

**Opinion of the Scientific Panel on Dietetic Products, Nutrition and Allergies  
on a request from the Commission related to a notification from WFA and  
the AWRI on albumin (egg white) used in the manufacture of wine  
pursuant to Article 6, paragraph 11 of Directive 2000/13/EC- for  
permanent exemption from labelling**

**(Request N° EFSA-Q-2006-155)**

**(Adopted on 15 October 2007 by written procedure)**

**SUMMARY**

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. The applicant provides information regarding egg albumin (egg white) used in the manufacture of wine. Detailed information is provided on manufacturing processes and on the detection and quantification of residual amounts of egg proteins, essentially ovalbumin and ovomucoid, in the fined wines.

A history of safe use regarding allergic reactions is claimed by the applicant. However, evidence for this claim is limited and adverse reactions due to the presence of egg protein residues in wine may have been under-reported. The analytical data presented regarding the presence of egg proteins in fined wines are inconclusive. The sensitivity and specificity of cell-based laboratory tests applied are not sufficient to draw conclusions. Clinical studies including a double blind placebo controlled food challenge involved five allergic adults. One patient presented respiratory symptoms both with a control unfined wine and with an egg-fined wine. However, the number of subjects tested as well as the clinical information provided is limited.

The Panel is aware that an adverse reaction occurred in another double blind placebo controlled food challenge study with an albumin (egg white) fined wine.

Taking into account all the information available, the Panel considers that wines fined with albumin (egg white) may trigger adverse reactions in susceptible individuals under the conditions of use stated by the applicant.

**KEY WORDS**

Wine, fining agents, egg albumin (egg white)

## **BACKGROUND**

In November 2003, the European Parliament and the Council adopted Directive 2003/89/EC<sup>1</sup> 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/EC<sup>2</sup>) 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 the Winemakers' Federation of Australia in the framework of the procedure laid down for temporary labelling exemptions 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: albumin (produced from egg) used as a fining agent in the production of wine.

## **ASSESSMENT**

Taking into account the numerous and well documented reports of allergic individuals reacting to egg albumin (egg white) and the prevalence of such allergy to egg proteins that has

---

<sup>1</sup> 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.

<sup>2</sup> 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.

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.

A dossier submitted by the Winemakers' Federation of Australia 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 was the basis for an earlier assessment of egg products by the NDA Panel (NDA, 2004b). The present opinion is based on an updated dossier from the same organization, with an application for permanent exemption. The updated dossier contains additional information and data mainly with regard to cell-based laboratory tests and a clinical study of allergenicity.

## **1. Preparation and characterisation of the fining agent and its use**

### ***1.1 Manufacturing process***

The manufacturing process of egg albumin (egg white) includes different steps, such as separation of the yolk and white, filtration, pasteurisation (55°C, 9.5 min), chilled storage, salt addition, in line filtration, filling and packaging, visual inspection, refrigerated storage, and release and dispatch under refrigeration.

### ***1.2 Characterisation of the fining agent***

Egg white contains predominantly ovalbumin with ovotransferrin, ovomucoid, and lysozyme (about 54%, 13%, 11% and 3.5% of dried egg white, respectively). Egg white is essentially purchased as a pasteurized frozen mixture or (more rarely) as fresh eggs that must be separated from their yolk prior to use.

The application contains general considerations on egg proteins but no specific information is provided on the origin or the quality control of the egg albumin (egg white) used in the fining process. It is claimed to comply with the general specifications for the use in food industry. It is specified that each batch of this material receives a certificate of analysis which is checked and recorded.

### ***1.3 Conditions of use and residual amounts in the fined wines***

#### ***1.3.1 Conditions of use***

The clarification (i.e. fining) of wine during winemaking is the process of removing undesirable substances such as proteins and phenolic and tannin compounds that would cloud the wine and cause bitterness and astringency. The most common application for egg white is the clarification of red wines in preparation for bottling. Egg white albumin is positively charged and will bind and adsorb negatively charged substances such as tannins to form a flocculent precipitate in wine. The removal of precipitate is performed by decanting, and may include centrifugation or filtration prior to bottling.

Typical amounts of egg white used are in the range of 20–100mg/L depending on the wine.

#### ***1.3.2 Residual amounts of egg albumin (egg white) in the fined wines***

A sandwich ELISA has been established for ovalbumin (egg albumin) detection in wine (limit of detection (LOD) 1µg/L). An inhibition ELISA was developed for ovomucoid detection in wine (same LOD of 1µg/L).

Twenty two commercially-available Australian wines fined with egg white were collected as well as 86 Australian wines fined without egg white, all made according to good manufacturing practice. Ovalbumin was undetectable in all wines after clarification.

Taking into account difficulties in performing ELISA assays in wines, the Panel notes that the LOD and quantification may not be reliable. In addition, although the application is for the use of egg white as fining agent, the analytical data presented essentially refer to ovalbumin (which is a major egg white protein and allergen but not the only one, representing about 54% of egg white). Ovomucoid was also analysed in the fined wines used for the laboratory cell-based and clinical studies, and was not detected (LOD for ovomucoid = 1µg/L).

## **2. Estimated exposure levels**

Based on the available statistics from a national food consumption survey conducted in Australia (Australian Bureau of Statistics, 1999), the applicant assumes a highest wine consumption rate of approximately 1L/d. Assuming a residual concentration of 1µg/L of ovalbumin in wine (LOD in section 1.3.2), this would correspond to a daily intake of 1 µg ovalbumin, which the applicant states to be below the lowest dose eliciting adverse reactions in egg allergic individuals as identified in a number of challenge studies (NDA, 2004a; FDA, 2006). The Panel notes that the amount ingested on a single occasion may be more relevant regarding food allergic reactions than average daily intake, and that no generally applicable threshold levels of intake for triggering allergic reactions have been defined for food allergens (NDA, 2004a, FDA 2006).

## **3. Evidence of non-allergenicity**

### ***3.1 History of non-allergenicity of wines fined with egg white***

#### *3.1.1 Literature search*

The applicant was unable to identify literature regarding adverse reactions to fining agents in wine. However, case reports on adverse reactions (including IgE mediated allergy or intolerance) following ingestion of wines attributed to biogenic amines such as histamine, or salicylates, sulfites, grape proteins or the yeast *Saccharomyces cerevisiae* have been identified (Vally and Thompson, 2003). The Panel notes that under-reporting of reactions caused by egg proteins after ingestion of wines may have occurred since consumers and health professionals may not be aware that egg white products are used in the wine making process.

#### *3.1.2 Historical evidence of safe use (allergy)*

The applicant states that no allergic reaction to egg albumin (egg white) used as proteinaceous processing aid in winemaking has been recorded in the Australian Wine Research Institute's database in the past 15 years, and also states that in the reported cases of allergy to wine, no relationship with egg albumin (egg white) has been established.

### ***3.2 Laboratory cell-based studies***

### 3.2.1 *Direct basophil activation test*

A basophil activation assay was performed (Stockley *et al.*, 2006; Rolland *et al.*, 2006) to assess the allergenicity of what the applicant considered a representative sample of commercially available Australian wines fined using different proteinaceous processing aids. Twenty two wines were fined with egg white. Two red and two white wines prepared without egg protein were selected as controls. The wines were characterized for their content of potential pharmacologically active substances such as biogenic amines or sulphites.

The principle, development and procedure of the basophil activation assay are extensively described in the application. Basophils are major effector blood cells involved in allergic reactions. In presence of the corresponding allergens, there is a cross-linking of specific IgE fixed on their membrane which provokes a degranulation of the sensitized cells. Basophil activation studies with egg white fined wines and control wines did not yield significantly different responses. However, the basophil donors had no or very low serum concentrations of specific IgE. Furthermore, the Panel notes that this test has not been validated for this purpose.

### 3.2.2 *Stripped basophil activation test*

A stripped basophil activation assay was performed by the applicant using sera from children with confirmed egg allergy and basophils from five non allergic adult donors.

Twenty egg allergic children (six females) were selected at the Royal Children's Hospital based on a clinical history of adverse reaction to egg in association with the demonstration of specific IgE to egg via RAST or skin prick testing (median age: 18 months; age range: six months to nine years). Specific anti-egg IgE RAST values ranged from 5kIU/L to 40kIU/L. The clinical manifestations experienced by the allergic children were mostly unknown. Sera collected from children allergic to eggs were used to sensitize blood basophils from non allergic donors. The challenge material consisted of 20 white wines which had been fined with egg white. No ovalbumin or ovomucoid residues were detected in those fined wines by ELISA (LOD = 1µg/L for ovalbumin and ovomucoid).

An activation of the stripped basophils was observed when the challenge was performed with egg white extract. After challenge with the wines fined with egg white, the activation was much lower and no difference was observed between the cells re-sensitised with the egg allergic sera or with the control sera. Both the direct basophile degranulation test and the stripped basophil activation test have not been validated.

## 3.3 *Clinical studies*

Subjects for the clinical study were recruited in Australia between January 2003 and February 2004 based on their clinical history of reaction to egg or as non-allergic control subjects. All participants were regular and moderate consumers of wine. Only limited clinical information was provided. Three males (26, 32, 54 years) and two females (24, 51 years) allergic to egg with a history of asthma, laryngeal oedema, generalised urticaria, facial angioedema and/or gastro intestinal symptoms were recruited. Specific anti-egg IgE levels in blood as measured by the CAP score were low (e.g. 0, 0, 2, 3 and unknown) and the skin prick test (SPT) reaction to egg extracts were 4mm, 4mm and 7mm wheal diameter and unknown for two

patients. The patients had not undergone a double blind placebo controlled food challenge (DBPCFC) prior to the study.

DBPCFCs were performed with wines fined with albumin (egg white) and with unfined wines in 16 subjects (five egg allergic patients and 11 non- allergic controls). One allergic subject (24 year old female with a specific IgE CAP score of 0 and a SPT of 4mm), showed an adverse reaction (i.e. respiratory symptom) which required treatment with salbutamol after challenge with an unfined control wine. The same individual also presented this symptom after consumption of an egg white-fined wine, but she recovered spontaneously.

The Panel is aware of a similar DBPCFC performed using wines fined with egg white (NDA, 2007) reporting an adverse reaction.

## **CONCLUSIONS**

Taking into account all the information available, the Panel considers that wines fined with albumin (egg white) may trigger adverse reactions in susceptible individuals under the conditions of use stated by the applicant.

## **DOCUMENTATION PROVIDED TO EFSA**

Dossier submitted by the Winemakers' Federation of Australia to the European Commission pursuant to Article 6 Paragraph 11 of Directive 2000/13/EC as amended by Directive 2003/89/EC, 29 September 2006.

## **REFERENCES**

FDA (Food and Drug Administration) (2006). Approaches to establish threshold for major food allergens and for gluten in food (Revised March 2006). US Department of Health and Human Services.

NDA (Scientific Panel on Dietetic Products, Nutrition and Allergies) (2004a). Opinion of the Scientific Panel on Dietetic Products, Nutrition and Allergies on a request from the Commission relating to the evaluation of allergenic foods for labelling purposes. The EFSA Journal 32, 1-197.

[http://www.efsa.europa.eu/en/science/nda/nda\\_opinions/food\\_allergy/341.html](http://www.efsa.europa.eu/en/science/nda/nda_opinions/food_allergy/341.html)

NDA (Scientific Panel on Dietetic Products, Nutrition and Allergies) (2004b). Opinion of the Scientific Panel on Dietetic Products, Nutrition and Allergies on a request from the Commission related to a notification from the Winemakers' Federation of Australia on milk products, egg products and fish products used in the manufacture of wine pursuant to Article 6 paragraph 11 of Directive 2000/13/EC. The EFSA Journal 134, 1-6.

[http://www.efsa.europa.eu/en/science/nda/nda\\_opinions/food\\_allergy/689.html](http://www.efsa.europa.eu/en/science/nda/nda_opinions/food_allergy/689.html)

NDA (Scientific Panel on Dietetic Products, Nutrition and Allergies) (2007). 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. *The EFSA Journal* 567, 1-7.

Rolland JM, Apostolou E, Deckert K, de Leon MP, Douglass JA, Glaspole IN, Bailey M, Stockley CS, O'hehir RE (2006). Potential food allergens in wine: Double-blind, placebo-controlled trial and basophil activation analysis. *Nutrition* 22: 882-888.

Stockley CS, O'Hehir RE, Rolland JM (2006). Is allergen labelling necessary for Australian wines ? *Wine Industry Journal* 21: 17-21.

Vally H and Thompson PJ (2003). Allergic and asthmatic reactions to alcoholic drinks. *Addiction Biology* 8: 3-11.

Vierk KA, Koehler KM, Fein SB, Street DA (2007). Prevalence of self-reported food allergy in American adults and use of food labels. *J Allergy Clin Immunol* 119: 1504-1510.

## **PANEL MEMBERS**

Jean-Louis Bresson, Albert Flynn, Marina Heinonen, Karin Hulshof, Hannu Korhonen, Pagona Lagiou, Martinus Løvik, Rosangela Marchelli, Ambroise Martin, Bevan Moseley, Andreu Palou, Hildegard Przyrembel, Seppo Salminen, John (Sean) J Strain, Stephan Strobel, Inge Tetens, Henk van den Berg, Hendrik van Loveren and Hans Verhagen.

## **ACKNOWLEDGEMENT**

The Scientific Panel on Dietetic Products, Nutrition and Allergies wishes to thank Taraneh Dean, Martin Stern, and Jean-Michel Wal for their contributions to the draft opinion.