

Occupational allergy in saffron workers

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Sensitization to the flower of saffron, a plant commonly grown in Spain for commercial purposes, and its clinical significance as an occupational allergen were studied. The prick test and RAST, with saffron pollen, stamen, and pistil extracts, were used to evaluate the cutaneous and specific antibody responses in the studied population. Provocation tests in patients with clinical findings were used to verify the implication of saffron components in these symptoms. Fifty saffron workers were evaluated. Three of them were sensitized to saffron pollen and stamen proteins, giving prick and RAST positive values. One patient presented asthma, showing a positive bronchial provocation test, and two patients rhinoconjunctivitis, showing positive conjunctival provocation tests. Of a general allergic population of 237, 10 patients also presented cutaneous test and IgE positive to saffron. Saffron allergens (from pollen and stamens) were characterized by SDS–PAGE immunoblotting. A relevant allergen of 15.5 kDa with profilinic nature was detected and further purified by high-resolution gel filtration chromatography. No allergenic components were demonstrated in pistils. Cross-reactivity of saffron extracts was evaluated by RAST inhibition with respect to other pollen species commonly causing sensitization in the same area of study. A significant degree of cross-reactivity was demonstrated between saffron and *Lolium*, *Salsola*, or *Olea*. The identification of the protein components involved in the cross-reactions was investigated by blot inhibition.

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Substances of vegetal origin frequently cause occupational allergy. Allergens extracted from wood (1), cereal flour (2), latex (3), castor bean (4), vegetable fibers (5), and glycolytic enzymes (6, 7) can cause sensitization in exposed workers, with prevalence values up to 40%, depending on the allergen nature and exposure level.

The saffron plant (*Crocus sativus*) belongs to the Iridaceae family, and it is commonly grown in the Castilla La Mancha, Aragón, and Murcia regions (central and southeastern Spain) for the pistils of its flowers, which yield the spice saffron. This spice is commonly used in cooking as well as in religious services. Saffron is picked during 3–4 weeks in October–November. It has become a family industry due to the low financial return of this crop.

As far as we know, there are no publications on the involvement of saffron plant components as an aeroallergen causing hypersensitivity reactions in a work or home environment. Only one case has been reported of contact dermatitis to safflower leaves and flowers used as an adulterant of the spice saffron (8).

Spain is the main world producer of saffron. However, it is also grown in several other Mediterranean countries, Asia, and the USA.

This work had the following three main objectives:

- 1) to conduct an epidemiologic study of cross-sectional prevalence of sensitization to saffron allergens among saffron-exposed workers
- 2) to determine the frequency of saffron-positive cutaneous response and saffron-specific IgE within the allergic population (unexposed to saffron) attending our allergy unit and complaining of episodes of rhinitis and/or asthma
- 3) to characterize the saffron allergens involved, as well as the degree of cross-reactivity between this extract and pollen from the plant species most frequently found in Ciudad Real province.

Material and methods

Patients

Group I (exposed workers) comprised a population of 50 saffron workers living in an important saffron

crop area in Ciudad Real province (200 km south of Madrid), who were studied by anamnesis and cutaneous tests. All 50 exposed workers were surveyed, and all of them answered the standardized questionnaire, which included nasoconjunctival and/or bronchial history in relation to saffron handling (October–November), exposure degree, development of sensitization during the rest of the year, and personal and family allergy history. The degree of exposure was determined by the amount of participation in the following activities: saffron picking, manual extraction of stigmas, and roasting of the stigmas.

Group II (unexposed patients) comprised 237 patients, who attended consecutively the allergy section from December 1994 to April 1995, being included in the respiratory allergy study whenever the anamnesis indicated respiratory allergy (rhinoconjunctivitis and/or asthma).

Sera from groups I and II patients, with positive skin tests to saffron pollen and stamen extracts were analyzed *in vitro* by the radioallergosorbent test (RAST) and immunoblotting, both extracts being used.

Sera from 10 healthy subjects with negative tests for saffron allergens were also studied by the same methods.

To determine the prevalence of sensitization to saffron extracts among groups I and II, we included the saffron pollen extract together with the routine battery of allergens used in our department (*Dermatophagoides pteronyssinus*, *D. farinae*, *Lepidoglyphus destructor*, *Lolium perenne*, *Cynodon dactylon*, *Olea europaea*, *Artemisia vulgaris*, *Plantago lanceolata*, *Chenopodium album*, *Salsola kali*, *Parietaria judaica*, *Alternaria alternata*, *Cladosporium herbarum*, *Aspergillus fumigatus*, and cat and dog dander).

Preparation of C. sativus stamens, pistils, and pollen extracts

C. sativus stamens, pistils, and pollen were collected in Ciudad Real province, in autumn 1993. Examination of purity revealed that 97% of pollen grains were *C. sativus*. Desiccated stamens and pistils were washed until no pollen grains were microscopically detected. Defatted materials were extracted according to Jiménez et al. (9).

Briefly, the raw materials were suspended in 0.1 M phosphate buffer, pH 8.0, at 15% w/v and stirred magnetically for 4 h at 4°C. The extract was clarified by centrifugation at 5600 g for 30 min, filtered through a 0.22- μ m pore diameter membrane, and dialyzed by tangential ultrafiltration with a 5-kDa cutoff. Finally, the extract was freeze-dried and stored until use. The same proce-

dure was followed with three different plant materials.

Skin prick test

The skin prick test was performed according to the Subcommittee on Skin Tests of the European Academy of Allergology and Clinical Immunology (10). Standardized lancets (Dome Hollister, Leverkusen, Germany) were used for prick tests.

Histamine chlorhydrate at 10 mg/ml and phenolated glycerol saline solution were used on positive and negative controls, respectively. The allergens used to test subjects from groups I and II were those described above.

On the basis of the sensitized population studied, 1 histamine equivalent prick (HEP) unit (defined as the allergen concentration eliciting a wheal equal to the one produced by histamine 10 mg/ml) was found to be equivalent to 4.6 mg/ml of allergen extract from stamens, and 1.7 mg/ml of allergen extract from pollen.

Conjunctival provocation test

This test was performed in two patients from group I, according to Jiménez et al. (9). Briefly, the concentrated extract for the conjunctival provocation test was prepared at 5 HEP/ml. Serial dilutions were prepared at 0.001, 0.005, 0.01, 0.05, 0.10, 0.5, and 1 HEP/ml.

The test was started by administering one drop of diluent into the conjunctival sac. Ocular symptoms were monitored after 20 min. If no reaction was observed within 20 min, the next concentration (0.001 HEP/ml) was applied to the other eye. The operation was repeated up to 1 HEP/ml concentration unless a positive response was detected. The conjunctival provocation test was considered positive under one of the following conditions:

- 1) chemosis
- 2) erythema >50% of the bulbar conjunctival area
- 3) erythema <50% of the bulbar conjunctival area plus itching or epiphora (11).

Specificity controls were performed in 10 healthy subjects and 10 allergic patients with different etiology, by the same schedule.

Bronchial provocation test (BPT)

The BPT was performed in one patient from group I who had bronchial symptoms upon exposure to saffron. A nonspecific bronchial reactivity test with methacholine was done by the procedure and concentrations proposed by Chatham et al. (12), recording vital capacity, FEV₁, and PEF values in

an ST-250 spirometer, Fukuda Sangyo Co. Dilutions were administered with a Hudson 1720 nebulizer with input and output flow rates of 7 l/min and 0.28–0.30 ml/min, respectively.

A specific BPT was performed with saffron pollen extract, basically according to Chai et al. (13), but with some modifications. The patient was tested when asymptomatic with FEV₁ more than 80% of predicted normal. He had not received any medication over the previous several days. A control challenge with phosphate-buffered saline (PBS) was carried out before antigen challenge. The patient was skin-tested with 10-fold dilutions of saffron pollen extract to determine a safe starting dose for the provocation test, and this concentration was a 0.01 HEP/ml extract. The patient inhaled the aerosolized allergen in progressively increasing concentrations (0.01, 0.05, 0.10, 0.5, and 1 HEP/ml) for 2 min at tidal volume. FEV₁ and PEF were measured after each inhalation and also 5, 10, 15, 30, and 60 min later. To assess the bronchial late response, the patient himself monitored PEF values, in parallel to spirometry recordings by means of a portable Mini-Wright type peak flow meter, for up to 24 h. A positive test was defined as a fall in FEV₁ of 20% or in PEF of 25% from the postdiluent value during early or late determinations.

Five asthmatic patients from group II (unexposed workers) and five asymptomatic subjects from the same group underwent the BPT with the same allergenic extract as the control group.

Two atopic patients with mild asthma were also challenged with the same extract as control subjects.

RAST

Solid-phase antigen was obtained by coupling the extract solution (2 mg/ml) to the 6-mm diameter CNB-activated paper disks, as described by Ceska & Lundqvist (14). RAST was performed by the method of Wide et al. (15) with Phadezym RAST reagents (Pharmacia Diagnostics AB), according to the manufacturer's instructions. This chromogenic immunoassay, based on the use of antihuman IgE- β -galactosidase conjugates, was considered positive when a value higher than 0.35 PRU/ml was obtained for the tested serum.

SDS-PAGE immunoblotting

Sodium dodecyl sulfate – polyacrylamide gel electrophoresis (SDS-PAGE) was carried out according to Laemmli (16). Polyacrylamide concentrations of 12.5% and 3% were used for separating and stacking gels, respectively. Proteins dissolved in 0.125 M HCl-Tris, pH 6.8, were dissociated with

0.1% SDS and 5% β -mercaptoethanol by treatment at 100°C for 5 min, 20 μ g protein being applied per lane.

After electrophoresis, the separated protein bands were electrophoretically transferred to PVDF membranes (Immobilon-P, Millipore), essentially according to Towbin et al. (17). Antigen-antibody binding was identified as described by Shen et al. (18). In sort, immunochemical staining was performed by first blocking the Immobilon membrane with 90% defatted dry milk in Tris-buffered saline (TBS) (1 h at 37°C). After three washes with TBS, blots were incubated with 2.5 ml of undiluted human serum (16 h at 4°C). The blots were washed three times with TBS and incubated with 2.5 ml of 1 : 25 peroxidase-conjugated rabbit immunoglobulins to human ϵ -chains (Dako, Glostrup, Denmark). Bound peroxidase was detected by the addition of 3 ml of freshly prepared 0.06% w/v of 4-chloro-1-naphthol solution (Bio-Rad Laboratories, Richmond, CA, USA) and 0.01% H₂O₂ in TBS. After 30 min, the blots were rinsed in distilled water and air-dried. Antigen-antibody binding was identified by purple staining. The amount of specific IgG bound to each strip was estimated in terms of optical density detected in the Bio-Image analyzer.

RAST inhibition

RAST inhibition was performed as previously reported (9). Fifty microliters of a serum pool from patients sensitized to saffron extract plus 50 μ l of four dilutions (10–0.01 mg/ml) of allergenic extracts (inhibitors in liquid phase) were added to solid-phase allergen (saffron coupled at 2 mg/ml in paper disks). The following allergenic extracts were used as inhibitors: saffron, *S. kali*, *L. perenne*, and *Olea europaea*.

SDS-PAGE immunoblotting inhibition

Aliquots of the undiluted serum pool from seven patients showing RAST values to saffron $\geq$ class 3 were incubated for 10 min with equal volumes of the different pollen extracts, at a final concentration of 0.1 mg protein/ml. Mixtures were then centrifuged and supernatants incubated with saffron pollen-blotted PVDF strips, followed by immunodetection, as described in the SDS-PAGE immunoblotting section.

Saffron profilin chromatographic purification

The first step in the profilin purification was affinity purification in PLP-CNBr-activated Sepharose column, by the method of Lindberg et al. (19). Briefly,

2 g of CNBr-activated Sepharose 4B (Pharmacia Biotech, Uppsala, Sweden) was swollen in 100 ml of 1 mM HCl for 15 min. Then, the resin was washed in a filtering funnel (porosity G3) with four additions of 100 ml of 1 mM HCl. Afterward, the resulting gel (about 7 ml) was suspended in two volumes of the coupling buffer (0.1 M KHCO₃, pH 8.3, 0.5 M KCl). An amount of 30 mg poly(L-proline) (PLP, *M_r* 5000, INC Biochemicals, Aurora, OH, USA) was dissolved in 2 ml of distilled water for 12 h. Nonsoluble material was discarded, and the supernatant was added to the resin suspended in coupling buffer. The mixture was left at room temperature for 2 h. Blockage of active groups was achieved by filtering the resin and resuspension in 10 ml of 0.5 M glycine, pH 8.5. Ligand excess was washed alternatively with 0.1 M acetate buffer, pH 4.0, and PBS, pH 8.3, both containing 0.5 M NaCl. The excess of blocking agent was washed with PBS. The resulting PLP-Sepharose conjugate was mounted onto a 1×24 cm column and was equilibrated with PBS. Chromatography was carried out at a flow of 10 ml/h, and 500 mg of *C. sativus* pollen extract was dissolved in 2.5 ml of PBS and applied to the column. A volume of 150 ml of the unretained fraction, containing most of the proteins, was collected. Afterward, the adsorbed fraction (mainly profilin) was eluted in 70 ml of 6 M urea in PBS.

Proteins coeluted with profilin were removed by gel filtration chromatography in the SMART™ System (Pharmacia, Sweden) on a Superdex 75 PC 3.2/30 column. Samples were applied at a concentration of 20 mg/ml in 0.05 M phosphate buffer, 0.15 M NaCl, pH 7.0 (PBS), and injected with a loop of 20 µl. A flow rate of 40 µl/min was maintained during the whole chromatographic process. The column was calibrated with a set of proteins of known molecular mass. Chromatography was done at 8°C.

Results

Patients

Survey evaluation of group I (50 exposed workers) revealed that eight subjects (16%) had nasal symptoms, three (6%) bronchial symptoms, and four (8%) cutaneous pruritus. The ages of these patients ranged from 6 to 75 years, with an average of 32.4. Of them, 10% had a history of allergy; 54% were female and 46% male. The age range of group II was 5–62 years, with a mean age of 28.6. The prick results for pistil extracts were negative in all subjects. RAST results of subjects sensitized to saffron pollen are outlined in Table 1, together with their symptomatic features.

Cutaneous tests

The prick test with saffron allergens was positive in three patients of group I (6%), being coincident with those showing allergic manifestations, one suffering from bronchial asthma and two from rhinoconjunctivitis (patients 1, 2, and 13 of Table 1). In all three patients, symptoms developed during excision of stigmas from the saffron flowers, a process which allows the inhalation of pollen and stamen particles. In patients 1 and 13, the appearance of symptoms of saffron allergy occurred 3 and 6 years, respectively, before pollinosis onset. Patient 2 was monosensitized. Patient 13, although only 12 years of age, was included in the exposed workers group because whole families do this kind of work.

Of the 237 allergic patients comprising group II (unexposed), 150 were positive to any pollen extract, and of those, 10 were also positive to saffron pollen extracts. The characteristics and clinical features of these 10 saffron-sensitized patients, together with the three saffron-allergic patients of group I, are presented in Table 1. The 10 patients positive to saffron (patients 3–12 in Table 1) accounted for 4.2% of group II, and all of them were also positive to pollens commonly found in the area (grasses, olive, Chenopodiaceae/Amaranthaceae, etc.).

Provocation tests

The three allergic patients had positive provocation tests. Conjunctival provocation tests in patients corresponding to patients 2 and 13 (Table 1) from group I were positive to an antigen concentration of 0.005 HEP.

The nonspecific BPT with methacholine were performed in the patient with asthma (no. 1, group I). The result was positive (PD₂₀=14 AU). A BPT to an antigen concentration of 0.05 HEP was also positive, with recordings decreasing to 37% and 51% for FEV₁ and PEF, respectively (Fig. 1). Fig. 1 shows the mediated asthmatic reaction after challenge with saffron pollen extract. Together with these decreasing values, the patient suffered from cough and sibilant dyspnea, which disappeared after inhalation of a 400-µg dose of salbutamol. No delayed reaction was detected by peak flow meter during the 24 h after BPT.

None of the control subjects had a positive response, even with the maximum dose assayed (0.05 HEP).

In vitro analysis

Fig. 2 shows IgE-binding fractions to saffron pollen extract, based on the molecular weight, detected

Table 1. Characteristics and RAST values of 13 patients sensitized to saffron pollen components

Patient no.	Age (years)	Sex	Symptoms	Other sensitizations	RAST values	RAST class
1*	23	M	R, A, C	Grass, olive, <i>Salsola</i> , cat dander	> 17.5	4
2*	18	M	R, C	None	0.9	2
3	15	F	R, C	Olive	13.2	3
4	21	F	R, C	Grass, olive, <i>Salsola</i> , <i>Artemisia</i> , <i>Plantago</i> , dog and cat dander	2.2	2
5	22	M	R, C, A	Grass, olive, <i>Salsola</i> , <i>Plantago</i> , cat dander	2.8	2
6	19	F	R, C	Grass, olive, Dpt	> 17.5	4
7	29	M	R, C, A	Grass, <i>Plantago</i> , <i>Salsola</i> , <i>Artemisia</i>	6.2	3
8	20	M	R, C, A	Olive, <i>Salsola</i> , <i>Alternaria</i>	13.5	3
9	26	F	R, C	Grass, olive, <i>Artemisia</i>	0.6	1
10	13	F	R, C	Grass, olive, Dpt, cat dander	3.4	2
11	25	F	R, C, A	Olive, <i>Salsola</i> , <i>Plantago</i>	0.9	2
12	32	M	R, C	Grass, olive, <i>Salsola</i>	2.4	2
13*	12	M	R, C	Grass, olive	> 17.5	4

A: asthma; R: rhinitis; C: conjunctivitis.

Dpt: *Dermatophagoides*.

* RAST values of patients with allergic symptoms (rhinitis or asthma/rhinitis).

by SDS-PAGE immunoblotting. No IgE-binding bands could be demonstrated by immunoblotting when normal sera from nonatopic and unexposed

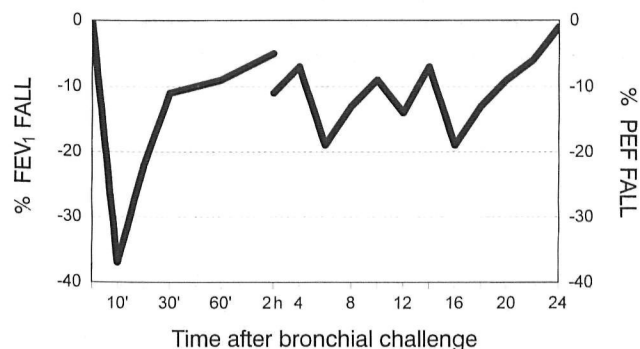


Fig. 1. FEV₁ and PEF recordings in patient 1 after inhalation of 0.05 HEP of saffron pollen extract.

subjects were analyzed. Fig. 3 shows IgE-binding fractions to saffron stamen extract based on the molecular weight; the presence of a 15.5-kDa IgE-binding component in all the sera studied could be demonstrated with both pollen and stamen extracts. A lower 14.0-kDa IgE-binding band was detected in seven of the studied sera.

For identification of the 15.5-kDa protein as profilin, its binding to PLP peptides was assayed by affinity chromatography on a PLP-CNBr-activated Sepharose column. The fraction retained was further chromatographed on a gel-filtration column (SMART System). The resulting profile is shown in Fig. 4. One main peak was obtained, corresponding to a mol. wt. of 15.5-kDa and containing two components with profilinic nature of about 15.5- and 14.0-kDa, respectively, as resolved by SDS-PAGE.

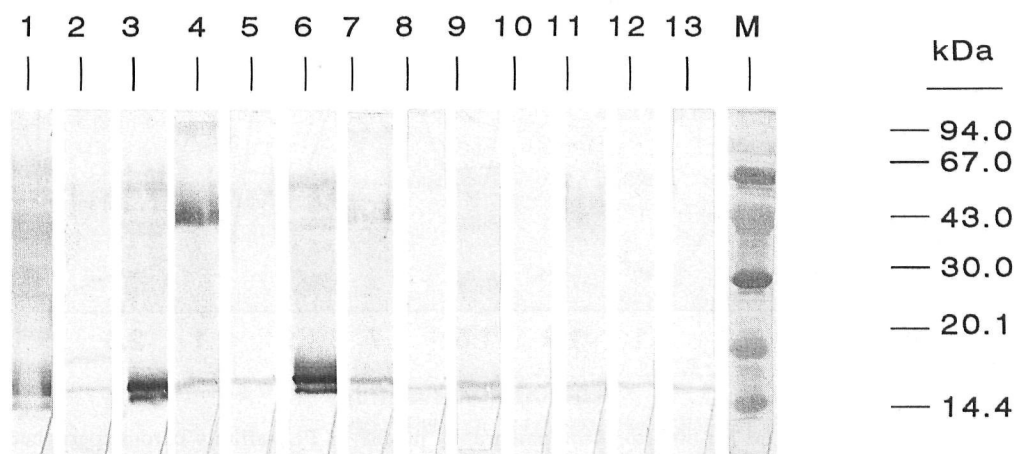


Fig. 2. SDS-PAGE immunoblotting with saffron pollen extract of 13 sensitized patients.

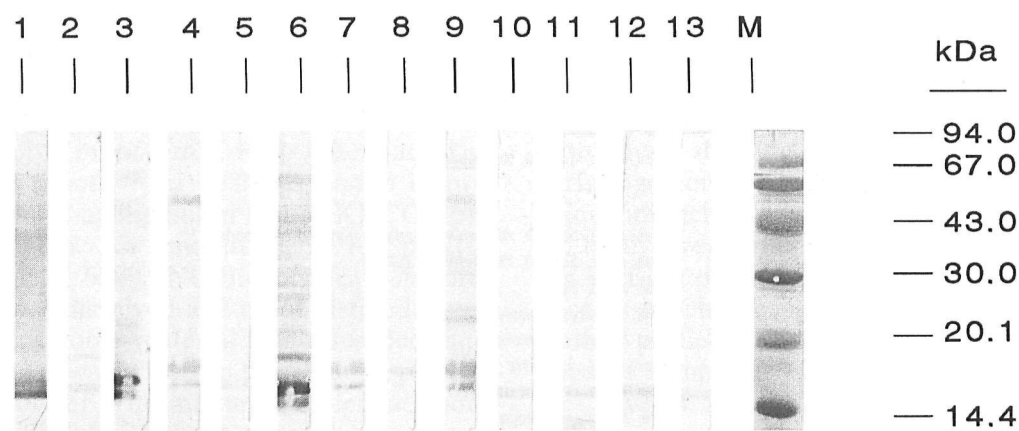


Fig. 3. SDS-PAGE immunoblotting with saffron stamen extract of 13 sensitized patients.

Fig. 5 shows the extent of IgE binding by purified profilin when human sera from eight of the patients sensitized to saffron extracts components (those with the most intensive IgE-binding pattern) were used.

Fig. 6 shows the inhibition curves with the saffron-allergenic solid-phase and other pollen allergens as inhibitors and a pooled serum from saffron-sensitized subjects with RAST values higher than or equal to class 3. The highest inhibitions obtained at 10 mg/ml concentration were 90, 47, 40, and 20% with saffron, *Olea*, *Salsola*, and *Lolium*, respectively,

as inhibitory liquid-phase allergens. Their respective slopes were 37, 9, 19, and 9.

SDS-PAGE immunoblotting inhibition was used to investigate the protein components involved in the overall inhibitory effect. As shown in Fig. 7, preincubation of the serum pool from saffron-sensitized patients (with RAST values $\geq$ class 3) with saffron pollen extract at 10 mg/ml completely blocked IgE binding. When *Lolium*, *Olea*, and *Salsola* pollen extracts were used as inhibitors, a significant degree of inhibition was reached, affecting several IgE-binding bands, mainly in the 27–67-kDa

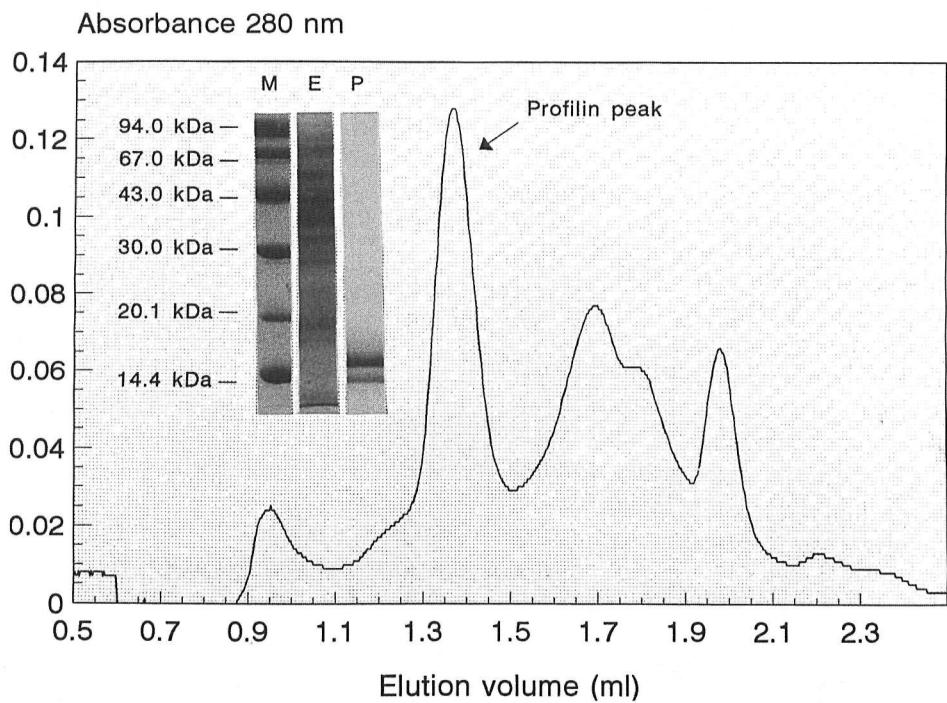


Fig. 4. Micropreparative high-resolution gel-filtration chromatography profile of PLP-affinity chromatographed saffron pollen extract. Main peak corresponding to 15.5-kDa purified profilin can be observed. M) SDS-PAGE of molecular weight markers; E) SDS-PAGE of saffron pollen extract; P) SDS-PAGE of main chromatographic peak of purified profilin.

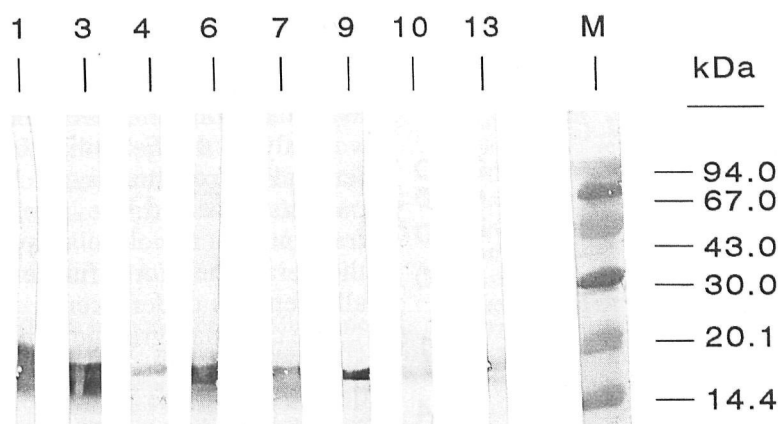


Fig. 5. SDS-PAGE immunoblotting with purified profilin of eight sensitized patients.

interval, with the exception of a 42-kDa band, which was not inhibited. A saffron IgE-binding component of 18.5 kDa was inhibited only by the *Olea* pollen extract. Finally, of the two profilin IgE-binding bands, the one with lower mol. wt. (14.0 kDa) was preferentially inhibited by the three studied extracts.

Discussion

Saffron is widely grown in Ciudad Real province. Due to the low profitability of this product, it is no longer of major industrial interest, but is a family labor activity. Saffron flowers are collected daily, and their stigmas are manually excised by all the members of the families involved. This is done indoors during the annual picking season (20–25 days), for 10–12 h a day (20), thus causing exposure to saffron-sensitizing elements such as pollen grains and stigma particles. The temporary character of saffron collection and its contribution as a mere supplement to family incomes entail noncontinuous exposure over the years, and the workers are children and adults without regular jobs (older than

65 years, housewives), whose saffron collection experience ranges from 2 to 47 years, with an average of 18.4.

In the present study, we observed that inhalation of saffron particles had sensitized 6% of the exposed workers, who showed IgE-mediated mechanisms and clinical expressions such as rhinoconjunctivitis (two cases) and asthma (one case).

Patients noted the onset of symptoms during the saffron season (October–November), and their remission once the collection season had ended. The occupational character of this sensitization seems to be demonstrated by the fact that these three patients noted clinical symptoms upon handling of saffron flowers, one of them (monosensitized to saffron) only during this activity, and the two others also in May and June due to their pollinosis.

The saffron-allergic worker suffering from asthma showed a 37% decrease of FEV₁ in the specific bronchial provocation test, followed by a complete recovery after 30 min (Fig. 1). This fact suggests an irritant reaction. However, these patients also exhibited positive cutaneous tests and specific IgE to saffron pollen, validating the specificity of the

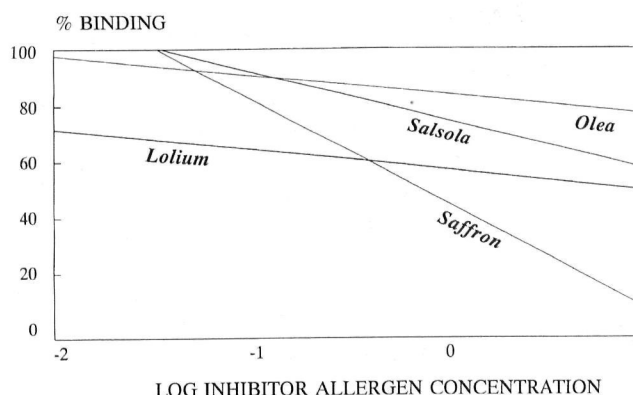


Fig. 6. RAST inhibition curves using saffron pollen extract in solid-phase and saffron, *S. kali*, *L. perenne*, and *O. europaea* as inhibitors.

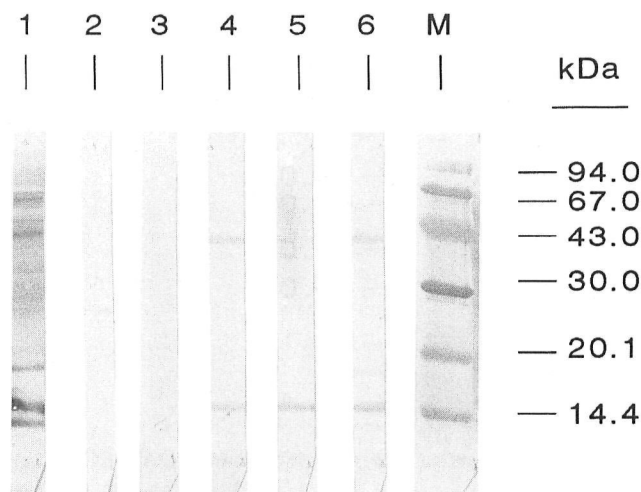


Fig. 7. SDS-PAGE immunoblotting inhibition of saffron pollen extract, using serum pool of patients showing RAST values to saffron $\geq$ class 3 and different pollen extracts as inhibitors. Lane 1) noninhibited saffron blot; 2) negative control, i.e., extract incubated with serum pool of unexposed patients; 3–6) blots inhibited with pollen extracts (at 0.1 mg/ml) of saffron, *Lolium*, *Olea*, and *Salsola*, respectively.

reaction. Besides, the bronchial provocation test was negative in five unexposed asthmatic patients and in five asymptomatic subjects, thus demonstrating the absence of pharmacologic or irritant mechanisms.

The continental climate of Ciudad Real province (dryness and extreme temperatures) causes a high pollinosis incidence, affecting 90% of patients suffering from rhinitis and/or asthma due to inhalant allergens. Therefore, we decided to study the incidence of sensitization to saffron pollen among the patients attending our allergy unit. Inclusion criteria were as follows: diagnosis of asthma and/or rhinitis, no previous exposure to saffron, and allergy to pollens commonly found in the area. We found that 4.2% of these patients had positive cutaneous tests. Since the saffron plant exhibits entomophilous pollination, these sensitizations could be due to cross-reactivity with other taxonomically related pollens (21).

Once the involvement of saffron pollen and stamen components in occupational allergy had been demonstrated by positive cutaneous tests and IgE determination by RAST (Table 1), we aimed to analyze the allergenic proteins and to study the cross-reactivity between saffron pollen and pollens from weeds to which the study population were sensitized.

SDS-PAGE immunoblotting of 13 sera – using saffron pollen extract – showed a very similar pattern to the SDS-PAGE immunoblotting with saffron stamen extract (Figs. 2 and 3), suggesting the presence of common proteins in both tissues.

Strikingly, the pistil (stigma), the part of the flower collected, unlike the stamen and pollen, does not cause sensitization, since the cutaneous test, RAST, and immunoblotting were negative.

Analysis of IgE-binding fractions of saffron pollen and saffron stamens in 13 sera from sensitized patients revealed the presence of one relevant fraction with a molecular weight of 15.5 kDa in all the sera. Therefore, further isolation of such an allergen was undertaken.

The chromatographic purification of this component and the subsequent immunoblotting analysis demonstrated the profilinic nature of this protein revealed as the main saffron allergen, as shown in Fig. 4 and 5. This relevant saffron allergen exhibited PLP-binding capacity, as it could be purified by affinity chromatography with this ligand, and it also presented mol. wts. in gel-filtration chromatography and SDS-PAGE (Fig. 4) concordant with the ones described for profilins (22). Finally, the doublet bands (15.5 and 14.0 kDa) obtained in SDS-PAGE could bind IgG from rabbit anti-sunflower profilin sera. This doublet band has also been found in some other profilins, being tentatively explained as differential glycosylated isoforms or as proteolytic derivatives (23), although they could also be natural profilin isoforms differing in some amino acid residue, one of them (15.5 kDa) showing a higher IgE reactivity, as already found for other isoallergens (24). All the data mentioned above corroborated the profilinic nature of the main allergen detected in saffron extracts. This allergen in the purified state retained its IgE-binding capacity (Fig. 5).

Although an important and reactive allergen, the relative proportion of the profilin in the total protein content of the saffron extract is low, since the bands were almost undetectable after SDS-PAGE staining of the whole extract (lane E, Fig. 4).

Profilinic proteins from other extracts have also been described as important allergens (17, 22, 25), being partly responsible for a large number of allergenic cross-reactivities.

The difference in slope values and Ag50, corresponding to the different linear regressions in RAST inhibition (Fig. 6), demonstrated a significant but low similarity in allergen composition between saffron pollen and pollen from other weeds. These results seemed to agree with the distant phylogenetic relationship between saffron and the other genera studied. However, a higher degree of inhibition was obtained by SDS-PAGE immunoblotting after preadsorption of the saffron-positive serum pool with the other pollen extracts analyzed (Fig. 7). It was demonstrated that several proteins in the 27–67-kDa range were mainly involved in the IgE-binding inhibition together with the saffron

profilin isoform of lower molecular weight (14.0 kDa), which should probably present higher homology than the other profilins included in the complete pollen extracts tested. A further search focused on that possible homology, as well as new cross-reactivity studies of various relevant allergenic pollens, should be undertaken in the near future.

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