

**Opinion of the Scientific Panel on Dietetic Products, Nutrition and Allergies
on a request from the Commission related to a notification from DWV and
VINIFLHOR on egg products used as fining agents in wine pursuant to
Article 6, paragraph 11 of Directive 2000/13/EC - for permanent exemption
from labelling**

(Request N° EFSA-Q-2006-157)

(Adopted on 15 October 2007 by written procedure)

SUMMARY

This joint notification deals with egg products that are used as fining agents for making wine and may partly remain in the finished products. The prevalence of allergy to egg proteins and particularly to albumin among the general population has been reported to be around 0.3% in adults.

Different wines from different geographic regions and varieties of grapes are considered. Limited information is provided regarding characterisation of egg products, i.e. egg white albumin with or without lysozyme, and their use as fining agents. SDS-PAGE electrophoresis and immunoblotting studies of the fining agent revealed IgG- and IgE-binding to non identified components.

Among 400 commercial wines tested with an ELISA assay, 14 were found to contain residues of egg white albumin. A history of safe use (allergy) of wines fined with egg white albumin is claimed by the applicant. However, under-reporting of reactions may have occurred because it is likely that consumers and health professionals are not aware of the possible presence of egg products in wine. Eight egg-allergic subjects were skin prick tested with egg white albumin-fined wines and five had a positive reaction. In a double blind placebo controlled food challenge study with eight egg allergic subjects, adverse reactions to fined wines were reported in two patients.

Taking into account the information provided, the Panel considers that wines fined with egg products may trigger adverse allergic reactions in susceptible individuals under the conditions of use stated by the applicant.

KEY WORDS

Wine, fining agents, egg products, egg white albumin/lysozyme.

BACKGROUND

In November 2003, the European Parliament and the Council adopted Directive 2003/89/EC¹ amending Directive 2000/13/EC, as regards indication of the ingredients present in foodstuffs.

Annex IIIa of the Directive specifies a list of food ingredients or substances that are known to trigger allergic reactions or intolerances in sensitive individuals for which no labelling exemptions are allowed. Whenever the listed ingredients/substances or their derivatives are used in the production of foodstuffs, they must be labelled.

Article 1, paragraph 11, subparagraph 2 of the Directive establishes a procedure allowing for temporary labelling exemption of derivatives from ingredients listed in Annex IIIa for which it has been scientifically established that it is not possible for them to cause adverse reactions. In accordance with this provision, submissions of request for temporary labelling exemption were notified to the Commission before 25 August 2004. The Commission, after consultation with the European Food Safety Authority, adopted a list (Directive 2005/26/EC²) of those ingredients which are temporarily excluded from Annex IIIa until 25 November 2007, pending the final results of the notified studies.

Consequently, applicants who submitted a dossier in 2004 on the basis of subparagraph 2, resulting in the inclusion of a product in the list of Directive 2005/26/EC, and who are seeking exclusion of that product from Annex IIIa beyond 25 November 2007 will have to submit a request enclosing the final results of the notified scientific studies. Therefore in the context of the permanent labelling exemption procedure, the European Food Safety Authority is asked to provide scientific opinions on the submissions in accordance with the present terms of reference.

TERMS OF REFERENCE

In accordance with Article 29 (1) (a) of Regulation (EC) N° 178/2002, the European Commission requests the European Food Safety Authority to evaluate the scientific data submitted by Deutscher Weinbauverband (DWV) and the Office National Interprofessionnel des Fruits, des Légumes, des Vins et de l'Horticulture (VINIFLHOR) in the framework of the procedure laid down in Article 6, paragraph 11 of Directive 2000/13/EC. On the basis of that evaluation, EFSA is requested to issue an opinion on the information provided, and particularly to consider the likelihood of adverse reactions triggered in susceptible individuals by the consumption of the following ingredients/substances used under the conditions specified by the applicant: egg products used as fining agents in wine.

ASSESSMENT

¹ Directive 2003/89/EC of the European Parliament and of the Council amending Directive 2000/13/EC as regards indication of the ingredients present in foodstuffs. OJ L 308. 25.11.2003, p. 15.

² Commission Directive 2005/26/EC of 21 March 2005 establishing a list of food ingredients or substances provisionally excluded from Annex IIIa of Directive 2000/13/EC of the European Parliament and of the Council. OJ L 75. 22.03.2005. p. 33-34.

Taking into account the numerous and well documented reports of allergic individuals reacting to egg albumin (egg white) and that the prevalence of such allergy to egg proteins has been reported to be around 0.3% in adults (NDA, 2004a; Vierk *et al.*, 2007), it is appropriate for the Panel to assess the likelihood of adverse reactions in allergic individuals consuming products where these proteins/allergens have been added during a manufacturing process.

Dossiers submitted by Deutscher Weinbauverband (DWV) and by the Office National Interprofessionnel de la Vigne et du Vin (ONIVINS) to the European Commission pursuant to Article 6, Paragraph 11 of Directive 2000/13/EC as amended by Directive 2003/89/EC for temporary exemption from labelling were the basis for earlier assessments of egg products by the NDA Panel (NDA, 2005a and 2005b). The present opinion is based on an updated dossier from DWV and VINIFLHOR (Office National Interprofessionnel des Fruits, des Légumes, des Vins et de l'Horticulture), with an application for permanent exemption. The updated joint application contains new information and data mainly with regard to analytical, animal and clinical studies.

1. Characterisation of the fining agent

The analytical data sheet submitted with the application provides limited information on the purity and characterisation of the commercial egg white albumin(s) used as fining agent for wine making. These agents comply with the International Oenology Codex standards.

Hen's egg white albumin is in the form of either fresh or frozen egg white, or as freeze-dried powder. Powdered egg white can be dissolved in a potassium carbonate solution. Two preparations exist: one without lysozyme, the other with lysozyme as preservative. Lysozyme has a bactericidal effect. It is extracted from egg white and used in the form of soluble hydrochloride. The addition of lysozyme to the wine is normally limited to 500mg/L.

Egg white albumin used as fining agent was characterised by the applicant using SDS-PAGE electrophoresis and, in parallel with Coomassie blue staining, different protein bands were revealed by western blotting using sera from egg allergic patients and from non-allergic controls. Sera from allergic patients were incompletely characterised. Tests were also performed using sera of rabbits or mice immunized with egg white albumin.

Purified rabbit IgG recognized a total of 14 bands, not all could be visualised by Coomassie Blue staining. The bands identified, i.e. at 40-55, 11-17 and 80kDa molecular weight, could correspond to ovalbumin, lysozyme and ovotransferrins respectively, but there were also bands not corresponding to identifiable components of egg proteins. Human IgE recognized three bands at around 31, 67 and 80kDa.

These observations may imply that bands not corresponding to pure egg white proteins may be related to degradation or aggregation products or other immunoreactive proteins in the starting materials. Thus, the egg white albumin which is used as fining agent may contain residual amounts of other proteins.

2. Conditions of use

The applicant describes the wine making process for different wines (red, white and rosé wines) that are produced in different European regions. The winemaking process can vary

according to region, vintage, colour of the wine and skill of the winemaker. However it follows the professional guidance documents, namely *Référentiel des pratiques oenologiques intégrées* (ITV France, 2001), and *Guide des bonnes pratiques hygiéniques filière vins* (Onivins, 2000). Conditions of processing are stated to be similar in France and in Germany.

The principle of fining is to mechanically remove insoluble and colloidal substances from wine. This aims to clarify and stabilize the wine, and to preserve and improve its flavour and taste.

In the acidic environment of must and wine albumin adsorbs phenolic compounds, particularly tannins that may affect wine colour and taste, leading to precipitates which are removed by sedimentation, filtration and/or centrifugation. The applicant states that filtration may not be applied to certain wines.

The amount of egg white albumin added to wine usually ranges from 50 to 150mg/L. Egg white albumin is particularly used for the clarification of red wines rich in tannins. According to the applicant, less than 2% of German wines are treated with egg white albumin. In France, 11% of wines are treated with egg white albumins. The total amount of egg white albumin used as fining agent in France is 100 tonnes per year.

3. Estimated level of exposure

The applicant estimates the wine consumption in France to be around 60L per person per year. In Germany the average consumption of wine was about 23L per person in 2005. These figures do not reflect the variability between individuals and geographic areas, neither the variations for a given individual over time. The Panel notes that intake on a single occasion may be more relevant regarding food allergic reactions than average daily or yearly intake, and that no generally applicable threshold levels of intake have been defined for food allergens (NDA, 2004).

No estimate of exposure to egg white albumin or lysozyme residues through fined wines is provided by the applicant.

4. Evidence of non-allergenicity

4.1 History of non-allergenicity of wines fined with egg products

The applicant provides a general review on food allergens and particularly on egg allergens. Regarding egg products used as processing aids and the products under consideration, reference to historical reviews is provided. The applicant states that the medical literature contains no indication of an allergenic potential of wine that has been fined with egg products but no justification for this statement is given. The strategy and the sources used for the literature search are not described. In addition, under-reporting of reactions caused by egg after consumption of wines may have occurred since consumers and health professionals may be unaware that egg and derived products are used in the wine making process.

4.2 Analytical studies

Two ELISA tests were developed and used for determination of egg products in panels of German and French wines. The studies performed using a competitive ELISA test have

recently been published (Weber *et al.*, 2007). The limit of detection (LOD) of the competitive ELISA for egg white albumin and lysozyme in wines were 20µg/L and 1µg/L, respectively. Analyses were performed directly in wines after appropriate dilution to reduce interferences with the wine. An immunometric (sandwich) ELISA for albumin was developed and performed on lyophilisates of fined wine with a claimed LOD of 1µg/L.

Egg white lysozyme (about 10-60µg/L) was detected in four out of five German wines fined with egg white albumin plus lysozyme at concentrations of 250 or 500mg/L. Egg white albumin (about 200µg/L) was detected in one wine fined with 200mg/L egg white albumin. Six industrial test wines, provided by Union des Oenologues de France, and fined with egg white albumin (30mg/L) were analyzed with the sandwich ELISA. The Panel notes that the applicant states that those wines were elaborated in industrial conditions but that the concentration of fining agent used is below the usual conditions (i.e. 50-150mg/L) as indicated in the manufacturing process (section 1). Three out of the six industrial test wines were found to be positive.

Six wines experimentally fined with albumin (100mg/L) were also found to contain detectable amounts of the fining agent. Four hundred commercial French wines were also analysed for the presence of egg white albumin using the sandwich ELISA. Fourteen were positive (> 1µg/L). Most positive wines were red wines (12 out of 14).

The Panel notes the difficulties in measuring residual fining agents in wine with the ELISAs because of interference with the wine matrix, which affects the reliability of the data provided.

4.3 *Animal studies*

The applicant presents animal studies using mice experimentally sensitized to egg white albumin and challenged with fining agents and fined wines, as confirmation of the presence of egg white albumin in fined wines.

Mice were intraperitoneally (i.p.) immunized with egg white albumin with or without lysozyme using alum as adjuvant. Hypersensitivity responses were observed after challenges with pure egg white albumin with or without lysozyme. Clinical reactions were also observed after challenges with lees and wines mixed with its lees from wines fined with albumin alone or albumin and lysozyme. Clinical reactions were also observed after one i.p. challenge with a decanted and filtered fined wine.

The Panel considers that animal studies confirm the presence of residual amounts of albumin in fined wines.

4.4 *Clinical studies*

4.4.1 *Wines tested*

Clinical studies were performed in France and Germany. They included specific IgE determination and skin prick testing and double blind placebo controlled food challenge (DBPCFC) with egg white albumin and/or with fined and unfined wines. German test wines were fined using egg white albumin at a concentration of 200mg/L for white wines and 400mg/L for red wines, or a fining agent containing both albumin and lysozyme at 500mg/L for

both red and white wines. French wines were experimentally fined using albumin and lysozyme at 100mg/L.

4.4.2 Recruitment of allergic patients

Five egg allergic female patients were recruited in Germany and three (two female) in France. No detailed information is provided regarding the French patients who had experienced anaphylaxis, generalised urticaria and/or oral allergy syndrome. The five German patients had specific anti egg IgE concentrations that ranged from 2.7 to more than 100kIU/L. The clinical manifestations included skin, respiratory and intestinal symptoms. No control DBPCFC with egg products were performed before the patients were challenged with egg white albumin and egg white albumin fined wines.

4.4.3 Skin testing

The eight patients mentioned above underwent skin testing with native egg white, the fining agents (egg white albumin and lysozyme), egg white albumin-fined wines and unfined wines. All five German patients had positive skin prick tests to egg white albumin and four to lysozyme. One French patient reacted to albumin. Test wines were six German wines and a French wine fined with egg white albumin alone or with albumin and lysozyme as described above. The applicant states that positive reactions were observed with five fined and two unfined wines, but no individual data were provided.

4.4.4 Double blind placebo controlled food challenge with fined and unfined wines

The test materials used for DBPCFC on the eight patients described above consisted of two French red wines and a German white wine fined as described in section 4.4.1.

One German egg allergic female showed no immediate reaction after a provocation test with a fined French wine. However, she reported worsening of eczema with erythematous papules and severe pruritus about five hours after the oral provocation. A French egg allergic individual, whose skin tests were negative with the fined wine, the fining agent, and who had a negative labial provocation test, reacted with pharyngeal and oesophageal discomfort after challenge with an egg fined red wine. She did not react to the unfined wine. A residual content of albumin (> 1µg/L) was detected in the fined wine responsible for the reaction.

CONCLUSIONS

Taking into account the information provided, the Panel considers that wines fined with egg products may trigger adverse allergic reactions in susceptible individuals under the conditions of use stated by the applicant.

DOCUMENTATION PROVIDED TO EFSA

Dossier submitted by Deutscher Weinbauverband (DWV) and the Office National Interprofessionnel des Fruits, des Légumes, des Vins et de l'Horticulture (VINIFLHOR) to the

European Commission pursuant to Article 6 paragraph 11 of Directive 2000/13/EC as amended by Directive 2003/89/EC, on 28 August 2006.

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