What's in a label?

How science is helping winemakers to respond to new EU rules concerning allergens in wine

By Leanne Hoxey, Creina Stockley*, Eric Wilkes and Dan Johnson
*Corresponding author. Email: creina.stockley@awri.com.au
The Australian Wine Research Institute, PO Box 197, Glen Osmond, SA 5064, Australia

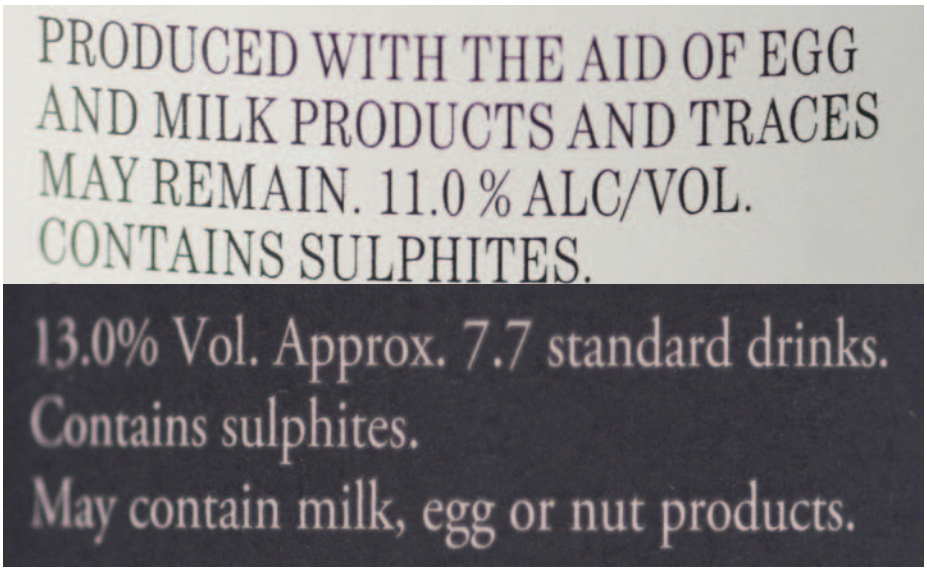
Mangaging director Dan Johnson

On 1 July 2012, new rules came into force regarding the labelling of potential allergens in wine exported to the European Union. The AWRI developed and validated a test for milk and egg residues to allow Australian producers to measure the levels of allergens in their wines and give consumers and policy-makers continued confidence in Australian wine. Through national and international collaborations, work is also under way to ensure that testing is equally robust overseas.

THE NEW RULES

From 1 July 2012, all wine entering the European Union (EU) became subject to EU laws regarding the labelling of allergens. These allergens include compounds found in milk and egg, which is relevant to winemakers given that both ingredients can be used as fining agents. The rules apply even though there is no evidence of life-threatening adverse reactions when consumers with allergies to milk or egg consume wine that has been processed using those compounds.

Until last July, wines entering the EU had been given an exemption from the rules. This 'derogation' had been extended twice to give wine researchers more time to gather data. Studies have now analysed egg- and milk-fined wines in detail; they have also assessed whether there is any risk of adverse reactions among consumers who are allergic to egg or milk.



Based on the evidence collected, the Organisation Internationale de la Vigne et du Vin (OIV) – the intergovernmental organisation concerned with the technical and scientific aspects of winemaking – made two submissions to the European Commission (EC) and the European Food Safety Authority (EFSA) asking for a permanent derogation.

The OIV argued that allergen labelling for milk and egg products was not necessary for wine, since research had shown no adverse reactions among consumers with confirmed allergies to egg or milk. Those consumers comprised 0.5-1.0 percent of the population, and hospital emergency department data had shown that anaphylactic reactions to egg and milk products were extremely rare. However, the OIV's submissions requesting a permanent derogation for wine were unsuccessful.

WHEN LABELS ARE NOT REQUIRED

The good news for Australian wine producers is that allergen labelling is not required for wines exported to the EU if residues of milk and egg remain below certain prescribed levels.

The OIV Resolution OIV-OENO 427-2010 for the methods of quantification of potentially allergenic residues of fining agent proteins in wine as written into EU law, means that no labelling is required if egg- or milk-fined wine has tested negative for egg and milk residues using an analysis technique with a limit of detection (LOD) of 0.25mg/L. The suggested threshold for adverse reactions to pure egg white

AT A GLANCE

- EU laws on the inclusion of milk and egg allergens on wine package labels have applied to all wines imported into the EU from 1 July 2012
- If tests for egg and milk residues return a negative result, then allergen labels are not required – but only certain tests can be used
- AWRI Commercial Services has worked with an Australian manufacturer to develop and validate tests that deliver reliable results that meet EU specifications
- Today, Australian wine producers are in a stronger position to comply with EU rules. Work is also under way to ensure a level playing field through the adoption of best practice guidelines and protocols.

and milk is generally much higher: approximately 1-2mg/L.

This resolution followed research by the OIV taskforce on allergens: a collaboration including a researcher from the AWRI and representatives from France, Germany and Italy. The role of the taskforce continues: it has coordinated additional research to gain a better understanding of residual protein in egg- and milk-fined wine and its significance for human health.

It is significant that taskforce studies have shown that no residues of ovalbumin (from egg) were detected in the wines they tested, which were made in accordance with good manufacturing practices, such as post-fining and filtration. Those wines had been treated with egg- or milk-fining agents. Similar results were obtained in different commercially-available white, red and rosé wines. This was regardless of the wine's physical or chemical characteristics, and the type and dosage of fining agent used.

FINDING THE RIGHT TEST

In order to ensure that Australian winemakers and consumers can have confidence in the data supplied, the AWRI set out to find a reliable test (or assay) for the detection of egg and milk residues.

Work intensified in 2010 when the AWRI recognised the need for industry to test for egg and milk. Initially, the information was required to satisfy certain vegan markets. It was also necessary for exporters to Canada to comply with guidelines from the Liquor Control Board of Ontario, which imposed an LOD for egg/milk residue of < 1.0ppm (note that mg/L and ppm are used as equivalent units in this article).

After consultation with several assay suppliers, enzyme-linked immunosorbent assay (ELISA) kits (manufactured by the Australian company ELISA Systems) were chosen to provide this analytical capability to industry.

At first, the assay was used as a qualitative screening test, with the capacity to develop the assay further to provide quantitative data. Two ELISA systems kits were chosen: Casein Residue ESCASPRD-48 and Enhanced Egg Residue ESEGG – 48. Casein is the protein most commonly associated with milk allergy.

QUALITATIVE RESULTS

From February 2011, AWRI Commercial Services began offering the qualitative ELISA assay to detect the presence or absence of casein and egg residues in wine. Over a 15-month period, 74 samples from across Australia (including five from New Zealand) were submitted for casein residue screening, and 56 samples from across Australia (including five from New Zealand) were submitted for egg residue screening.

Only one sample returned a positive result for milk residue (being > LOD of 1.0ppm). This was found in a juice sample that received a milk addition of 707ppm. A total of six samples returned a positive result for egg residue (being > LOD of 1.0ppm); in these cases the fining regime was not known.

ASSAY VALIDATION AND DEVELOPMENT

To meet the requirements of Australian wine producers and exporters, the AWRI worked closely with the assay manufacturer to further validate and develop the testing kits.

In 2012, the OIV agreed on an LOD for allergen assays of 0.25ppm and a limit of quantification (LOQ) of 0.5ppm for both casein and egg white. In response to this – and its impact on labelling and testing – the AWRI started work to validate ELISA assays at the required LOD and LOQ levels.

Since there was debate regarding which residue component the limits applied to

Table 1.

Casein Residue ESCASPRD-48	LOD	Enhanced Egg Residue ESEGG – 48	LOD
Skim milk powder	0.108ppm	Egg powder	0.268ppm
Total milk protein	0.035ppm	Total egg white protein	0.075ppm
Casein	0.028ppm	Ovalbumin	0.037ppm

Table 2.

Casein Residue ESCASPRD-48	LOQ	Enhanced Egg Residue ESEGG – 48	LOQ
Skim milk powder	1.0ppm	Egg powder	0.9ppm
Total milk protein	0.32ppm	Total egg white protein	0.25ppm
Casein	0.26ppm	Ovalbumin	0.13ppm

Table 3.

Positive total egg white protein residue breakdown			
Sample type	Egg result ppm	Fining addition	Winery processing
2011 Cabernet Sauvignon	1.04	75ppm egg white	Centrifuged only
2011 Cabernet/Shiraz/Merlot	2.72	0.9ppm egg albumin	Racked only
Unknown	1.05	Unknown	Coarse racking only

Table 4.

Negative total milk protein residue breakdown		
Sample type	Fining addition	Winery processing
Wine	< 1ppm – 30ppm milk < 1ppm – 200ppm casein 16ppm – 300ppm skim milk powder No additions made (10 wines)	Various wine processing (settling/racking/centrifuging) followed by filtration
Juice	200 – 350ppm milk	Milk added to juice, then centrifuged to ferment

('milk products' or 'casein'; 'egg products' or 'ovalbumin' or 'ovomucoid'), the AWRI met with ELISA Systems and the parties agreed to refine the interpretation of data generated using the kits.

The milk residue kit was adapted to report 'total milk protein', rather than 'casein'. The egg residue kit was adapted to report 'total egg white protein', rather than 'ovomucoid'. Both kits were then validated using skim milk powder and egg powder.

LODs were determined by averaging a large number of blank replicates across numerous assays, then adding three times (3x) the standard deviation (SD), (See Table 1).

The uncertainty of measurement (UOM) was calculated from the data of seven replicates from several different assays on different days. From this, it was estimated that the uncertainty of measurement at two times (2x) SD was equal to ± 0.1ppm for both kits.

The LOQ was then determined by spiking wines in triplicate at various levels over several assays, and assessing the lowest level at which the UOM of 0.1ppm could be met (see Table 2).

In summary, the qualitative assay met the OIV specifications of a LOD of 0.25ppm and a LOQ of 0.5ppm for both ELISA kits.

OUTCOMES FOR INDUSTRY

From June 2012 to May 2013, further details were obtained from wineries to evaluate the impact of fining regimes and processing techniques. Of the 521 samples submitted for allergen testing in that period, processing details were obtained in 90 cases. The results are summarised below.

Egg residue

Of the 521 samples submitted, 394 samples were tested only for total egg white protein residue. Of these, 20 samples (5%) returned positive results (> LOQ of 0.25ppm) ranging from 0.27-3.73ppm. The samples came predominantly from red wines. Table 3 summarises the fining regime and processing techniques used in three of the wines that tested positive. This suggests that filtration (or lack of it) may affect the result.

There were 374 samples that returned a negative result (< 0.25ppm). In these cases, fining regime details were provided for 37 wines; of which 35 wines had egg added. Two of the wines had no egg added at all and were tested to confirm its absence. Significantly, none of the 35 wines that were filtered (using various standard winery protocols after egg fining) returned a positive result.

Milk residue

All samples (521) were tested for

total milk protein residue, with just three (0.6%) returning positive results (> LOQ of 0.25ppm ranging from 0.27ppm – > 5ppm). Unfortunately, no information was provided about the fining regimes in these three samples.

Of the 518 samples that returned a negative result, fining regimes were provided for 59 wines and 14 ferment samples that had milk added (Table 4).

Of those wines where processing information was available, 10 had no milk added and were tested to confirm its absence. None of the other 49 wines that were filtered (using various standard winery protocols) after milk fining returned a positive result.

This research showed that filtered wines did not return a positive result (> 0.25ppm) for either egg or milk. Furthermore, there were no false positive results returned for the 12 samples that were listed as being processed without adding egg or milk.

TOWARDS A LEVEL PLAYING FIELD

Further research has revealed that manufacturing, processing and filtration techniques can affect on the way that allergen-testing results are interpreted and extrapolated.

The European Food Safety Authority (EFSA) has stated that there is no standard or uniform winemaking practice, no code of good manufacturing practice, and there are no best practice guidelines. EFSA has also noted that microfiltration and bentonite treatment are not mandatory steps and that this may significantly affect fining agent residues.

As a result, the OIV has established a set of 'good fining practice guidelines'. These apply to egg- and milk-fined wine and may soon be adopted into EU law. The OIV has summarised the key issues in a working document (available at http://www.oiv.int/oiv/info/enguidesoiv) and, although it is not yet official, it serves as a useful resource for winemakers. It covers the definition of good manufacturing and fining practices, criteria for analysis methods, and the scientific background. This working document can also be used to work towards harmonisation of international legislation.

In the short term, members of the OIV taskforce are working collaboratively with industry and test manufacturers to conduct further studies. Through a 'ring test' involving 10 overseas laboratories, two commercially-available testing kits and wine producers, further data will be generated to supply even greater certainty to laboratories, as well as wine exporters regarding the reliability of test results.

The study will give confidence to regulatory authorities in the EU, Canada, Australia and New Zealand that the legislation is valid and that consumers are not at risk. The samples under study will have a full winemaking history. The study will also comply with the OIV's new guidelines regarding microfiltration and bentonite treatment.

The study will continue work at the AWRI, in collaboration with its partners, to ensure that research informs appropriate decision making, contributing constructively to debate, protecting consumers and ensuring fairness for Australian wine producers.

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