

**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 milk (casein) 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-2006-158)

(Adopted on 6 July 2007)

SUMMARY

This joint notification deals with cow's milk derived casein, which is used as fining agent for making wine and may partly remain in the finished products. The prevalence of allergic reactions to milk proteins and particularly to casein among the general population of adults has been reported to be around 1%. Several different wines from different geographic regions and varieties of grapes are considered.

Limited information is provided on the characterization of casein and its use as fining agent. SDS-PAGE electrophoresis and immunoblotting studies revealed several IgE-binding components whose molecular weights did not correspond to those of caseins. ELISA assays were developed (limit of detection 0.1-0.3mg/L), but the quantification of casein was unreliable due to interferences with the wine matrix.

Among 400 commercial wines, 13 were found to contain residues of casein. A history of safe use (allergy) of wines fined with casein 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 milk products in wine. Five milk allergic human subjects were skin prick tested with casein-fined wines and one had a positive reaction with clinical manifestations. In a double blind placebo controlled food challenge study with six milk allergic subjects no adverse reactions to casein-fined wines were reported. However, the number of subjects tested as well as the clinical information was limited. The Panel is aware that an adverse reaction occurred in another double blind placebo controlled food challenge study with casein-fined wines.

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

KEY WORDS

Wine, fining agents, milk products, casein.

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: milk (casein) 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 that the prevalence of allergic reactions to milk proteins and particularly to casein among the general population of adults has been reported to be around 1% (NDA, 2004), it is appropriate for the Panel to assess the likelihood of adverse reactions in allergic individuals consuming products where milk (casein) products have been used during the 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 2000/89/EC for temporary exemption from labelling were the basis for earlier assessments of milk (casein) products by the NDA Panel (NDA, 2005a; NDA, 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 studies, animal studies and clinical studies.

1. Characterisation of the fining agent

According to the technical datasheet, the soluble casein product used contains 80-85% food grade acid casein and 15-20% potassium carbonate and complies with the International Oenology Codex standards.

Casein used as fining agent was characterized by the applicant using SDS-PAGE electrophoresis and, in parallel with Coomassie blue staining, different separated protein bands were revealed by western blotting using sera of milk 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 casein. Purified rabbit IgG recognized a total of 16 bands, most but not all corresponding to Coomassie Blue staining.

Human IgE recognized 6 bands at 22, 54, 67, 74, 130 and 155 kDa.

These observations may imply that bands of high molecular weight not related to pure caseins (20-30kDa) may be related to aggregates or other immunoreactive proteins in the starting material. Thus, the casein (including potassium caseinate) which is used as fining agent is a mixture of several proteins and may contain residual amounts of other proteins (e.g. whey proteins) as impurities.

2. Conditions of use

The applicant describes the wine making process for different wines (red, white and rosé wines) that are produced in different 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). The applicant states that the conditions of processing are 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 flavor and taste. Casein adsorbs phenolic compounds, particularly tannins that may affect wine colour and taste.

Casein coagulates and precipitates in the acidic environment of must and wine, and is removed usually by sedimentation, filtration and/or centrifugation. However the applicant states that filtration may not be applied to certain wines, including some French wines.

The amount of casein added to wine usually ranges from 10 to 100 g/hL.

Cow's milk casein is particularly used for the treatment of astringency and for clarification of white and rosé wines. According to the applicant, up to 20% of wines in Germany are treated with casein. In France, 41% of wines are treated with caseins or a mix containing casein. The total amount of casein used as fining agent in France is 815 tons per year.

3. Estimated level of exposure

The applicant estimates the wine consumption in France to be around 60 litres per person per year. In Germany the average consumption of wine was about 23 L per person in 2005. These figures do not reflect variability between individuals and geographical areas, neither 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 casein residues through fined wines is provided by the applicant.

4. Evidence of non-allergenicity

4.1 History of non-allergenicity of the product

The applicant provides a general review on food allergens and particularly on milk allergens. Regarding milk products used as processing aids in this application and regarding the specific finished products that are concerned, reference to historical reviews is provided. The applicant states that "in the medical literature, there is no indication of an allergenic potential of wine that has been fined with milk 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 milk after ingestion of wines may have occurred since consumers and health professionals may be unaware that milk and derived products are used in the wine making process.

The Panel is aware of the publication by Stockley *et al.* (2006), where an adverse reaction to wine fined with milk following a DBPCFC is reported. The applicant has not mentioned this case report in its review despite the publication by Stockley is quoted in another section.

4.2 Analytical studies

Two ELISA tests were developed and used for determination of casein residues in samples 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 casein in wines was 0.1 to 0.3 mg/L. Analysis was performed directly in wines after appropriate dilution to reduce interferences with the wine. An immunometric (sandwich)

ELISA was developed and performed on lyophilisates of wine fined with casein with a claimed LOD of 4µg/L.

No residual casein was detected with the competitive ELISA in 5 German wines fined with casein at concentrations of 6-30 or 10-50 g/hL depending on the wine. A wine experimentally fined with casein (0.5 or 1 g/L) in France was found positive (above 4µg/L) with the sandwich ELISA. Four hundred commercial French wines were also analysed for the presence of casein using this test. Thirteen were positive for casein, including 3 organic wines. Most positive wines were red wines (10 out of 13). The Panel notes the two orders of magnitude difference between the LODs of the two ELISA methods and the difficulties in measuring residual fining agents in wine because of interference with the wine matrix, which affect the reliability of the data provided.

4.3 *Animal studies*

The applicant presented animal studies using mice experimentally sensitized to casein and challenged with fining agents and fined wines. The fined wines did not elicit an allergic reaction in sensitised mice. However the Panel considers that those studies are inconclusive due to weaknesses in the study design i.e., low number of animals used and insufficient number of positive controls.

4.4 *Clinical studies*

Clinical studies were performed in France and Germany. They included specific IgE determination, skin prick testing and double blind placebo controlled food challenge (DBPCFC) with casein and/or with fined and unfined wines. Six German test wines were fined using a concentration of casein of 30g/hL for white wines and 50g/hL for red wines. One French white wine was experimentally fined using a concentration of casein of 50 g/hL.

4.4.1 *Recruitment of allergic patients*

Five milk allergic patients were recruited in Germany and one in France. All were females. Specific anti-milk IgE concentrations ranged from 0.20 to more than 100 kIU/L and all had a history of positive skin prick tests to milk. The clinical manifestations included skin, respiratory and intestinal symptoms. Clinical information is lacking for one patient. No control DBPCFC with milk were performed before the patients underwent the tests with casein and casein fined wines.

4.4.2 *Skin testing*

Five allergic patients underwent skin testing and three patients underwent atopy patch tests with native milk, the fining agent (casein), wines fined with casein and unfined wines. In four out of five patients positive SPT were observed with both native milk and the fining agent (i.e. casein) at different dilutions. Eczematous skin lesions were observed in one patient after an atopy patch test.

Test wines were six German wines and a French wine fined with casein as described above. Positive reactions were observed with fined and unfined wines.

4.4.3 DBPCFC with fined and unfined wines

The test materials used for DBPCFC on milk allergic patients were a French white wine and a German white wine experimentally fined with casein as described above.

The applicant states that none of the six milk allergic individuals presented an adverse reaction to the ingestion of 200 ml of either fined or unfined wine. The Panel notes the small number of patients and limited clinical information in the study. The Panel is aware of a similar DBPCFC performed using wines fined with milk (Stockley *et al.*, 2006; Rolland *et al.*, 2006) and reporting an adverse reaction.

CONCLUSIONS

Taking into account the information available, the Panel considers that wines fined with milk (casein) may trigger adverse 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.

REFERENCES

ITV France (2001). Référentiel des pratiques oenologiques intégrées.

NDA (Scientific Panel on Dietetic Products, Nutrition and Allergies) (2004). 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

NDA (Scientific Panel on Dietetic Products, Nutrition and Allergies) (2005a). Opinion of the NDA Panel related to a notification from DWV 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 185, 1-7.

http://www.efsa.europa.eu/en/science/nda/nda_opinions/food_allergy/840.html

NDA (Scientific Panel on Dietetic Products, Nutrition and Allergies) (2005b). Opinion of the NDA Panel related to a notification from ONIVINS on milk products, egg products and fish products used as fining agents in wines pursuant to Article 6 paragraph 11 of Directive 2000/13/EC. The EFSA Journal 184, 1-5.

http://www.efsa.europa.eu/en/science/nda/nda_opinions/food_allergy/841.html

ONIVINS (2000). Guide des Bonnes Pratiques Hygiéniques filière vin.

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.

Weber P, Steinhart H, Paschke A (2007). Investigation of the Allergenic Potential of Wines Fined with Various Proteinogenic Fining Agents by ELISA. *J Agric Food Chem*, 55: 3127-3133.

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.