

# Allergenicity of Wine Containing Processing Aids: A Double-Blind, Placebo-Controlled Food Challenge

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## ■ Abstract

*Background:* The European Union requires allergenic food ingredients to appear on labels in order to protect allergic consumers.

*Objective:* To determine whether traces of egg-, milk-, and fish-derived processing aids used in winemaking might elicit clinical reactions in food-allergic patients.

*Methods:* Five German wines were fined with a high dose of egg albumin, lysozyme, milk casein, fish gelatin, or isinglass, and filtered. Fourteen adults with allergy to egg (n=5), milk (n=5), or fish (n=4) were included. Skin prick tests were performed with fining agents, and fined and unfined wines. All patients underwent double-blind placebo-controlled food challenges with fined and unfined wines.

*Results:* Skin prick tests were positive to hen's egg (n=5), ovalbumin (n=5), lysozyme (n=4), cow's milk (n=5), casein (n=4), and cod (n=3), but not to isinglass or fish gelatin (n=0). Positive skin prick test results were observed for wines fined with albumin (n=3), lysozyme (n=2), casein (n=1), gelatin (n=0), and isinglass (n=3), and for unfined wines (n=1-2 in each patient group), with no significant differences between groups. Seventy-five percent of skin test-positive patients had specific immunoglobulin E to other allergens present in wine (eg, carbohydrates). The provocation test revealed no reactions to fined or unfined wines.

*Conclusions:* Although concentrated fining agents containing ovalbumin, lysozyme, and casein were allergenic in the skin prick test, no patient reacted adversely in the provocation test to fined wine. Wines treated with fining agents at commercial concentrations appear not to present a risk to allergic individuals when filtered.

**Key words:** Food allergy. Allergen labeling. Wine. Processing aids. Fining agent. Albumin. Lysozyme. Casein. Fish gelatin. Isinglass.

## ■ Resumen

*Antecedentes:* La Unión Europea requiere que los ingredientes alergénicos alimentarios aparezcan en las etiquetas para proteger a los consumidores alérgicos.

*Objetivos:* Determinar si las trazas de aditivos alimentarios derivados de huevo, leche y pescado empleados en la elaboración del vino podrían desencadenar reacciones clínicas en pacientes con alergia alimentaria.

*Métodos:* Cinco vinos alemanes fueron clarificados con altas dosis de albúmina de huevo, lisozima, caseína láctea, gelatina de pescado, o mica, y filtrados. Se incluyeron 14 adultos alérgicos a huevo (n=5), a leche (n=5), o pescado (n=4). Se realizaron pruebas cutáneas con los agentes de clarificado, y con vinos clarificados y no clarificados. Todos los pacientes llevaron a cabo una provocación alimentaria doble ciego controlada con placebo con vinos clarificados y no clarificados.

*Resultados:* Las pruebas cutáneas fueron positivas para el huevo de gallina (n=5), ovoalbúmina (n=5), lisozima (n=4), leche de vaca (n=5), caseína (n=4), y bacalao (n=3), pero no a la mica o a la gelatina de pescado (n=0). Se observaron pruebas cutáneas positivas para los vinos clarificados con albúmina (n=3), lisozima (n=2), caseína (n=1), gelatina (n=0), y mica (n=3), y para los vinos no clarificados (n=1-2 en cada grupo de pacientes), con diferencias no significativas entre los grupos. Setenta y cinco por ciento de los pacientes con pruebas cutáneas positivas tuvieron inmunoglobulina E específica a otros alérgenos presentes en el vino (p. ej. carbohidratos). La provocación reveló la ausencia de reacciones al vino clarificado y al no clarificado.

*Conclusiones:* Aunque los agentes clarificantes que contenían ovoalbúmina, lisozima, y caseína fueron alergénicos en la prueba cutánea, ningún paciente reaccionó adversamente en la provocación al vino clarificado. Los vinos tratados con agentes clarificantes a las concentraciones comerciales parece que no presentan riesgo para los individuos alérgicos cuando se filtran.

**Palabras clave:** Alergia alimentaria. Etiquetado de alérgenos. Vino. Aditivos alimentarios. Agentes clarificantes. Albúmina. Lisozima. Caseína. Gelatina de pescado. Mica.

## Introduction

Food allergy is an increasingly prevalent health problem in Western countries [1,2]. It can manifest as life-threatening anaphylactic shock, with about 150 food-allergy-related deaths per year in the USA alone [3]. In order to achieve a high level of protection for food-allergic individuals, the listing of all allergic ingredients in processed food became mandatory as a result of legislation passed in the European Union (EU) in 2003 [4,5]. Consequently, allergens such as hen's egg, cow's milk, or fish must be indicated on the label of food products when they or their derivatives are used in the production of foodstuffs. This provision also applies to alcoholic beverages, such as wine [6].

During the wine fining process, products from hen's egg, cow's milk, and fish are used as processing aids, and traces may remain in the final product. Fining serves to remove insoluble and colloidal substances, as well as astringent compounds such as tannins. Several fining agents can be used, including albumin and lysozyme (extracted from hen's egg), cow's milk casein, fish gelatin, and isinglass, a collagen preparation from the swim bladder of certain fish. These fining agents coagulate with the particles and colloids present in the wine, resulting in flocculation and sedimentation of those substances, which can then be filtered. Estimates show that up to 20% of German wines are clarified with casein, about 7% with isinglass, and 2% with egg white (ovalbumin) [7].

Anaphylactic reactions following wine consumption have not been attributed to fining agents containing egg, milk, or fish in the scientific literature. *In vitro* data on the allergenicity of fined German wines using a new enzyme-linked immunosorbent assay (ELISA) have revealed undetectable *in vitro* allergen levels for milk and fish proteins. For egg white and lysozyme, concentrations were at the level of detectability, which was estimated to be 0.2 ppm and 0.01 ppm, respectively [8]. Thus, allergic reactions to wines treated with these fining agents could not be excluded.

The objective of this study was to examine whether traces of allergen-containing processing aids in wine can elicit allergic reactions in susceptible individuals. We performed a double-blind, placebo-controlled food challenge (DBPCFC) in adult patients who were allergic to egg, milk, and fish using German wines fined with a high dose of agents derived from these proteins. We show that, although the allergenicity of the fining agents ovalbumin, lysozyme, and casein was proven by skin prick tests (SPTs), there was no increased skin test reactivity to fined wines in our patients. Furthermore, all patients tolerated fined wines in the provocation test.

## Materials and Methods

### Fining Agents

The fining agents tested in this study were hen's egg albumin, hen's egg lysozyme, 2 commercial casein fining agents (potassium caseinate 1 and 2), 3 isinglass fining agents (isinglass 1, 2, and 3), and fish gelatin from the producers (Erbslöh Getränke-Technologie GmbH, Geisenheim,

Germany and E. Begerow GmbH & Co., Langenlohnheim, Germany).

### Wines

The wines investigated were representative of 5 different wine-growing regions in Germany and included 4 white wine varieties (Riesling Mosel, Riesling Rheingau, Pinot blanc Pfalz, Pinot gris Baden) and 1 red wine variety (Dornfelder Rheinhessen). In order to ensure that reactions were detected, even at threshold levels, each wine was fined separately with each agent at a dose 5 times higher than that used in commercially available wines, with the exception of lysozyme, for which only twice the amount could be added according to EU regulations (Table 1). Wines were filtered after fining according to the standard process. The allergen content of the filtered wines was determined and found to be equal to the detection level of the ELISA for egg albumin and egg lysozyme, and estimated to be 0.2 ppm (Riesling Rheingau only) and 0.01 ppm (all white wines), respectively [8]. For isinglass, fish gelatin, and potassium caseinate, the allergen content was below the level of detection for all the wines tested. For each wine, controls with no fining agent were provided as a placebo. In order to exclude intolerance, histamine and sulfite levels were tested and found to be in a range of 1.0 to 1.7 mg/L and 70 to 99 mg/L, respectively. A recent study has shown that there was no correlation between wine intolerance and histamine content up to 13.8 mg/L [9].

Table 1. Fining Agents and Doses

Fining Agent	Recommended Dose/hL of Wine	Excess Dose/hL of Wine
Egg albumin	4 g	20 g
Lysozyme	25 g	50 g
Potassium caseinate	6 g	30 g
Isinglass	50 mL	250 mL
Fish gelatin	10 g	50 g

### Patients

Screening the results of *in vitro* allergy tests from the Department of Dermatology and Allergy (approximately 35 000 outpatient visits per year) of our institution revealed 261 adults with specific immunoglobulin (Ig) E antibodies to food from January 2004 until May 2006 [10]. After reviewing the patients' medical records, we contacted those with a possible food allergy and interviewed them by telephone. Twenty-three out of 78 patients sensitized to the allergens of interest reported clinical symptoms after ingestion of the specific food. Thirteen of those 23 patients agreed to participate in the study. With the support of an allergy patient organization (Deutscher Allergie- und Asthmabund, DAAB), 1 additional patient was included. Thus, the study sample was composed of 14 patients (12 women, 2 men) with a median age of 47 years (range, 26 to 71 years) and food allergy confirmed by positive SPT results and/or appropriate specific IgE levels as measured by

the ImmunoCAP system (Phadia, Freiburg, Germany) (Tables 2-4) [11]. There were 5 egg-allergic female patients, 5 milk-allergic female patients, and 2 females and 2 males with an allergy to fish. The clinical history of allergy was clear-cut in all patients except patient 9, who had a previously unclear food allergy, and in whom a cumulative dose of 0.1 mL of milk in

a DBPCFC produced oral allergy syndrome and angioedema of the lip and tongue. The other patients' records revealed that they had reacted to 1 drop (n=5), 1 spoonful or forkful (n=2), a few spoonfuls or forkfuls (n=5), or 1 meal (n=1) containing the offending food, and that they had reacted several times (2-5 times [n=2], 6-10 times [n=2], and >10 times [n=9]).

Table 2. Clinical Characteristics of Hen's Egg-Allergic Patients

Patient no.	Age, y	Sex	Total IgE (kU <sub>A</sub> /L)	Egg White-Specific IgE (kU <sub>A</sub> /L)	Reported Symptoms After Allergen Ingestion	Atopic Diseases	SPT Reactions to Egg White (Wheal Size, mm)	History of Intolerance to Wine	
								Red wine	White wine
1	50	Female	4383	>100	OAS P FI U	A aE aR	19	-	Dys
2	31	Female	1321	2.66	OAS ED Dys	A aE aR	15	P Dys	P Dys
3	56	Female	4864	70	OAS P U ED N V AP D Cj Dys H	A aE aR	25	-	-
4	69	Female	588	9.57	OAS P FI U ED N Cj H	aE	10	-	-
5	56	Female	10835	33.6	OAS P U ED N V AP D C Cj	A aE aR	17	-	-

Abbreviations: A, bronchial asthma; aE, atopic eczema; AP, abdominal pain; aR, allergic rhinoconjunctivitis; C, cough or dysphonia; Cj, conjunctivitis; D, diarrhea; Dys, dyspnea; ED, eczema, deterioration; FI, flushing; H, hypotension; Ig, immunoglobulin; N, nausea; OAS, oral allergy syndrome; P, pruritus; SPT, skin prick test; T, tachycardia; U, urticaria; V, vomiting.

Table 3. Clinical Characteristics of Cow's Milk-Allergic Patients

Patient no.	Age, y	Sex	Total IgE (kU <sub>A</sub> /L)	Milk Specific IgE (kU <sub>A</sub> /L)	Reported Symptoms After Allergen Ingestion	Atopic Diseases	SPT Reactions to Cow Milk (Wheal Size, mm)	History of Intolerance to Wine	
								Red wine	White wine
6	46	Female	31929	17.0	ED D	A aE aR	8	-	ED
7	48	Female	442	0.2	OAS N V AP D R C Dys	A aE aR	4	R Cj	N
8	31	Female	1321	49.7	OAS P U D R Dys	A aE aR	10	P Dys	P Dys
9	56	Female	4864	>100	OAS P FI ED N Dys	A aE aR	15	-	-
10	26	Female	9647	87.8	OAS P FI ED R Cj	E	8	P FI	-

Abbreviations: A, bronchial asthma; aE, atopic eczema; AP, abdominal pain; aR, allergic rhinoconjunctivitis; C, cough or dysphonia; Cj, conjunctivitis; D, diarrhea; Dys, dyspnea; ED, eczema, deterioration; FI, flushing; H, hypotension; IgE, immunoglobulin E; N, nausea; OAS, oral allergy syndrome; P, pruritus; R, rhinorrhea; SPT, skin prick test; T, tachycardia; U, urticaria; V, vomiting.

Table 4. Clinical Characteristics of Fish-Allergic Patients

Patient no.	Age, y	Sex	Total IgE (kU <sub>A</sub> /L)	Cod Specific IgE (kU <sub>A</sub> /L)	Reported Symptoms After Allergen Ingestion	Atopic Diseases	SPT Reactions to Fish (Wheal Size, mm)		History of Intolerance to Wine	
							Cod	Tuna	Red wine	White wine
11	46	Female	31 929	9.63	OAS P F T	A aE aR	7	3	–	ED
12	41	Male	685	1.31	OAS P N Dys	A aR	15	8	Dysph	Dysph
13	36	Male	12 622	4.42	OAS	A aE aR	8	3	–	–
14	71	Female	5 652	0.8	P F U ED Cj	aE aR	0	0	P N	–

Abbreviations: A, bronchial asthma; aE, atopic eczema; AP, abdominal pain; aR, allergic rhinoconjunctivitis; C, cough or dysphonia; Cj, conjunctivitis; D, diarrhea; Dys, dyspnea; Dysph, dysphagia; ED, eczema, deterioration; Fl, flushing; H, hypotension; IgE, immunoglobulin E; N, nausea; OAS, oral allergy syndrome; P, pruritus; R, rhinorrhea; SPT, skin prick test; T, tachycardia; U, urticaria; V, vomiting.

All patients reported moderate to strong reactions occurring between 1 and 30 minutes (median 5 min) after ingestion of the relevant food. The symptoms elicited by the offending foods are listed in Table 2. Ten patients reported a wheal-and-flare reaction (contact urticaria) after direct skin contact with the allergen. Patients 1-5, 7-10, and 13 developed their food allergy in adulthood between 1.5 and 30 years ago, whereas patients 6, 11, 12, and 14 developed their allergy during childhood. For nearly all of the patients (13 of 14), the most recent reaction had occurred within the last year. Only patient 1, who had reacted >10 times, had not had a reaction for 10 years. Twelve patients were sensitized to inhalant allergens (birch pollen, grass pollen, and/or house dust mite). Five patients were confident they had always tolerated wine, whereas 9 patients reported occasional previous mild symptoms of pruritus, skin irritation, and shortness of breath rated as mild following the consumption of more than 1 glass of wine.

Prior to the study, ethical approval was obtained and written consent given by the patients. Two men and 3 women aged between 29 and 42 years (mean, 33 years) with (n=2) and without (n=3) allergic rhinoconjunctivitis and sensitizations to grass pollen, but not to known wine allergens, served as SPT controls.

#### SPT and Determination of Specific IgE Antibodies

SPTs were performed in duplicate on the volar surface of both forearms on 2 separate days. On day 1, SPTs were performed with food allergens, the fining agent in different dilution steps, and some of the fined and unfined wines, including those selected for challenge. On day 2, the remaining wines were tested. For patients allergic to egg, milk, and fish, we tested 7, 6, and 10 fined and 5 unfined (control) wines (Figure 2A, 2B, and 2C) to ensure that all wines and fining agents were represented. The wines selected included those with allergen contents at detection levels [8]. In addition, the respective fining agents were tested in different logarithmic dilutions. SPTs were performed using a 1-mm single peak lancet (Diaprx GmbH, Wesel, Germany) and reactions

were read after 20 minutes. Wheals larger than 3 mm with a surrounding flare were regarded as positive. Histamine 0.1% served as a positive control and physiological saline solution as a negative control. Specific IgE antibodies to food allergens, cross-reactive carbohydrate determinants (CCD) (MUXF3), and insect venom were determined using Phadia UniCAP FEIA (Freiburg, Germany). The levels of specific IgE antibodies at the beginning of the study are given in Tables 2-4.

#### DBPCFC

The food challenge with fined and unfined wines was performed double-blind according to European guidelines [12] on 2 days, with the placebo challenge and active challenge separated by at least 48 hours. Fined wine was not visually discernible from unfined wine. Successive doses were administered in 4 steps at 30-minute time intervals. After a labial challenge with 1 drop of wine, 1 mL of wine was followed by 10 mL and the rest of the dose of 189 mL for women and 289 mL for men. Medication that might influence the test results had been discontinued. The wines for the food challenge were Riesling Rheingau fined with ovalbumin, Riesling Mosel fined with casein, and Pinot blanc fined with isinglass. For the ovalbumin-fined Riesling Rheingau, allergen content at the detection level had been demonstrated [8]. The challenges were regarded as positive after observation of any of the following clinical reactions: erythema, wheal, exanthema, exacerbation of atopic eczema, angioedema, rhinorrhea, vomiting, diarrhea, abdominal pain, dyspnea, bronchospasm, bronchial asthma, drop in blood pressure, tachycardia, or cardiovascular shock. Subjective symptoms such as oral discomfort, pruritus, nausea, dyspnea, or headache were also recorded. In the cases with no reaction, women tolerated a cumulative dose of 200 mL and men 300 mL.

#### Statistical Analysis

Statistical analyses were performed using Microsoft Excel for Windows. Mean values are given. The Fisher exact test

was used for comparison. A *P* value of <.05 was considered statistically significant.

## Results

### SPT With Fining Agents

SPT results for ovalbumin in varying dilutions were positive in all 5 egg-allergic patients. The SPT result for lysozyme was also positive in 4 cases, and negative in 1.

Five milk-allergic patients were tested for the casein-derived fining agents with casein undiluted and diluted 1:10 and 1:100. In 4 cases, the SPT result was positive to casein, and in 1 case it was negative.

None of the 4 fish-allergic patients had a positive reaction to isinglass or to fish gelatin.

### SPT With Wines

The SPT with all test wines fined with ovalbumin and lysozyme was performed in 3 of 5 egg-allergic patients (3 patients × 7 fined wines). One patient could not be tested because of eczema on both forearms, another refused to take part after having experienced an anaphylactic reaction due to the SPT with egg protein, egg fining agents, and Riesling Rheingau (Table 2, patient 5, Figure 1A). She developed angioedema, pruritus, conjunctivitis, eyelid edema, and rhinorrhea 30 minutes after the SPT, which resulted in strongly positive reactions (wheal diameters >12 mm) to albumin, lysozyme, and egg proteins, but not to wine. The

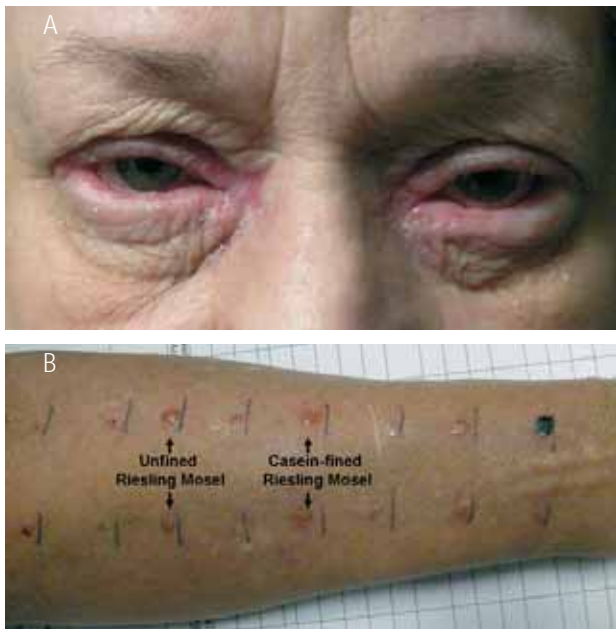


Figure 1. A, Skin testing with hen's egg proteins in patient 5 resulted in rhinoconjunctivitis, swelling of the eyelids, periorbital pruritus, and angioedema. B, The result of the skin prick test in patient 9 was positive to undiluted casein-fined as well as to unfined control Riesling Mosel with no significant differences.

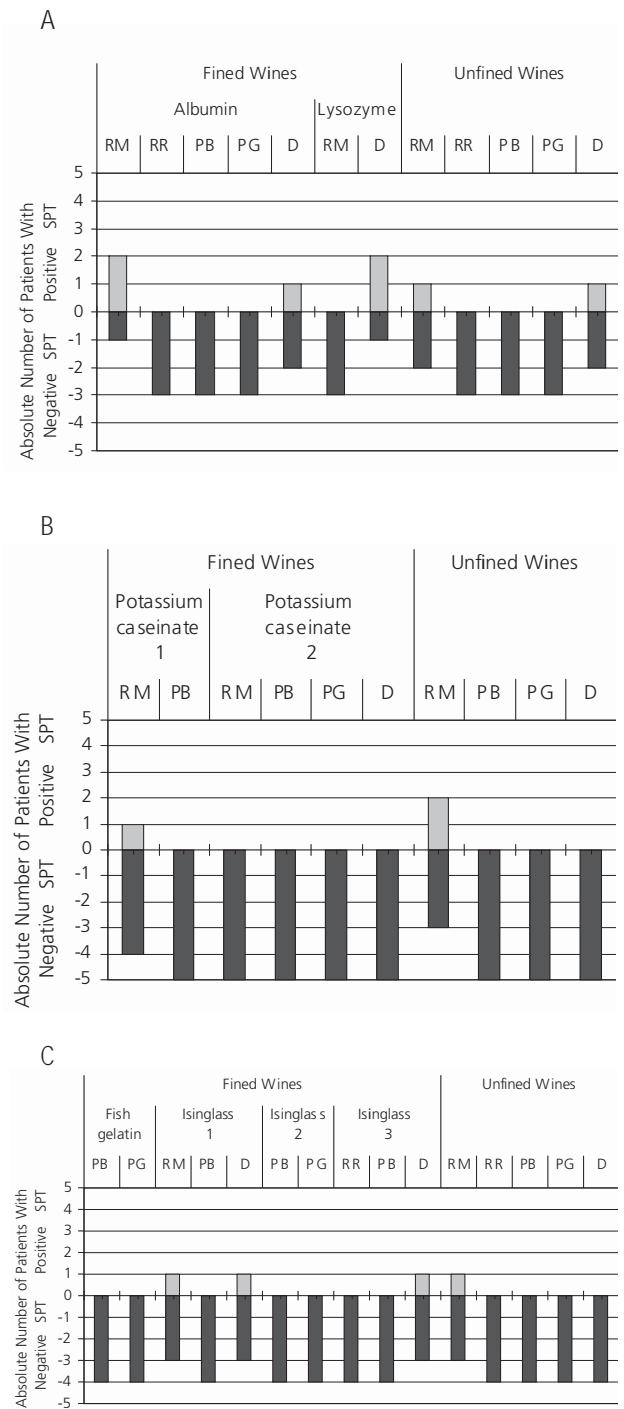


Figure 2. Skin prick test results with fined and unfined wines in patients allergic to egg, milk, and fish. The absolute number of patients with positive skin prick test reactions to wines fined with ovalbumin or lysozyme (A), casein (B), and fish protein (gelatin, 3 commercial isinglass products)-containing fining agents (C) and their respective controls is shown on the upper half of the graph, whereas the absolute number of patients who did not react is depicted on the lower half. RM indicates Riesling Mosel; RR, Riesling Rheingau; PB, Pinot blanc; PG, Pinot gris; D, Dornfelder.

reaction was treated immediately with 4 mg of dimethindene maleate and 50 mg of prednisolone maleate intravenously, and it subsided 1 hour later. The food challenge was repeated blinded 2 weeks later with fined and unfined wines, but with no preceding SPT. This time, the patient tolerated 200 mL of both wines without symptoms. After unblinding, it was revealed that the wine used in the first challenge was the unfined one; therefore, the reaction was probably due to the SPT.

Of the remaining 21 skin prick tests, 16 showed no reactions to fined wines (Figure 2A). Patients 3 and 4 reacted to wines fined with albumin (egg white below detection level) and patients 2 and 4 to a wine fined with lysozyme (lysozyme levels estimated to be 0.01 ppm). The SPT with the respective unfined wines elicited 13 negative reactions and 2 positive reactions in patient 4. The difference between fined and unfined wines was not significant ( $P > .49$ ). The only wine with egg white allergen content at the detection level (Riesling Rheingau, 0.2 ppm) did not elicit a positive SPT response.

The SPT with casein-fined wines was performed in all 5 patients (Figure 2B). In 29 of 30 tests (5 patients  $\times$  6 fined wines), the SPT result for fined wines was negative. Patient 9 showed a positive SPT result for fined Riesling Mosel, and patients 8 and 9 for unfined Riesling Mosel, with no significant differences ( $P > .35$ ) (Figures 1B and 2B).

All 4 fish-allergic patients were tested with Pinot blanc and Pinot gris fined with fish gelatin, as well as Riesling Mosel, Riesling Rheingau, Pinot blanc, Pinot gris, and Dornfelder fined with isinglass. In 37 of 40 tests (4 patients  $\times$  10 fined wines), the SPT to fined wines was negative. In patients 11 and 13, the SPT results were positive to isinglass-fined wines, and patient 13 had a positive SPT result to an unfined wine, whereas the other 19 SPT results for unfined wines were negative (Figure 2C). There was no significant difference between fined and unfined wines ( $P > .56$ ). Patients 1, 3-6, 9, 11, and 13 also had specific IgE levels between 1.7 kU<sub>A</sub>/L and 28 kU<sub>A</sub>/L to cross-reactive carbohydrate determinants, grapes, and/or hymenoptera venom. Sensitizations to such allergens may be a better explanation for positive skin test results with wine in atopic patients.

The SPT results for all wines were negative in all 5 non-food-sensitized controls.

### DBPCFC

Five egg-allergic patients, 5 milk-allergic patients, and 4 fish-allergic patients underwent DBPCFC with both the fined and unfined wines; they all received the total cumulative dose (200 mL for women and 300 mL for men). None of the 14 individuals challenged in the DBPCFC showed a clinical reaction to fined wines or to unfined wines. No subjective symptoms were reported.

## Discussion

Changes in EU legislation aim to guarantee the safety of allergic consumers by listing all allergenic ingredients. It remains unknown whether European wines fined with agents containing egg protein, milk protein, and fish protein have to be labeled, as their allergenicity has not yet been examined.

The results of this study demonstrate that wines treated with fining agents containing egg, milk, or fish were tolerated by patients who were allergic to egg, milk, or fish. A limitation of the study is that patients were included without confirming their allergy by DBPCFC. Hence, tolerance could not be excluded. However, a DBPCFC is often rejected in adult patients with a clear-cut food allergy—that is, patients who have reacted several times after accidental food intake—and was not indicated in most patients in our study because of previous anaphylaxis [12]. Only patients with a clear-cut history of food allergy and relevant demonstrable IgE-mediated sensitization to the allergens of interest were enrolled in this low-dose oral challenge test, which was performed according to a consensus protocol [13]. All patients were highly atopic and most suffered from the triad of atopic eczema, allergic rhinoconjunctivitis, and bronchial asthma. The number of patients identified with allergy to egg, milk, or fish was surprisingly low considering that patients were screened over a 2.5-year period. This result was similar to that of a report in Munich in 1990, in which 5 patients with clinically relevant cow-milk allergy were identified over a 16-year period [14]. In an Australian study [15], DBPCFCs were conducted with a panel of commercially available Australian fined wines. Five egg-sensitive patients, 1 milk-sensitive patient, and 10 fish-sensitive patients were included over a 3-year period. This again suggests a low number of milk- and egg-sensitive adult patients and a higher prevalence of fish sensitivity in coastal regions. Milk and egg allergies are common in infants; however, most of them develop tolerance during early childhood [16]. An overall prevalence of food allergy in adults of 2.6% has been reported in a German population sample [17]. Investigations on the prevalence of food hypersensitivity in adults reveal prevalence rates of 0.1%, 0.3%, and 0.2% to hen's egg, cow's milk, and codfish, respectively [18].

In this study, positive SPT reactions to egg albumin, egg lysozyme, and milk casein demonstrated the allergenicity of the concentrated wine fining products. Nevertheless, the fish-containing fining agents gelatin and isinglass, even in their concentrated form, did not elicit SPT reactions in our patients. These data strongly indicate that gelatin and isinglass will not produce clinical reactions in much more diluted solutions, such as those present in wine. This is also consistent with data on the allergenic potential of fish gelatin in DBPCFC studies, which conclude that neither codfish gelatin nor tuna skin gelatin seems to present a risk to sensitized individuals [19,20].

In SPT with nonstandardized matrices such as wine, nonspecific test reactions may occur, especially in patients with long-standing atopic eczema, as described by Brockow et al for orange juice in 58% of tested patients [21]. In the present study, there were few positive SPT reactions with wines fined with albumin, lysozyme, casein, and isinglass, or unfined wines, and the differences were not significant. No specific reaction to fining agents in wines was demonstrable in the SPT. However, given the highly atopic status of the patients, other proteins contained in wines, such as bacterial or yeast products, or cross-reacting proteins could yield positive SPT results in sensitized patients. In fact, 75% of patients with a positive SPT result for wine had specific IgE to grape, cross-reactive carbohydrate determinants, or insect venom. This could be a possible alternative explanation [22,23].

The DBPCFC is considered the gold standard diagnostic test for detecting food allergy [12]. The lowest threshold levels to egg white range from 1 mg to 2 mg [24]. In consideration of the estimated allergen content of the wine used in our study, this would correspond to 1.2 L of Riesling Rheingau [8]. In a DBPCFC, Morisset et al observed that 5.6% of egg-allergic patients reacted to 15 mg of egg white and 1.7% of milk-allergic patients reacted to 0.3 mL of milk [25]. In the present study, in which 14 patients with allergy to hen's egg, cow's milk, or fish were challenged with white wines fined with a 5-fold higher dose of fining agents than normally used, the no observable adverse event level based on reactions was 200-300 mL of wine. In 1 egg-allergic patient, the result of DBPCFC to albumin-fined wine was negative, despite an anaphylactic reaction occurring after SPT with egg protein and egg-derived fining agents, thus indicating a severe allergy [26]. It should be noted that filtration techniques may differ between producers and countries, and the results of this study should not be transferred to unfiltered wines, where higher traces of fining agents may be present.

The only comparable data to those of the present study come from an Australian group [15], who performed 67 DBPCFCs with a single 100-mL dose of commercial fined and unfined wines in patients with a history of allergy and corresponding specific IgE or positive SPT results for peanut (n=10), fish (n=10), egg (n=5), or milk (n=1). However, no SPTs were performed with fining agents or wines, and the allergen levels of the wines tested in the challenge were not provided. After challenge, the authors observed an 11% decrease in the forced expiratory volume in 1 second (FEV<sub>1</sub>) to egg-fined wine, but also a 22% decrease in FEV<sub>1</sub> to unfined wine in the same patient, a 13% decrease in FEV<sub>1</sub> to peanut-fined wine, and lip numbness to unfined wine. The relevance of these symptoms remains unclear, as other proteins in wine (eg, grape proteins) could have caused these reactions [23]. However, it should be noted that 1 patient reported a "lump in the throat" after ingestion of milk-fined wine, and mild itch in the repeat challenge, but no reaction to unfined wine. Thus, an initial allergic reaction to milk-fined wine in this patient cannot be excluded. Subjective symptoms lacking objective reactions after administration of very low doses of allergen have also been described in other studies [27]. No further information on the wine triggering the repeated reactions was available. It is possible that the wine may not have been filtered, resulting in less clearance of proteins and a high allergen content.

In conclusion, despite the allergenic potential of ovalbumin, lysozyme, and casein in undiluted fining agents as demonstrated by SPT, none of the highly allergic patients reacted adversely in the oral wine provocation with the consumption of 200 or 300 mL of wine. Thus, the risk of allergic reactions elicited by traces of fining agents in wines following filtration appears to be negligible.

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## References

1. Ring J, Brockow K, Behrendt H. Adverse reactions to foods. *J Chromatogr B Biomed Sci Appl.* 2001;756:3-10.
2. Sampson HA. Update on food allergy. *J Allergy Clin Immunol.* 2004;113:805-19.
3. Sampson HA. Anaphylaxis and emergency treatment. *Pediatrics.* 2003;111:1601-8.
4. European Commission. Directive 2000/13/EC of the European Parliament and of the Council of 20 March 2000 on the approximation of the laws of the Member States relating to the labelling, presentation and advertising of foodstuffs. *Official Journal of the European Commission.* 2000;L109:29-42.
5. European Commission. Directive 2003/89/EC of the European Parliament and of the Council of 10 November 2003 amending Directive 2000/13/EC as regards indication of the ingredients present in foodstuffs. *Official Journal of the European Union.* 2003;L308:15-18.
6. Sicherer SH, Sampson HA. 9. Food allergy. *J Allergy Clin Immunol.* 2006;117:S470-5.
7. Deutscher Weinbauverband: [www.dwv-online.de](http://www.dwv-online.de)
8. Weber P, Steinhart H, Paschke A. Investigation of the allergenic potential of wines fined with various proteinogenic fining agents by ELISA. *J Agric Food Chem.* 2007;55:3127-33.
9. Kanny G, Gerbaux V, Olszewski A, Fremont S, Empereur F, Nabet F, Cabanis JC, Moneret-Vautrin DA. No correlation between wine intolerance and histamine content of wine. *J Allergy Clin Immunol.* 2001;107:375-8.
10. Sampson HA. Utility of food-specific IgE concentrations in predicting symptomatic food allergy. *J Allergy Clin Immunol.* 2001;107:891-6.
11. Bruijnzeel-Koomen C, Ortolani C, Aas K, Bindslev-Jensen C, Bjorksten B, Moneret-Vautrin D, Wuthrich B. Adverse reactions to food. *European Academy of Allergy and Clinical Immunology Subcommittee. Allergy.* 1995;50:623-35.
12. Bindslev-Jensen C, Ballmer-Weber BK, Bengtsson U, Blanco C, Ebner C, Hourihane J, Knulst AC, Moneret-Vautrin DA, Nekam K, Niggemann B, Osterballe M, Ortolani C, Ring J, Schnopp C, Werfel T. Standardization of food challenges in patients with immediate reactions to foods--position paper from the European Academy of Allergy and Clinical Immunology. *Allergy.* 2004;59:690-7.
13. Taylor SL, Hefle SL, Bindslev-Jensen C, Atkins FM, Andre C, Bruijnzeel-Koomen C, Burks AW, Bush RK, Ebisawa M, Eigenmann PA, Host A, Hourihane JO, Isolauri E, Hill DJ, Knulst A, Lack G, Sampson HA, Moneret-Vautrin DA, Rance F, Vadas PA, Yunginger JW, Zeiger RS, Salminen JW, Madsen C, Abbott P. A consensus protocol for the determination of the threshold doses for allergenic foods: how much is too much? *Clin Exp Allergy.* 2004;34:689-95.
14. Jessberger B, Rakoski J. Kuhmilchallergie beim Erwachsenen. *Allergologie.* 1991;14:133-6.
15. Rolland JM, Apostolou E, Deckert K, de Leon MP, Douglass JA,

- Glaspole IN, Bailey M, Stockley CS, O'Hehir RE. Potential food allergens in wine: Double-blind, placebo-controlled trial and basophil activation analysis. *Nutrition*. 2006;22:882-8.
16. Sampson HA. Food allergy. Part 2: diagnosis and management. *J Allergy Clin Immunol*. 1999;103:981-9.
  17. Zuberbier T, Edenharter G, Worm M, Ehlers I, Reimann S, Hantke T, Roehr CC, Bergmann KE, Niggemann B. Prevalence of adverse reactions to food in Germany - a population study. *Allergy*. 2004;59:338-45.
  18. Osterballe M, Hansen TK, Mortz CG, Host A, Bindslev-Jensen C. The prevalence of food hypersensitivity in an unselected population of children and adults. *Pediatr Allergy Immunol*. 2005;16:567-73.
  19. Hansen TK, Poulsen LK, Stahl Skov P, Hefle SL, Hlywka JJ, Taylor SL, Bindslev-Jensen U, Bindslev-Jensen C. A randomized, double-blinded, placebo-controlled oral challenge study to evaluate the allergenicity of commercial, food-grade fish gelatin. *Food Chem Toxicol*. 2004;42:2037-44.
  20. Andre F, Cavagna S, Andre C. Gelatin prepared from tuna skin: a risk factor for fish allergy or sensitization? *Int Arch Allergy Immunol*. 2003;130:17-24.
  21. Brockow K, Hautmann C, Fotisch K, Rakoski J, Borelli S, Vieths S, Ring J. Orange-induced skin lesions in patients with atopic eczema: evidence for a non-IgE-mediated mechanism. *Acta Derm Venereol*. 2003;83:44-8.
  22. Armentia A, Pineda F, Fernandez S. Wine-induced anaphylaxis and sensitization to hymenoptera venom. *N Engl J Med*. 2007;357:719-20.
  23. Sbornik M, Rakoski J, Mempel M, Ollert M, Ring J. IgE-mediated type-I-allergy against red wine and grapes. *Allergy*. 2007;62:1339-40.
  24. Moneret-Vautrin DA, Kanny G. Update on threshold doses of food allergens: implications for patients and the food industry. *Curr Opin Allergy Clin Immunol*. 2004;4:215-9.
  25. Morisset M, Moneret-Vautrin DA, Kanny G, Guenard L, Beaudouin E, Flabbee J, Hatahet R. Thresholds of clinical reactivity to milk, egg, peanut and sesame in immunoglobulin E-dependent allergies: evaluation by double-blind or single-blind placebo-controlled oral challenges. *Clin Exp Allergy*. 2003;33:1046-51.
  26. Liccardi G, D'Amato G, Canonica GW, Salzillo A, Piccolo A, Passalacqua G. Systemic reactions from skin testing: literature review. *J Investig Allergol Clin Immunol*. 2006;16:75-8.
  27. Hourihane JB, Kilburn SA, Nordlee JA, Hefle SL, Taylor SL, Warner JO. An evaluation of the sensitivity of subjects with peanut allergy to very low doses of peanut protein: a randomized, double-blind, placebo-controlled food challenge study. *J Allergy Clin Immunol*. 1997;100:596-600.

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