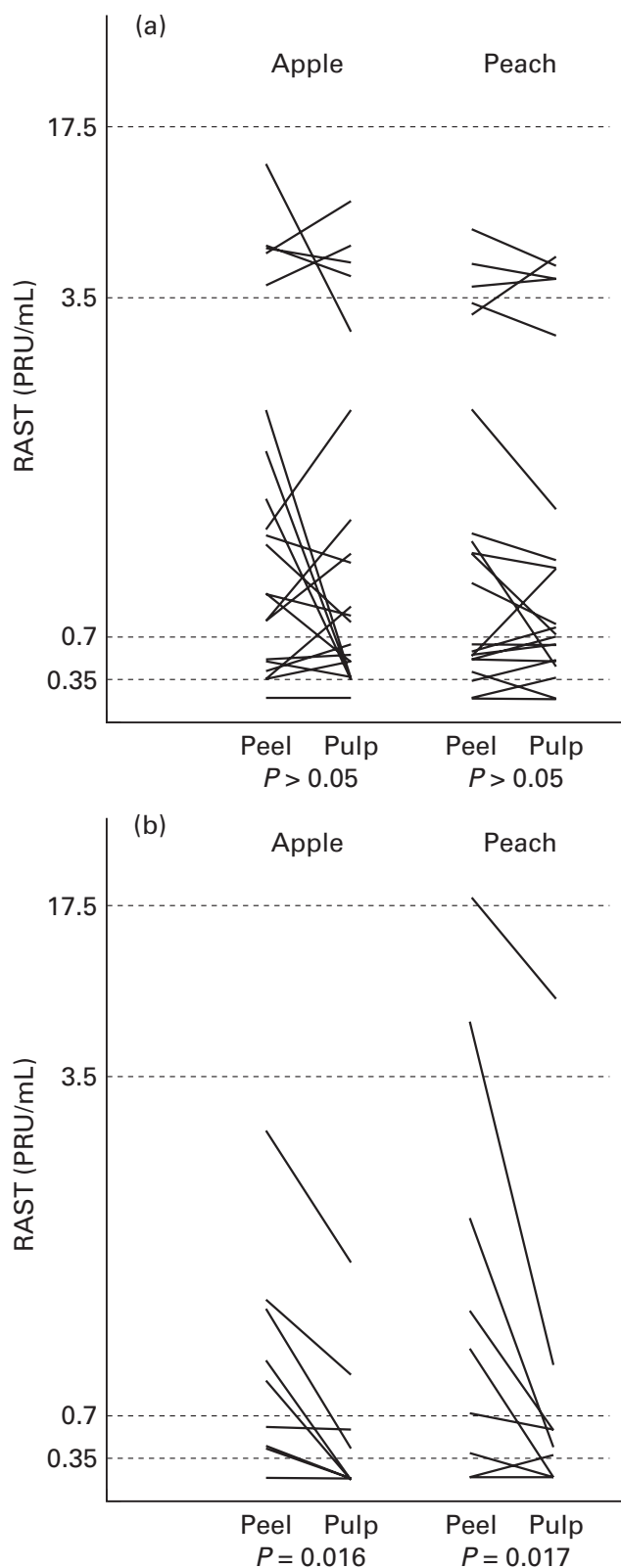


Fig. 4. RAST with peels and pulps of apple and peach. (a) F&P patients, (b) F patients. Statistics: Wilcoxon test for paired data.

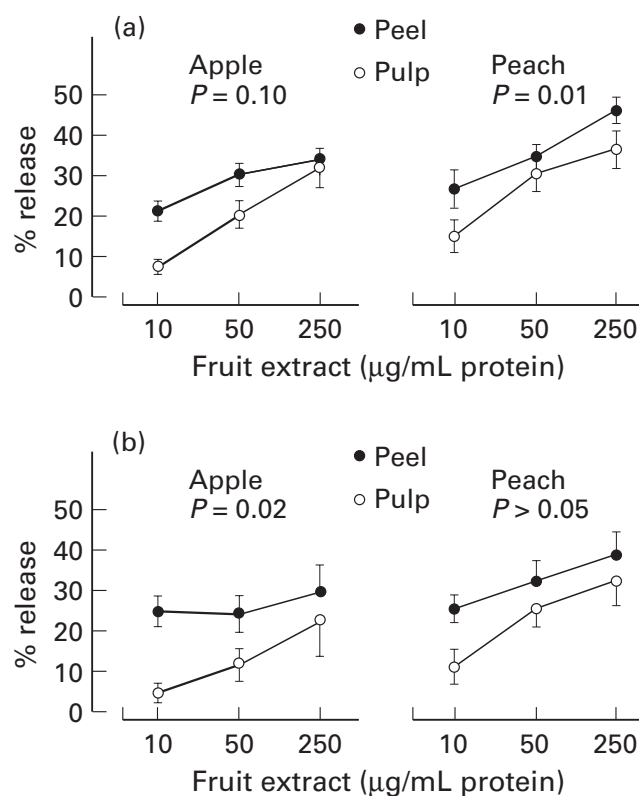


Fig. 5. Histamine release with peels and pulps of apple and peach. (a) F&P patients, (b) F patients. At each concentration tested mean value and standard error of the mean are given. No significant release (<10%) was observed in the control group. The variable analysed was the area under the curve of release. Statistics: Wilcoxon test for paired data.

F & P, 7F). SPTs with peels of apple, pear, and peach induced significantly higher responses than the pulps, both in F & P and F patients (Fig. 3). RAST to apple and peach peel was significantly higher than RAST to these fruit pulps in the F patients, but no significant differences were observed in the F & P group (Fig. 4). The area under the curve of histamine release was larger for peels of apple and peach in the two groups of patients, but only the peach peel area in F & P patients, and the apple peel area in F individuals were statistically larger than their respective pulps (Fig. 5).

RAST inhibition assays were performed in F & P and F patients separately. Equal volumes of sera from patients 3, 4, 5, 6, 7, 9, 15, 16, 17, 20, 21, and 22 of the F & P group, and patients 23, 27, 28, 29, 31, and 33 of the F group were pooled for the inhibition assays. Each fruit peel RAST was inhibited by itself and by its pulp, and viceversa (Fig. 6). An important inhibition ranging from 60 to 97% was observed in all the assays performed. With the exception of the inhibition of peach peel RAST by peach pulp in F & P

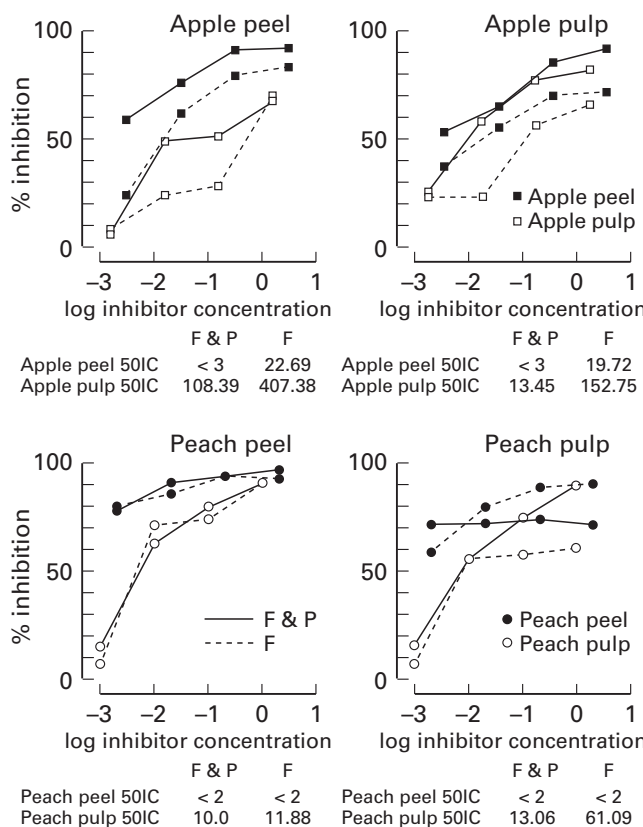


Fig. 6. RAST inhibition. The fruit used as solid phase is given at the top of each panel. The inhibition assays were performed in duplicate. Mean values are presented in the figure. SE were $\leq 8\%$. 50IC, 50% inhibitory concentration ($\mu\text{g/mL}$).

patients all the inhibition lines presented similar slopes ($t < 2.78$, $P < 0.05$, 4 degrees of freedom). The 50% inhibitory concentrations (50IC) for pulps were always higher (three- to sevenfold in F & P, six- to 30-fold in F) than those of the peels. The behaviour of F & P and F groups in the 'peach peel' panel was (very) similar, but in the 'apple peel' and 'apple pulp' panels a different behaviour could be observed: 50IC are always higher (three- to 11-fold) in the F group.

Discussion

In this study we aimed to compare the allergenicity of peels and pulps of three Rosaceae fruits. The approach included the study of the clinical reactivity when the fruits were eaten with the peel and without it, and the responses in SPTs, HRTs and RASTs to peels and pulps tested separately. The clinical reactivity was established by the medical history according to restrictive criteria: all the patients included had presented at least two immediate reactions with the involved fruit (whole fruit, pulp or both) and had a strongly positive

SPT response to it. The assessment of the clinical response by an oral challenge test was not included in the protocol due to the lack of a standardized procedure [2], the good concordance between history and open challenge with peach [15], and the severity of the reactions reported by some patients [8].

We have previously reported that allergy to Rosaceae fruits observed in patients without a related pollen allergy differs in terms of clinical presentation and allergens involved, to the pattern found in subjects with a combined grass pollinosis [8]. For this reason, in this study, the clinical reactivity and the responses to SPTs, HRTs, RASTs, and RAST inhibition are analysed in F & P and F groups separately.

The analysis of the clinical data suggests that adverse reactions appear more frequently and are more severe when fruits are eaten with the peel. More than 40% of the patients allergic to apples and pears tolerated the ingestion of the pulps of these fruits, and they only presented reactions when eating them with the peel. These clinical data are congruent with the results of SPTs for the three fruits studied. Apple and peach, the two most relevant fruits, were chosen for further evaluation by HRTs, RASTs, and RAST inhibition. The results of HRTs and RASTs are not always in agreement with SPTs. The higher sensitivity of SPTs with fresh fruits [16], the extraction procedure (for HRTs and RASTs), the binding of fruit allergens to the solid phase of RAST, and the different target of these three tests (the skin mast cell, the basophil, and the serum IgE) could explain the discrepancies. Additionally, the lack of statistical significance of some peel–pulp comparisons can be related to the low number of patients evaluated in some tests. As the P -value is dependent on the sample size, all $P \leq 0.10$ values are given in this study (in our opinion they should not be overlooked).

The RAST inhibition results suggest that peels and pulps of apple and peach share allergenic epitopes. The shared epitopes in pulps might be present in lesser quantities or might have a lower affinity for anti-peel IgE antibodies, thus explaining the (three to 30 times) higher 50IC of pulp extracts. However, specific epitopes of peel, pulp or both, might exist. The different slopes of the inhibition lines of peach peel and pulp (Fig. 6) might indicate the existence of peach peel specific epitopes. These results are in agreement with the findings of Leonart *et al.* [17]: they found some common allergens to peach peel and pulp, and one allergen exclusive to peach peel.

Although performed with great care, both SPTs with the peel of fresh fruits and peel extracts may have been contaminated by pulp. However, pulp SPTs and pulp extracts were not at all contaminated by peel. Fortunately, pulps turned out to be less allergenic than peels. Therefore, a contamination of peel-related test materials by pulp would

result in a reduction of peel allergenicity, but, even in this unfavourable situation, our results suggest that peels are more allergenic than pulps.

There is very little information in the literature concerning the allergenicity of peels and pulps of Rosaceae fruits. Pastorello *et al.* [15] studied cross-reactivity among Prunoidae fruits with extracts prepared with peels 'for their higher allergenicity': no data were given to support this statement. Similarly to us, Lleonart *et al.* [17] and Kleine-Tebbe *et al.* [18] found a higher allergenicity of peels of peach and apple, respectively. However, neither of these two papers gave reason for the separate analysis of peel and pulp, and no information concerning clinical reactivity or SPTs to peel and pulp was available either. Kleine-Tebbe *et al.* [18] found that the concentration needed to obtain a 10% HR with apple peel extract was 2.3 times lower than for apple pulp extract. Lleonart *et al.* [17] found a higher level of specific IgE to peach peel by means of ELISA, and they identified in the immunoblotting IgE-binding components common to peel and pulp, and a doublet of 8–10 kDa exclusive to peach peel.

Interestingly, some of the Rosaceae fruit allergens identified up to now belong to different groups of proteins related to pathogenesis [19–23]. As the peel of the fruit is a protective barrier, its content in these defence proteins might be different (quantitatively or qualitatively) to that of the pulp, thus explaining the peel's higher allergenicity. Recently, Pastorello *et al.* [19] have identified a <10 kDa major allergen of peach as a lipid-transfer protein (LTP). LTPs are plant defence proteins located in the cell wall and involved in cutin biosynthesis and surface wall formation. They are mainly located in the outer epidermal layer [24]. These characteristics make them good candidates to be involved in the higher allergenicity of peels of Rosaceae fruits.

In this study we have shown that peels of apple, peach and pear have a higher allergenicity than pulps, which is clinically relevant. This fact should be taken into account in the clinical evaluation of patients (medical history, SPTs with fresh fruits, and oral challenges), and in the production of fruit-diagnostic materials (for *in vivo* and *in vitro* tests). Further research is now needed to study the allergenic composition of peel and pulps of Rosaceae fruits.

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