

**Opinion of the Scientific Panel on Dietetic Products, Nutrition and Allergies
on a request from the Commission related to a notification from WFA and
AWRI on milk 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-2006-153)

(Adopted on 6 July 2007)

SUMMARY

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%. The applicant provides information regarding milk and products thereof used in the manufacture of wine. Detailed information is provided on manufacturing processes and on the detection/quantification of residual amounts of milk proteins, essentially casein, in the final wines.

A history of safe use with regard to allergic reactions is claimed by the applicant. However, evidence for this claim is limited and adverse reactions due to the presence of milk residues in wine may have been under-reported. The analytical data presented regarding the presence of milk proteins in fined wines are inconclusive. The sensitivity and specificity of cell-based laboratory tests applied were not sufficient to draw conclusions. Clinical studies including double blind placebo controlled food challenges only included one milk allergic patient, who presented a mild adverse reaction when challenged with wines fined with milk.

Taking into account the information provided, the Panel considers that wines fined with milk (e.g. skimmed milk powder) and products thereof may trigger adverse reactions in susceptible individuals under the conditions of use stated by the applicant.

KEY WORDS

Milk, skimmed milk powder

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/EC) 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 and the Australian Wine Research Institute 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 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 used as fining agent in the production of wine.

ASSESSMENT

Taking into account that the prevalence of allergic reactions to milk proteins among the general population of adults has been reported to be around 1% (NDA, 2004a), it is appropriate for the Panel to assess the likelihood of adverse reactions in allergic individuals consuming products where milk (e.g. skimmed milk powder) has been added during the manufacturing process.

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.

A dossier submitted by the Winemakers' Federation of Australia (WFA) to the European Commission pursuant to Article 6, Paragraph 11 of Directive 2000/13/EC as amended by Directive 2000/89/EC on 13 May 2004 for temporary exemption from labelling was the basis for an earlier assessment of milk by the NDA Panel (NDA, 2004b).

The present opinion is based on an updated dossier from the same organization and the Australian Wine Research Institute (AWRI), with an application for permanent exemption. The updated dossier contains additional information and data mainly regarding cell-based laboratory studies and clinical studies of allergenicity.

1. Characterization of the fining agent and its use

1.1 Characterization of the fining agent

No specific detailed information is provided by the applicant on the origin or the quality control of the skimmed milk powder 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.

1.2 Conditions of use and residual amounts in the fined wines

1.2.1 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. Casein, the major milk protein, is positively charged and will bind and adsorb negatively charged substances. Casein precipitates quickly in the acidic environment of wine. When bentonite is added to the wine, it is intended to increase the separation of casein from the wine. The removal of precipitates is performed by sedimentation, decanting, and usually centrifugation or filtration prior to bottling. The typical amount of skimmed milk powder added ranges from 20–200 mg /L depending on the wine. The skimmed milk powder is mixed with water and slowly incorporated into the wine.

1.2.2 Residual amounts of milk in the fined wines

A sensitive and specific ELISA has been established for casein detection in wine (limit of detection (LOD) 8 µg/L). This test uses monoclonal and polyclonal antibodies to casein specially prepared for the intended use by Australian Wine Research Institute.

Thirty four commercially-available Australian white wines fined with milk were collected as well as 74 Australian wines fined without milk or casein. The average amount of skimmed milk powder added during winemaking was ca. 452 mg/L but ranged from 1 to 5500 mg/L. Casein was undetectable (i.e. < 8 µg/L) in all wines after clarification.

Taking into account difficulties in performing this assay in wines, the Panel notes that the LOD and quantification may not be reliable. In addition, although the application is for the use of milk as a fining agent, the analytical data essentially refer to casein (which is the major milk protein but not the only one, representing about 80% of milk proteins), and mainly to α -casein, which represents only about 50% of the casein in milk.

2. Estimated level of exposure

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 1 L/d. Assuming a residual concentration of 8 µg/L of casein in wine (LOD in section 1.2.2), this would correspond to a daily intake of 7.49 µg casein, which the applicant states to be below the lowest dose eliciting adverse reactions in milk allergic individuals as identified in a number of challenge studies (NDA, 2004a; FDA, 2006). The Panel notes that intake on a single occasion may be more relevant regarding food allergic reactions than daily intake, and that no generally applicable threshold levels of intake have been defined for food allergens (NDA, 2004a).

3. Evidence of non-allergenicity

3.1 History of non-allergenicity of the product

3.1.1 Literature search

The applicant performed a literature search using one database and did not to identify reports 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, salicylates or sulphites, grape proteins or alcoholic fermentation wine yeast *Saccharomyces cerevisiae* have been identified (Vally and Thompson, 2003; ref. for *Saccharomyces*). The Panel notes that 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.

3.1.2 Historical evidence of safe use (allergy)

The applicant states that no allergic reaction to milk 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 allergic reactions to wine, no relationship with milk allergens has been established.

3.2 Laboratory cell-based studies

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

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 the presence of the corresponding allergens, there is a cross-linking of specific IgE fixed on their membrane which provokes a degranulation of the sensitized basophils. None of the wines fined with milk tested gave a statistically significantly higher activation of the blood basophils from a milk allergic patient than did the control wines. However, the results are difficult to interpret. The basophil donor (a milk allergic individual) did not exhibit

specific IgE in the serum as measured with the CAP test and this could explain the negative results obtained in the basophil activation assay. Even if this test may have the potential to assess allergenicity in terms of biological responses to milk fined wines, i.e. the ability to activate sensitized cells, the Panel notes that this test has not been validated for that purpose.

3.2.1 Basophil activation study using sera from children allergic to milk

A stripped basophil activation assay was performed by the applicant using sera from children with confirmed milk allergy and basophils from 5 non allergic adult donors.

Twenty milk allergic children (7 females and 13 males) were selected at the Royal Children's Hospital based on a clinical history of reaction to milk in association with the demonstration of specific IgE to milk via RAST or skin prick testing (median age: 12-13 months; age range: 7 months to 14 years). Specific anti-milk IgE RAST values ranged from 5 to 50 kIU/L. The clinical manifestations of the children allergic to milk included urticaria, vomiting, rash, eczema, and coughing. Sera were also collected from 5 non-allergic subjects as controls. Sera collected from children allergic to milk were used to sensitize blood basophils from non-allergic donors. The challenge material was 20 white wines which had been fined with skimmed milk powder (up to 200 mg/L). No casein residues were detected in those fined wines by ELISA.

The activation of stripped basophils sensitised with sera from milk allergic individuals after stimulation with milk-fined wines was not significantly different than after stimulation with milk.

The Panel agrees with the conclusion of the applicant that stripped basophil activation by the wines fined with milk was no different in magnitude to stripped basophil activation by the control milk extracts, which suggests that residual milk proteins may be present in wine fined with milk.

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 allergic reaction to milk or as non-allergic control subjects. Only one patient allergic to milk (19 year old male) with a history of laryngeal oedema, generalised urticaria, facial angioedema was recruited. Specific anti-milk IgE in blood was not detectable as measured by the CAP system, and the wheal diameter after skin prick testing was 15 mm. Eleven non-allergic subjects were recruited as controls.

Double-blind, placebo-controlled food challenges were performed with wines fined with milk or a milk protein such as casein and with unfined wines in 12 subjects (1 milk allergic and 11 non-allergic). None of the 12 subjects challenged with the control wines showed an adverse reaction. The one milk-allergic subject reported a "slight lump in the throat" following ingestion of a wine fined with milk, which did not occur with the control challenge. A repeated, double-blind challenge with the same wine fined with milk resulted in itch.

CONCLUSIONS

Taking into account the information provided, the Panel considers that wines fined with milk (e.g. skimmed milk powder) and products thereof 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 (WFA) and the Australian Wine Research Institute (AWRI) to the European Commission pursuant to Article 6 Paragraph 11 of Directive 2000/13/EC as amended by Directive 2000/89/EC, 29 September 2006.

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