



FIVS GOOD FINING PRACTICES

**Guidelines for the fining of wine using proteinaceous agents
with allergenic potential**

October 2016

This document is intended as a guide only; it should not be relied upon as legal advice or used as a substitute for legal advice.

Table of Contents

1. PURPOSE	3
2. REGULATORY BACKGROUND	3
2.1 Distinguishing between Food Additives and Processing Aids	3
2.1.1 Good Manufacturing Practice (GMP)	3
2.1.2 Food Additive	3
2.1.3 Processing Aid	4
2.1.4 Decision Tree for Distinguishing Between Food Additives and Processing Aids.....	4
2.1.5 Principles for distinguishing Food Additives from Processing Aids	5
3. GOOD FINING PRACTICE GUIDELINES FOR WINE.....	5
3.1 Principles.....	5
3.2 Methods of Analysis.....	7
4. FOOD SAFETY CONTROL MEASURES FOR WINE FINING	7
4.1 Validation Steps	7
4.1.1 Risk; and Pre-Validation Tasks	7
4.1.2 Approach	7
4.1.3 Parameters and Decision Criteria:.....	7
4.1.4 Background information for validation; and/or the need for further studies?	8
4.1.5 Analysis of results.....	8
4.1.6 Documents and decisions supporting the validation of control measures	8
4.1.7 Conclusion	8
4.2 Monitoring	8
4.3 Verification Steps	9
Annex 1 -- Fining Agents - Technical Aspects	10
Test Sampling	10
Mixing	10
Addition of Fining Agents to White and Red Wine	10
Milk, casein, sodium and potassium caseinate	11
Egg-derived products	12
Isinglass	12
Annex 2 -- Historical and empirical evidence concerning the risks associated with consumption of fined wines by allergic individuals	13
Annex 3 -- Summary of scientific data documenting the risks associated with consumption of fined wines by allergic individuals	19
Annex 4a -- Summary of data indicating that residual protein is negligible using routine and readily available test methods in commercial wines fined according to internationally agreed best practices.....	21
Annex 4b -- Additional Recent Scientific Publications on Fining Agent Residues in Wine	24
Annex 5 -- The ability of the fining procedures elaborated in internationally agreed best practices to consistently achieve the desired outcomes	26
Annex 6 -- Checklist - GMP Principles for Effective Allergen Management	28

1. PURPOSE

This document sets out the regulatory background to the labelling requirements for use of food additives and processing aids that are, or contain food allergens. It then outlines guidance on internationally agreed best fining practices for winemaking, together with the validation procedures, scientific and empirical data that have been used to demonstrate that these practices remove from the final wine product residual levels of egg, fish, milk proteins used as fining agents in winemaking.

2. REGULATORY BACKGROUND

The legal basis for regulatory oversight of all substances used in food processing is set by individual jurisdictions. In the Wine sector they almost invariably draw on recommendations made by Codex Alimentarius and by the International Organisation for Vine and Wine (OIV), and in these guidelines particular reference is made to:

- **Codex:** [Codex Guidelines for the Validation of Food Safety Control Measures, CAC/GL69-2008](#)
[Codex Guidelines on Substances used as Processing Aids, CAC/GL 75-2010](#)
[Codex General Standard for the Labelling of Prepackaged Foods, CODEX STAN 1-1985](#)
- **OIV:** [OIV Methods of Analysis, http://www.oiv.int/public/medias/2551/oiv-ma-as315-23.pdf](#)

2.1 Distinguishing between Food Additives and Processing Aids

2.1.1 Good Manufacturing Practice (GMP)

The use of allergenic fining agents under GMP, and a risk-based decision to label for the presence of allergens, exists within a framework of quality management systems at a manufacturing site level.

The industry best practice approach to allergen risk management is through a Hazard Analysis and Critical Control Point (HACCP) programme and this involves evaluating the hazards associated with a significant part of the 'lifecycle' of the product. It includes the production of raw materials, assessing every manufacturing and process control step, through to transport and trade of bulk wine, and to the labelling and packaging of the final product. The critical points where potential allergens can be introduced into products during manufacture should be identified, and a system established to monitor these critical control points, to ensure that unintentional cross contact is minimised, and that cleaning and sanitation procedures are validated for efficacy.

A Checklist of GMP Principles for Effective Allergen Management is at Annex 6.

2.1.2 Food Additive

"Food additive" is defined in the Codex Alimentarius Commission Procedural Manual, Twenty-fourth edition Section 1 as:

any substance not normally consumed as a food by itself and not normally used as a typical ingredient of the food, whether or not it has nutritive value, the intentional addition of which to food for a technological (including organoleptic) purpose in the manufacture, processing, preparation, treatment, packing, packaging, transport or holding of such food results, or may be reasonably expected to result, (directly or indirectly) in it or its by-products becoming a component of or otherwise affecting the characteristics of such foods. The term

does not include “contaminants” or substances added to food for maintaining or improving nutritional qualities.

2.1.3 Processing Aid

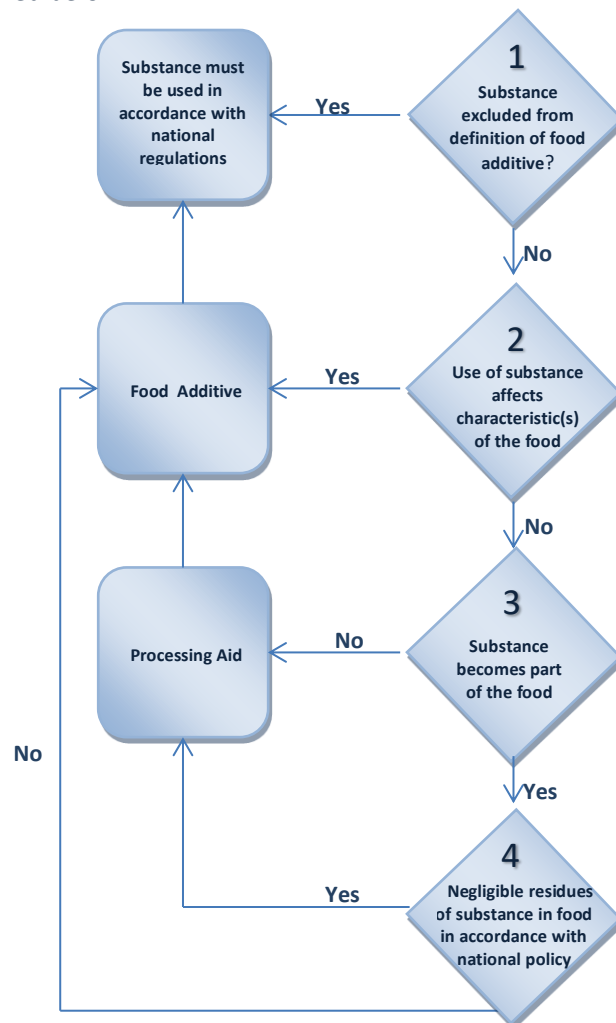
A food processing aid is defined by the Codex Alimentarius Commission as:

any substance or material, not including apparatus or utensils, and not consumed as a food ingredient by itself, intentionally used in the processing of raw materials, foods or its ingredients, to fulfill a certain technological purpose during treatment or processing and which may result in the non-intentional but unavoidable presence of residues or derivatives in the final product.

Processing aids fall outside the regulatory definitions of "food additive" and food "ingredient". As a result, processing aids are not required by most jurisdictions to be declared on prepackaged food labels.

2.1.4 Decision Tree for Distinguishing Between Food Additives and Processing Aids

The following decision tree that can be used to distinguish between food additives and processing aids, based on the Codex definition of “food additive”, and the questions in the tree should be answered by taking into account the principles outlined below:



2.1.5 Principles for distinguishing Food Additives from Processing Aids

Question 1: Does the Codex definition of “food additive” exclude the substance in question?

Principle 1: Egg, fish and milk fining agents are defined as processing aids in most jurisdictions but may not be specifically excluded in some from the definition of “food additive”.

Question 2: Does use of the substance affect one or more characteristics of the food?

Principle 2: A substance is a “food additive” if its presence continues to have a technological function on the finished wine. Thus eggs, milk and fish products used as fining agents would most likely be viewed as processing aids rather than additives provided that no residues of the fining agent or its by-products were found on analysis to be in the finished wine.

Question 3: Does the substance become part of the wine?

Principle 3: A substance is a “food additive” if it or its by-product(s) becomes part of the wine, unless it can be demonstrated that there are no residues of the substance in the finished product.

3. GOOD FINING PRACTICE GUIDELINES FOR WINE

3.1 Principles

Fining is the winemaking process, either before and/or after the fermentation process, to remove unwanted insoluble particles and un-dissolved microscopic particles (colloidal material) from the juice or wine.

Fining involves the addition of adsorptive or reactive material to reduce or eliminate the presence of certain less desirable wine components.

Fining agents are added to modify a wine’s clarity, colour, texture or flavour or to ensure that a wine remains in a particular stable state for a given period of time. Fining materials serve no ongoing purpose in the finished product and are designed to be removed entirely from the treated wine as part of the fining process, see Annex 1: Fining Agents – Technical Aspects.

The effectiveness of a given fining agent depends on the agent itself, on its method of preparation and addition, and on the levels of addition together with characteristics of the wine such as pH, metal content, temperature, presence of CO₂ and prior wine treatments.

In addition to the principles outlined, winemakers should maintain traceability throughout the wine production process by recording the batch from which each sample of fining material is drawn, and to obtaining documented evidence from suppliers of the fining agents used, see Section 2.1.1 on Good Manufacturing Practice.

FIVS has developed 9 principles which have been adopted by the OIV in [Resolution Oeno 520-2014](#):

1. Fining agents shall be free from undesirable taints and must conform to all applicable regulations. They should be stored in a cool, dry environment in sealed containers, or in other recommended storage conditions as advised by the manufacturers.

2. It is recommended that laboratory-scale trial runs be conducted prior to treatment of wine in the winery.

3. The laboratory trial runs are conducted in order to reproduce, as far as possible, the treatment conditions to be used in the winery; special attention should be paid to the batch of fining agent to be used, the method of its preparation and addition to the wine, and to the temperature of the laboratory sample with respect to the total volume of wine normally fined in a winery. Preparative protocols (hydration, concentration, etc.) for protein fining agents used in the laboratory and winery should be similar, if not identical.
4. The volume of distilled, de-ionised or other potable water used to dissolve or disperse the fining agent should be minimised so as to avoid overly diluting the wine (applicable regulations must be met).
5. The quantity of fining agent used is the smallest amount needed to achieve the desired result as stipulated by the winemaker's sensory and/or analytical evaluation, and in no case shall the amount exceed that which is specified in the applicable standards and regulations.
6. Thorough and adequate mixing of the fining agent into the juice or wine should be ensured, and sufficient contact time should be observed for the material to react prior to subsequent racking and/or filtration.
7. Industry recognized best practice filtration methods (including fine filtration using diatom powder and cellulose fibres and/or pre-bottling filtration through at most a 0.65 µm and preferably a smaller, 0.45 µm membrane filter, or performing treatments of equivalent effect) should be used to remove insoluble protein fining agents. **If the treated wine is simply racked off the lees** remaining from the fining treatment and bottled without filtration, or if a less rigorous filtration or other technique for removal of the lees is applied, an **analysis must always be conducted prior to bottling** to confirm the absence of detectable residual fining agent. However, even in the case of filtration, it is recommended to analyse filtered or unfiltered wines to confirm that no residual of fining potential allergenic agent(s) can be detected.
8. The fining process shall be routinely monitored after it has been carried out and following the removal of residues. In general, this will entail analysis of a sample of fined wine, using a sufficiently sensitive method of analysis, for the fining agent in question. The frequency of sampling should be adequate to ensure that the fining processes are being conducted in such a way that no detectable residue of allergen remains in the treated wine.

Appropriate corrective action (e.g. appropriate filtration) must be taken where the analysis of wines indicates the presence of residual fining agents, or without appropriate corrective action the product labels must reflect the presence of allergens.

9. Verification should be conducted at regular intervals, in the form of a review of the means of monitoring the fining processes, at a frequency that is adequate to ensure that these processes are being conducted in such a way as not to leave detectable fining agent residues. Verification should also ensure that adequate and timely corrective actions are taken where evidence is obtained that indicates the potential for the presence of residual fining agents in a treated wine (e.g. through false positive results).

According to OIV:

“While regulations do not provide a specific threshold level, typical analytical methods for food allergens can detect residues in the low parts per million (ppm) range. If these methods do not detect any allergenic protein in the wine, then it could be considered that no residue above the detection limit is present.”

3.2 Methods of Analysis

OIV's recommended Methods of Analysis are set out at: <http://www.oiv.int/public/medias/2551/oiv-ma-as315-23.pdf>

4. FOOD SAFETY CONTROL MEASURES FOR WINE FINING¹

A study has been conducted by an economic operator on the available scientific and empirical data to determine whether internationally agreed best practices for the fining of wine do indeed present a sufficiently robust control measure to ensure that there are negligible (if any) residual fining agents in treated wines and thus that they are not present in the final product at levels which pose a risk to consumers with food allergies, see Annex 5.

The method followed in this evaluation and its outcome are set out below:

4.1 Validation Steps

4.1.1 Risk; and Pre-Validation Tasks

- a. **Hazard:** The presence of residual allergenic protein in wines fined with milk, eggs and egg derivatives.
- b. **Food safety outcome required:** No residual fining agents (using routine and readily available test methods) in wines fined according to internationally agreed best practices.
- c. **Control measure to be validated:** The fining and filtration processes applied to wines.

4.1.2 Approach

Historical and empirical evidence and the outcome of recent scientific studies in the public domain are at Annexes 2-5.

4.1.3 Parameters and Decision Criteria:

- a. Parameters:
 - i. The amount of protein used to fine a wine should be the minimum amount required to produce the desired outcomes.
 - ii. Wines should be fined according to internationally agreed best practices.
- b. Decision Criteria:

The control measure for fined wines must be validated to determine whether any allergenic protein can be found (generally by using routine and readily available test methods with adequate sensitivity) in a wine fined according to internationally agreed best practices.

¹ Based on the Codex Alimentarius Commission "Guidelines For The Validation of Food Safety Control Measures" - CAC/GL 69 -2008

4.1.4 Background information for validation; and/or the need for further studies?

- a. Historical and empirical evidence concerning the risks associated with consumption of fined wines by allergic individuals are summarised in Annex 2. They show that such risks are very small and are based on a number of factors: literature reviews; emerging information on the tolerance of allergic individuals to small amounts of allergenic protein; the experience of allergy clinics; clinical trial data; and consumer complaints information received by retailers and other organizations over many years.
- b. Scientific data documenting the risks associated with consumption of fined wines by allergic individuals are summarized in Annex 3. They show that none of the 49 subjects in clinical trials had any significant or life-threatening adverse reaction to a protein-fined wine, and that only one of 49 subjects had a mild skin condition adverse reaction.
- c. Information is presented in Annex 4 to show that residues of casein and ovalbumin may be below 0.07 and 0.002 mg/L (ppm), respectively, in the final wine, when the proteinaceous wine fining agents casein and egg white are used according to GMP in winemaking. Such levels are not likely to trigger adverse reactions in milk- or egg-allergic individuals, respectively, which comprise approximately 1% or less of the adult population.

The available scientific literature and data relating to fining of wine have been reviewed to determine their pertinence to the internationally agreed best practices. The information is believed to be sufficient to validate the control measures indicated without the need for further studies.

4.1.5 Analysis of results

Information on the likelihood of achieving desired outcomes, provided that best practices are followed, is at Annex 5.

4.1.6 Documents and decisions supporting the validation of control measures

All analyses, data, and decisions are presented in the Annexes to this text.

4.1.7 Conclusion

- a. Data from scientific studies, as well as historical and empirical evidence, indicate that fining wine according to internationally agreed best practices leaves no detectable residual levels of protein from fish, eggs or milk food allergens in the finished wine product using readily available analytical methods of sufficient sensitivity.
- b. These data can be used by economic operators to establish a programme of monitoring for residual fining agents in treated wines, see Annex 5.

4.2 Monitoring

Filtered Wines:

Routine, periodic monitoring of the fining process should be conducted. In general, this will entail analysis of a sample of fined wine using a sufficiently sensitive method of analysis for the fining agent in question.

The frequency of sampling should be adequate to give confidence that the fining processes are being conducted so as to leave no detectable fining agent residues in the treated wine.

Racked Wines:

Analysis should always be conducted on fined wines that are intended to be bottled without filtration, to ensure that no residues of fining agents are detected.

Corrective action must be taken to remove allergenic residues where the analysis of such wines indicates their presence in their final product, or labels must reflect their presence in a “Contains:” statement.

4.3 Verification Steps

Verification should be conducted at regular intervals. It should consist of a review designed to ensure that monitoring is careful and consistent, at a frequency that is adequate to give confidence that the fining processes are being conducted so as to leave no detectable fining agent residues in the treated wine.

Verification should also ensure that adequate and timely corrective actions are taken where evidence indicates the potential for the presence of residual fining agents in a treated wine, i.e., through false positive results.

References

Codex Procedural Manual

<http://www.fao.org/fao-who-codexalimentarius/procedures-strategies/procedural-manual/en/>

HACCP - Adopted by Codex

<http://www.fao.org/docrep/005/y1579e/y1579e03.htm>

FARRP - Components of an Effective Allergen Control Plan: A Framework for Food Processors

<http://farrp.unl.edu/allergen-control-food-industry>

Food Drink EU - Guidance on Food Allergen Management for Food Manufacturers

http://www.fooddrinkurope.eu/uploads/press-releases_documents/temp_file_FINAL_Allergen_A4_web1.pdf

Australian Food and Grocery Council - Food Industry Guide to Allergen Management and Food Labelling

http://allergenbureau.net/wp-content/uploads/2013/11/Allergen_Guide_2007.pdf

FIVS is an international federation serving trade associations and companies in the alcohol beverage industry from around the world. It provides a forum for its members to work collaboratively on legal and policy issues and communicates Federation views to national governments and international organizations.

Annex 1 -- Fining Agents - Technical Aspects

The purpose of adding a fining agent to wine can be three-fold: to “soften” or reduce its astringency and/or bitterness; to clarify and remove proteins capable of haze formation; and/or to stabilize and reduce the colour by the adsorption and precipitation of polymeric phenolic compounds and tannins. The fining agent reacts with wine components either chemically or physically, to form a new complex that can be separated from the wine.

Fining agents may bind with substances either through:

- Electrical interaction – the fining agent and substance(s) to be removed are of opposite charge and come together forming larger particles which settle in wine;
- Bond formation – the chemical bond is formed between the substance(s) to be removed and the fining agent;
- Absorption and adsorption – the substance(s) to be removed are either caught within the structure of the fining agent, or bind on the surface of the fining agent.

Test Sampling

Fining should be carried out only when necessary and using lower rather than higher levels of fining agent addition, as it is possible to remove desirable aroma and flavour characteristics from the wine with excessive additions. It is important, however, that sufficient fining agent is added when the prime purpose of fining is to achieve stability and/or to remove undesirable sensory characteristics.

Different fining agents react differently with different wines², and even with the same wine. Therefore, sample testing, which involves adding varying amounts of a fining agent to small wine samples, is strongly recommended to determine the outcome of the specific fining material used and the optimum dosage to avoid over- or under-fining. The test samples are assessed for organoleptic quality, and the treatment is scaled up proportionately for the larger, production batch of wine.

Mixing

Powdered fining agents should be rehydrated in water before addition to wine, and the added fining agents must be thoroughly mixed throughout the wine. This can be achieved by constant stirring and slow addition, or incorporating the fining agent addition into a racking procedure for larger wine batches.

Addition of Fining Agents to White and Red Wine

According to international research concerning the presence of residual potentially allergenic proteinaceous fining agents in wine, it could be concluded that if residual fining agents cannot be detected using an analytical method with a limit of quantification of 1 mg/L, those agents are not present in the final product at levels which pose a risk to consumers with food allergies, see Annexes 2 to 5.

² Every wine is different in composition and will react differently to the same fining agent. The effectiveness of a fining agent will depend on the agent used, the preparations, the method of addition to the wine, the dosage, the wine's pH and metal content, the temperature, the dissolved CO₂ level, and any previous wine treatment.

Type of Wine	Fining Agent	Typical Addition (Mg/L)	Characteristics ³
White Wine	Isinglass	10-25 ⁴ 20-50 ⁵ 6-10 ⁶	Good clarity. Intensifies yellow colour. Light flakes, bulky, settles slowly
	Milk, Casein, sodium and potassium caseinate.	50-500 ⁷	Good clarification. Treats and prevents oxidation. No over- fining. Mainly used before alcoholic fermentation
Red Wine	Egg derived products	30-150 ⁸	Very good fining agent for tannic wines with some age. Tends not to remove protective colloids.
	Milk, Casein, sodium and potassium caseinate.	50-250 ⁹	Good clarification. Treats and prevents oxidation. No over- fining.

Milk, casein, sodium and potassium caseinate

Because wines differ in their composition, there is no set recommendation on the amount of casein to be used in fining. From the winemaker's perspective, it is important that no detectible protein remains in the wine after the fining/ clarification, as the presence of residual fining agent could lead to visual protein precipitates that necessitate further remedial processes. Excessive casein fining can also cause milky/cheesy aromas. Therefore, most fining processes are based on laboratory trials of individual batches of wine.

Casein is difficult to mix into the juice/wine as it is relatively insoluble in acidic solutions and should be mixed in water with a pH value above 8 or made alkaline prior to mixing. Potassium caseinate is usually used in preference to casein itself, as it can be dissolved directly in water. Either form is less effective when stirred into wine directly. Casein binds to the material to be removed from the wine before flocculating and precipitating quickly in the acidic environment. Slow and thorough mixing is important.

³ Canadian Vintners Association (2012)

⁴ Ribéreau-Gayon et al. (2000)

⁵ Wine Australia (2008)

⁶ New Zealand Wine Growers (2008)

⁷ Results of new studies to evaluate the potential allergenicity of wine made using proteinaceous processing aids (OIV 2010)

⁸ *Ibid*

⁹ *Ibid*

Casein is often introduced to the bottom of the vessel at fining and the wine is then agitated. This prevents clumps forming on the surface of the wine. After the fining agent has settled, the wine is either racked or preferably filtered.

Egg-derived products

Egg white can be used for fining (when necessary) when the wine is in barrel or prior to bottling. The resulting coagulum settles over the days following treatment and is separated from the wine by racking or preferably filtration.

Isinglass

Isinglass is a pure form of collagen, which is derived from the dried swim bladders of certain tropical and subtropical fish. Only minimal residual amounts of the allergenic fish protein, parvalbumin, have been detected in commercially produced isinglass, as it is not a component of the swim bladder and adventitious parvalbumin tends to be removed in the production process.

In the wine production process, isinglass is added prior to fermentation to remove phenolic compounds from white juice, or immediately post fermentation to remove yeast, phenolic and tannin compounds from white wine. The typical usage level is 10-25 mg/L for white wines, and the protein is subsequently removed by sedimentation and filtration. Isinglass is seldom used to fine red and rosé wines.

Annex 2 -- Historical and empirical evidence concerning the risks associated with consumption of fined wines by allergic individuals

The literature is almost completely lacking reports of genuine allergic reactions following the ingestion of wine. A comprehensive literature search has identified only a few case reports of severe adverse reactions to wine ingestion largely anecdotally attributed to biogenic amines such as histamine, salicylates or sulphites, and an alcoholic fermentation wine yeast, *Saccharomyces cerevisiae* (Clayton or Busse 1980, Littlewood et al. 1988, Clayton and Busse, 1989, Alibrandi et al. 1990, Kortekangas-Savolainen et al. 1994, Vally et al., 1999 and 2000, Kanny et al., 2001, Borghesan et al., 2004), in addition to grape proteins (Pastorello et al. 2003, Borghesan et al. 2004, Kalogeromitros et al. 2005). Concerning Vally et al. (1999 and 2000), however, there was not a significant and independent association between adverse reactions to wine and IgE-mediated allergies to eggs, fish or milk in the Asthma Foundations of Australia survey, which were asked as separate questions (personal communication with Dr Hassan Vally, PhD MAppEpid, on 31 January 2005), while Borghesan et al. (2004) suggest grape protein as the probable allergen given that the individual did not have a history of egg, fish or milk allergies but did have a history of IgE-mediated adverse reactions to red and white grapes. While IgE-mediated adverse reactions to grape proteins have been described in the literature these are, however, extremely uncommon.

The question of a reaction due to fining agents has not, however, been specifically considered in the literature and there is no published literature available on the concentration of these processing aids in the finished wine. If, however, the dose of a proteinaceous processing aid used in winemaking ranges between 1-50 mg/L (Ribéreau-Gayon et al. 1998) and is followed by further fining or clarification, it is likely that only ng-µg/L of a processing aid would reside in the finished wine. This level is 100-1000-fold less than the doses eliciting a reaction in previously conducted clinical trials (Hourihane et al. 1997b, Sicherer et al. 2000, Hourihane 2001). The 'gold standard' or definitive test for determining whether a patient is allergic to a particular product is a double-blind placebo-controlled food challenge (DBPCFC) (Bock et al. 1988).

There is also accumulating evidence to suggest that the majority of allergic individuals can tolerate small amounts of allergy-causing protein, although the threshold amount or dose varies among individuals and also among sources of the same protein/allergen (Hourihane 2001, Hefle and Taylor 2002, Taylor et al. 2002). For example, for sulphur dioxide, usually the threshold dose is considered to be 10 mg/L in sensitive individuals, which reflects existing international legislation (Vally and Thompson 2001). It has been clinically demonstrated, however, that sulphur dioxide will generally only elicit an adverse reaction in sulphite-sensitive asthmatics, which comprise approximately 1.7% of all asthmatics. Steroid-dependent asthmatics are most at risk of an adverse reaction (Vally and Thompson 2001). In a challenge study to determine a peanut protein threshold in sensitive individuals, the lowest dose to elicit a mild, non-threatening adverse reaction was observed to be 2 mg, although 50% of subjects could tolerate up to 50 mg (Hourihane et al. 1997b).

From a review of DBPCFC undertaken over the past 30 years in milk allergic adults, the maximum dose of milk powder/casein at which was tolerated was 14.1 g of milk powder or *ca.* 3 g of casein (Bernstein et al. 1982). Other subjects could only tolerate a doses of 105 mg milk powder (*ca.* 90 mg casein) up to 50 g milk powder (*ca.* 1.5 g casein) (Olalde et al 1989, Pastorello et al. 1989, Norgaard et al. 1992, Lam et al. 2008).

In an extensive food challenge study to determine an egg and milk protein threshold in 267 and 117 sensitive individuals, respectively, while some subjects (11% and 25%, respectively) reacted to doses of 100 mg, the majority of sensitive individuals could tolerate this dose (Sicherer et al. 2000, Hourihane et al. 2001), which would contain approximately 3 g casein and approximately 5.51 mg ovalbumin. Indeed it has been suggested that the threshold dose eliciting an adverse reaction in 1 in 1 million subjects with egg allergy is 0.002 mg or 2 µg and 1 in 100 patients is 3.4 mg (Bindslev-Jensen et al. 2002).

Furthermore, no purported allergic reaction to the use of egg, fish or milk as a proteinaceous processing aid in winemaking has been recorded in the database of The Australian Wine Research Institute (AWRI) in the past 20 years. Approximately 250 information requests are recorded annually, and include a record of all potential adverse effects reported to the AWRI's Health and Regulatory Information Manager from March 1991 to March 2011 by wine consumers and by wine companies on behalf of wine consumers. Similarly, The Alfred Hospital (Victoria, Australia) allergy specialists have not encountered any patients with allergic reactions attributable to a proteinaceous processing aid consumed in wine (personal communication with Professor Robyn O'Hehir, FRACP PhD).

References

- Alibrandi B., Parodi A., Varadlo G. Purpura due to ethanol. *N Engl J Med*, 322:702; 1990
- Bernstein, Day JH, Welsh A. 1982. Double-blind food challenge in the diagnosis of food sensitivity in the adult, *J Allergy Clin Immunol.*, 70(3):205-10
- C. Bindslev-Jensen, B. K. Ballmer-Weber, U. Bengtsson, C. Blanco, C. Ebner, J. Hourihane, A. C. Knulst, D. A. Moneret-Vautrin, K. Nekam, B. Niggemann, M. Osterballe, C. Ortolani, J. Ring, C. Schnopp, T. Werfel Standardization of food challenges in patients with immediate reactions to foods – position paper from the European Academy of Allergology and Clinical Immunology. *Allergy* 2004; 59: 690–697
- Bock, S.A.; Sampson, H.A.; Atkins, F.M.; Zeiger, R.S.; Lehrer, S.; Sachs, M.; Bush, R.K.; Metcalfe, D.D. (1988) Double-blind, placebo-controlled food challenge (DBPCFC) as an office procedure: a manual. *J. Allergy Clin. Immunol.* 82(6):986-97.
- Borghesan F., Basso D., Chieco Bianchi F., Favero E., Plebani M. Allergy to wine. *Allergy*, 59:1135-6; 2004
- Clayton D.E., Busse W. Anaphylaxis to wine. *Clin Allergy*, 10:341; 1980
- Hefle S.L., Taylor S.L. How much food is too much? Threshold doses for allergenic foods. *Curr. Allergy Asthma Rep*, 2(1):63-6; 2002.
- Hourihane J.O'B., Kilburn S.A., Nordlee J.A., Hefle S.L., Taylor S.L., Warner J.O. An evaluation of the sensitivity of subjects with peanut allergy to very low doses of peanut protein: a randomized double blind, placebo-controlled food challenge study. *J Allergy Clin Immunol*, 100(5): 596–600; 1997a.
- Hourihane J.O'B., Kilburn S.A., Dean T.P., Warner J.O. Clinical characteristics of peanut allergy. *Clin Exp Allergy*, 27: 634-639; 1997b.
- Hourihane, J.O'B. (2001) The threshold concept in food safety and its applicability to food allergy. *Allergy* 56 Suppl. 67: 86-90.
- Kalogeromitros D.C., Makris M.P., Gregoriou S.G., Mousatou V.G., Lyris N.G., Tarassi K.E., et al. Grape anaphylaxis: a study of 11 adult onset cases. *Allergy Asthma Proc*, 26:53-8; 2005.
- Kanny G., Gerbaux V., Olazewski A., Fremont S., Empereur F., Nabet F., Cabanis J.C., Moneret-Vautrin D.A. No correlation between wine intolerance and histamine content of wine. *J Allergy Clin Immunol*, 107:375-8; 2001.

- Kortekangas-Savolainen O., Savolainen J., Lantto R., Kalimo K. The immediate hypersensitivity to bakery, brewery and wine products in yeast-sensitive atopic dermatitis patients. *Clin Exp Allergy*, 24:836-42; 1994.
- Lam H-Y., Van Hoffen E., Michelsen A., Guikers K., Van der Tas C. H. W., Bruijnzeel-Koomen C. A. F. M., Knulst A. C. 2008. Cow's milk allergy in adults is rare but severe both casein and whey proteins are involved, *Clin. Exp. Allergy*, 38 (6):995 - 1002
- Littlewood J.T., Gibb C., Glover V., Sandler M., Davies P.T., Rose F.C. Red wine as a cause of migraine. *Lancet* 1:558-9; 1988,
- Norgaard A, Bernard H, Wal JM, Peltre G, Skov PS. 1996, Allergenicity of individual cow milk proteins in DBPCFC-positive milk allergic adults. *J Allergy Clin Immunol*, 97:237
- Osterballe M, Hansen TK, Mortz CG, A. Høst A and Bindslev-Jensen C. 2005. The prevalence of food hypersensitivity in an unselected population of children and adults *Pediatr Allergy Immunol* 16: 567–573
- Pastorello EA; Stocchi, L; Pravettoni, V; Bigi, A; Schilke, ML; Incorvaia, C; Zanussi, C. 1989. Role of the elimination diet in adults with food allergy. *J. Allergy Clin. Immunol.* 84:475-483.
- Pastorello EA, Farioli L, Pravettoni V, et al. Identification of grape and wine allergens as an endochitinase 4, a lipid-transfer protein, and a thaumatin. *J Allergy Clin Immunol*, 111(2):350-9; 2003,
- Ribereau-Gayon P., Glories Y., Maujean A., Dubourdieu D. *Handbook of Enology. Volume 2: The chemistry of wine stabilisation and treatments.* Chichester, John Wiley and Sons Ltd; 1998.
- Sicherer S.H., Sampson H.A. Peanut and tree nut allergy. *Curr Opin Pediatr*, 12: 567-573; 2000.
- Taylor, S.L.; Hefle, S.L; Bindslev-Jensen, C.; Bock, S.A.; Burks, A.W. Jr; Christie, L.; Hill, D.J.; Host, A.; Hourihane, J.O.; Lack, G.; Metcalfe, D.D.; Moneret-Vautrin, D.A.; Vadas, P.A.; Rance, F.; Skrypec, D.J.; Trautman, T.A.; Yman, I.M.; Zeiger, R.S. (2002) Factors affecting the determination of threshold doses for allergenic foods: how much is too much? *J. Allergy Clin. Immunol.* 109(1):24-30.
- Vally H., Carr A., El-Saleh J., Thompson P. Wine-induced asthma: a placebo-controlled assessment of its pathogenesis. *Journal of Allergy and Clinical Immunology*, 103: 41–6; 1999.
- Vally H., Thompson P.J. Role of sulfite additives in wine induced asthma: single dose and cumulative dose studies. *Thorax*, 56:763-9; 2001.
- These observations are further supported by data submitted by the Liquor Control Board of Ontario (LCBO) to the United States Alcohol and Tobacco Tax and Trade Bureau (TTB) in response to its Notice of Proposed Rulemaking No. 62 71 FR 42329 – July 26, 2006:

December 19, 2006

Mr. John Manfreda
Administrator
Alcohol and Tobacco Tax and Trade Bureau
U.S. Treasury
650 Massachusetts Avenue,
NW Washington, DC 20226

Dear Mr. Manfreda,

Re: Comments in Response to Notice No. 62 71 FR 42329 – July 26, 2006

We appreciate the opportunity to comment on the proposed regulations on “Major Food Allergen Labeling for Wines, Distilled Spirits and Malt Beverages”.

As the importer of record of beverage alcohol in the province of Ontario, we are concerned that the proposed regulations could mislead the consumer and will not provide the consumer with adequate information as to the correct identity and quality of the products.

The proposed labeling requirement for allergens is mandated of the fact that processing aids are used and designed to be absent from the final product, and if used and removed according to good manufacturing practice, the final concentration of these substances in the wine, if present at all, is likely to be extremely low due to the removal of precipitates through the clarification process.

There is no published literature available on the concentration of these proteinaceous fining agents in the finished wine; however, there are commercially available assays to measure their concentration in foods – ELISA and PCR. Unfortunately the lower level of sensitivity of both of these assays is generally at the mg/L level, which is approximately 100-fold higher than the likely level of processing aid residue in wine when GMP is adopted.

Furthermore, there is no reliable scientific data on the human threshold limits to sensitivity of these potential allergens, other than the study published by J. M. Rolland, et. al., *Nutrition* 22 (2006), 882-885 from Monash University, Melbourne, Australia.

The justification for your proposed regulations relies heavily on anecdotal evidence of adverse allergic reactions. In this respect, we believe that we can provide you with substantial, objective information from our consumer complaint database regarding whether wine that we import poses an allergen risk.

The LCBO is a provincial government enterprise reporting to the Minister of Infrastructure Renewal. It is one of the world’s largest retailers of beverage alcohol, importing products from over sixty countries world wide with a retail network of more than 800 stores in the province of Ontario, Canada. Net sales in 2005/06 were at \$3.68 billion CDN which represented 51.2% of the Ontario beverage market share. Total volume sales for the same year were 388,733,000 litres of which 14% represented spirits, 29% wine, 49% beer and 8% ready to drink beverages. During this same period, more than \$240 million of revenue was due to USA beverage alcohol sales, of which approximately 46% was from wine.

On a given year the LCBO retails either through its stores or through private stock/direct delivery/virtual offer programs more than 12,000 brands of beverage alcohol products of which approximately 75% represents wines, 10% spirits and the balance beer and ready-to-drink products. One of the primary reasons of this

amazing selection of products is the demographics of our consumer base, which represents a multicultural society of more than 100 nationalities.

The LCBO is committed to retailing products of good quality, authentic and free of any contaminants, and as such all products listed by the LCBO are stringently evaluated for taste and appearance and chemically tested by its state-of-the-art Quality Assurance testing facilities.

Quality Assurance is also responsible for monitoring and investigating all customer complaints.

LCBO classifies customer complaints into two categories; complaints of a general nature and complaints requiring investigation. Complaints of a general nature are open bottles returned to an LCBO retail outlet for reasons of off taste, off odour, off colour, foreign matter or other, e.g., faulty package. The customer is issued a refund for their purchase and the complaint information is keyed into our Point of Sale (POS) system. Complaint data is transmitted nightly to our corporate mainframe and reconciled on a weekly basis. Statistical reports comparing the ratio of total complaints received, by Stock Keeping Unit (SKU), to the actual sales are generated and reviewed to identify possible product quality problems. Complaints requiring investigation are complaints of alleged illness, personal injury or property damage. Retail staff notifies Quality Assurance immediately upon receipt of the complaint and arrangements are made to have the customer's sample forwarded for investigation. The steps taken to investigate the complaint are dependent on the nature of the complaint and the condition of the sample. Sensory evaluation, laboratory and packaging testing may be conducted. The customer is provided with a written report at the conclusion of the complaint.

In reviewing our Customer Complaint data base year-to-date since the year 2000, we have recorded 486,535 customer complaints. Of the total number of complaints, 1,344 (0.28%) were investigated by QA, of which 337 (0.07%) were of an alleged illness related nature.

One (1) complaint was specifically identified as an allergic reaction confirmed by a physician at a hospital emergency. The product consumed was a liquor type (Amaro Feltsina Ramazzotte). This product contains a mixture of several herbs, including "chinarinde", a source of quinine.

The possible side effects of quinine are well documented. The symptoms described by this customer, swelling of the lips & face and hives, are the classic symptoms of an allergic reaction to quinine.

Considering our total volume sales, the demographics of our customer base and the large selection of products we retail, we can postulate that the lack of any substantiated adverse allergic reactions to wine products in the last approximately six years, provides strong evidence that legally permitted additives and processing aids for wine-making, present virtually no risk of severe adverse reaction such as anaphylaxis.

As a consequence of the lack of data available on the residual of processing aids in wine and the inability to accurately and sensitively measure the residual at present as well as the lack of data on harm (human threshold limits to sensitivity), such regulations would be technically of no additional value to consumers and practically impossible to enforce at any level.

In order to avoid unnecessary expenses at all levels, we would suggest a delay in the implementation of such legislation until all of the above concerns are reasonably addressed.

Thank you for allowing us to submit our comments and we appreciate the granting of the extension on the comment period.

We would be happy to respond to any questions you may have as related to our comments. Sincerely yours,

George Soleas, M.Sc., Ph.D., MCIC
Vice President, Quality Assurance

c.c. Mr. Bob Peter, President & Chief Operating Officer, LCBO
Mr. Bob Downey, Senior VP, Sales & Marketing, LCBO
Mr. John Salminen, Chief, Chemical Health Hazard Assessment Division, Health Canada
Ms. Carla Barry, National Manager, Fair Labelling Practices Program, CFIA
Mr. Dan Paszkowski, President, Canadian Vintners Association

Annex 3 -- Summary of scientific data documenting the risks associated with consumption of fined wines by allergic individuals

Research groups in Australia, France and Germany (2006-2009) all undertook a complementary double-blind placebo- controlled clinical study to determine if egg/fish/milk-allergic consumers elicited a positive reaction on consumption of a wine made with any of these particular proteinaceous processing aids. The groups included the:

- Department of Asthma, Immunology and Respiratory Medicine, The Alfred Hospital (Victoria, Australia) and The Australian Wine Research Institute (South Australia, Australia)
- Technical University of Munich, Clinic of Dermatology and Allergology Biederstein and the University of Hamburg, Department of Chemistry, Institute of Food Chemistry (Germany)

Food related allergies affect 1-2% of the adult population as allergies to egg and milk observed in 6%-8% of infants and children usually resolve by four years of age. This low adult prevalence of egg and milk allergies is reflected in the small number of subjects able to be recruited for the study in Australia, France and Germany. In total, only 26 Australian and 23 French/German allergic subjects could be recruited for the studies. This small size shows or suggests that the size of the potential problem is small. In particular, only one milk-allergic subject was recruited in Australia and only five egg-allergic subjects. In all countries, the diagnosis of IgE-mediated food allergy was confirmed by a clinical allergist based on a history of adverse reactions and anaphylaxis and corresponding demonstration of specific IgE to allergens of fish, egg and/or milk using, for example, the immunoCAP fluoroenzyme system and/or by skin-prick testing (wheal ≥ 4 mm in diameter).

No life-threatening adverse reactions such as asthma (constriction of bronchioles), laryngeal edema (swelling of the throat) and anaphylactic shock (blood pressure decrease, cardiac arrhythmia and multiple organ failure) were experienced by any of the subjects on consumption of protein fined-wine. Subjective, mild clinical symptoms were recorded by a small number of subjects. For example, in Australia, the one milk allergic subject gave a subjective 'lump or tickle in the throat' response to a milk-fined wine, one of the five egg allergic subjects had transient reduced lung function (22% and 11%, respectively, reduced FEV₁) which resolved immediately to both the egg-fined and the unfined control wine. Clinical assessment suggested that this subject had unstable asthma triggered by the spirometric manoeuvre, resulting in non-specific airway reactivity. No adverse reactions to casein-fined wine were observed in the six German milk-allergic subjects.

In Germany only one of the eight egg-allergic subjects had a skin condition adverse reaction to a French egg- fined wine, which resolved on treatment. Also, two egg-allergic subjects had a subjective adverse reaction - one 'laryngeal/pharyngeal discomfort' to an egg-fined wine although a subsequent skin prick test was negative and one oropharyngeal pruritis (itching) which was subsequently shown to contain residual egg white. In addition, one French egg allergic patient had a subjective reaction to a French egg-fined wine.

In the Australian clinical study, the subjects were challenged with 100 mL (one Australian standard drink) on two occasions, separated by at least 7 days; a fined wine and an unfined control wine. The subjects were then monitored for 2 h post challenge in the hospital and then by daily diary for a further 6 days for adverse reactions.

Similar to the Australian clinical study, in the French and German clinical studies, the subjects were challenged with protein-fined and unfined control wines on two occasions, separated by at least 2 days. On each occasion, however, successive doses from 1 drop to a total of 300 mL (three Australian standard drinks) for men and 200 mL (two Australian standard drinks) for women were administered in four steps at 30 minute intervals. The challenge ceased immediately if any subjects experienced an adverse reaction. The subjects were then monitored

for 2 hour post challenge in the hospital/research department, and then by daily diary for a further 2 days for adverse reactions. Subjects also underwent skin prick tests to casein and egg white and to the protein-fined and unfined control wines. One German egg allergic subject initially had an anaphylactic- related adverse reaction to the skin prick test with egg protein but on subsequent retesting with a German egg-fined wine, however, had no adverse reaction. Only one French egg allergic subject had a positive skin prick test with egg protein but on subsequent retesting with a French egg-fined wine, however, had no adverse reaction.

In summary, none of the 49 Australian and French/German subjects had a clinically significant or life-threatening adverse reaction to a protein-fined wine -- only one of 49 subjects had a mild skin condition adverse reaction. There is accumulating evidence to suggest that the majority of allergic individuals can tolerate small amounts of allergy-causing protein, although the threshold amount or dose varies among individuals and also among sources of the same protein/allergen (Hourihane 2001, Hefle and Taylor 2002, Taylor et al. 2002). In a challenge study to determine an egg and milk protein threshold in sensitive individuals, while some subjects (11% and 25%, respectively) reacted to doses of 100 mg, the majority of sensitive individuals could tolerate this dose (Sicherer et al. 2000, Hourihane et al. 2001). A recent literature review suggests that the threshold dose eliciting an adverse reaction in 1 in 1 million subjects with egg allergy was 0.002 mg or 2 µg and 1 in 100 patients was 3.4 mg (Bindslev-Jensen et al. 2002).

The highest amount of residual egg white protein in a German wine, which was actually fined with 5-times the amount of egg white recommended by the manufacturer, was only 0.02 mg/L. No residual milk protein was found in any of the Australia, French and German wines analysed.

Relevant references by the Australian, French and German research groups are:

Kirschner S, Belloni B, Kugler C, Ring J, Brockow K. Allergenicity of wine containing processing aids: a double-blind, placebo-controlled food challenge. *J Investig Allergol Clin Immunol*. 2009; 19(3):210-7.

Lifrani A, Dos Santos J, Dubarry M, Rautureau M, Blachier F, Tome D. Development of animal models and sandwich-ELISA tests to detect the allergenicity and antigenicity of fining agent residues in wines. *J Agric Food Chem*. 2009; 57(2):525-34.

Rolland JM, Apostolou E, Deckert K, de Leon MP, Douglass JA, Glaspole IN, Bailey M, Stockley CS, O'Hehir RE. Potential food allergens in wine: double-blind, placebo-controlled trial and basophil activation analysis. *Nutrition*. 2006; 22(9):882-8.

Sabine Hildebrandt, Hartmut D. Kratzin, Raphaël Schaller, Rodolphe Fritsché, Hans Steinhart, and Angelika Paschke : In Vitro Determination of the Allergenic Potential of Technologically Altered Hen's Egg J. *Agric. Food Chem.*, 2008, 56 (5), 1727-1733.

Annex 4a -- Summary of data indicating that residual protein is negligible using routine and readily available test methods in commercial wines fined according to internationally agreed best practices.

A systematic review of the scientific literature supports that the known thresholds for adverse reactions to egg white are approximately 1 to 2 mg and for milk protein (such as casein) are approximately 100 µg. Accordingly it has been suggested, that to guarantee the safety of 95% of allergic consumers, on the basis of 100 g of food (100 mL wine), the detection limits of any analytical methods should be equal to or exceed a sensitivity of 10 mg/L for egg white and 1 mg/L for milk proteins. In addition, considering consumption of 1 L of wine on a heavy drinking occasion, the quantity of total protein ingested would be approximately 1 mg. Most likely, however, the maximum ingestion of wine in short period of time would be limited to 500 mL corresponding to 0.5 mg of proteins.

Research groups in Australia and Germany have all undertaken complementary analytical and clinical research programmes in order to determine the allergenic potential of protein fined-wine. The groups are:

- Monash University (Victoria, Australia) and The Australian Wine Research Institute (South Australia, Australia)
- Research Institute Geisenheim, Section of Oenology and Wine Technology and the University of Hamburg, Department of Chemistry, Institute of Food Chemistry (Germany)

For example, each research group developed an analytical method such as a specific and sensitive ELISA to determine if there were residuals of the allergenic processing aids remaining in the final wine product. The wines analysed were either commercially available or made specifically for the studies with differing additions of processing aid. The German analytical and clinical studies were broadly based on the initial Australian study.

No residual processing aid was found in any of the 153 Australian wines analysed, however, a small amount of residual egg and milk processing aid was found in a small percentage (6 % and 1 %, respectively) of the 400 French and 56 German wines. Specifically, residual protein (approximately 0.02 mg/L or 20 µg/L) was found in one egg-white fined wine which had been fined with 5-times the recommended dosage and in seven wines treated with 25 or 50 g lysozyme; 50 g is twice the recommended dosage (Weber et al. 2007). Of the 9% of French wines that were organic, that is, where the wines were not filtered after fining, 13.5 % contained residual casein or egg white protein compared with only 5.5 % of the non-organic wines.

The commercially-available Australian wines were all made according to Good Winemaking Practice, that is, were fined and then filtered. The German wines were made like their commercially-available equivalents but had specific amounts of casein, dried egg white or lysozyme added at a dosage within the manufacturer's recommendation or up to five-times higher than recommended or twice for lysozyme. The wines were then further fined with bentonite and then filtered. The French wines were also commercially-available.

The lower limits of detection in the Australian-developed ELISA specific for the casein and ovalbumin proteins were 8 and 1 µg/L, respectively (Rolland et al. 2008). The lower limits of detection in the German-developed ELISA specific for the both casein and egg white proteins was 400 µg/L and the lower limit of detection for lysozyme was 5 µg/L.

These results suggest that adhering to a specific amount of addition for casein and egg white, followed by further fining with bentonite which absorbs positively charged proteins, and filtration are important for removing residual protein from wine. Alternatively or in addition, the wine tannins form cross links with protein leading to protein precipitation, such that precipitated proteins are readily removed by filtration.

In a subsequent but as yet unpublished study of German white wines treated with different fining agents and processes including casein and ovalbumin/hen's egg white were investigated by indirect ELISA. Analytical techniques such as sensitive indirect ELISA and immunoblotting methods are considered to be unequivocal measure of potentially allergenic protein residues.

No residues of casein and ovalbumin were detectable in the wines treated with common concentrations of these substances and by good manufacturing practice (GMP). Double doses of ovalbumin in the fining process, however, led to detectable residues of ovalbumin in the wine. The limit of detection of the analyses is 70 µg/L (70 ppb or 0.07 ppm) for casein and 2 µg/L (2 ppb or 0.002 ppm) for ovalbumin. These detection limits are much less than the proposed clinical threshold levels of the BfR paper (Statement No. 002/2010 des BfR of 29. July 2009) given at 100 ppm to 10 ppm allergenic food and 10 ppm to 1 ppm allergenic protein, respectively. The fining agents in this study were used at maximum doses according to legislation and at double the maximum doses as a worst case scenario: casein 40/80g/hL and hen's egg white 110/220g/hL. The fining agents remained 24 h in the wine before being racked. The wine then passed through pasteurization and filtering processes. In addition, different commercially-available Australian white wines labeled with "May contain" milk and/or egg or without labeling of allergens were also investigated by the indirect ELISA. No residues of casein and ovalbumin were detectable in the commercially-available white wines.

These results mean that the proteinaceous wine fining agents casein and egg white used according to GMP in winemaking show that there may only be residues of casein and ovalbumin below 0.07 and 0.002 mg/L (ppm), respectively, in the final wine product.

Hence they are not likely to trigger adverse reactions in milk or egg allergic individuals, respectively, which comprise approximately 1% or less of the adult population.

Relevant references from the Australian and German research groups are:

Rolland JM, Apostolou E, de Leon MP, Stockley CS, O'Hehir RE. Specific and sensitive enzyme-linked immunosorbent assays for analysis of residual allergenic food proteins in commercial bottled wine fined with egg white, milk, and nongrape-derived tannins. J Agric Food Chem. 2008; 56(2):349-54.

Weber P, Steinhart H, Paschke A. Investigation of the allergenic potential of wines fined with various proteinogenic fining agents by ELISA. J Agric Food Chem. 2007; 55(8):3127-33.

Weber, P., Kratzin, H., Brockow, K., Ring, J. Steinhart, H., Paschke, A. Lysozyme in wine: A risk evaluation for consumers allergic to hen's egg, Molecular Nutrition and Food Research 2009, (53): 1469-1477.

Weber, P., Steinhart, H., Paschke, A. Determination of the bovine food allergen casein in white wines by quantitative indirect ELISA, SDS-PAGE, Western-Blot and Immunostaining, Journal of Agricultural and Food Chemistry 2009; (57):8399-8405.

Weber, P., Steinhart, H., Paschke, A. Competitive indirect ELISA for the determination of parvalbumins from fish species in food gelatins and isinglass with PARV-19 anti parvalbumin antibodies, Journal of Agricultural and Food Chemistry 2009; (57): 11328-11334.

Weber, P., Steinhart, H., Paschke, A. Characterization, antigenicity and detection of fish gelatine and isinglass used as processing aids in wines, Food Additives and Contaminants 2010; (3): 273-282.

Weber, P., Steinhart, H., Paschke, A. Allergic potential of fining agent residues in German wines related to their dosage and an ordinary bentonite treatment, Agro Food industry High Tech 2007: 18 (5), 22-24.

Annex 4b -- Additional Recent Scientific Publications on Fining Agent Residues in Wine

The following papers have been published recently on the subject of determining residual fining agents in wines treated with protein-based fining agents. The general theme is of a pursuit for increasingly sensitive methods, but the papers also provide additional evidence of the following:

- the negligible quantities (if any) of protein in commercially fined wines,
- the importance of filtration procedures (such as those in the good fining practice guidelines above) to remove completely or reduce the fining agent residues in treated wines to the lowest technologically practical level, and
- The efficacy (in the case of egg proteins) of commercial ELISA test kits to determine residual protein in fined wines.

1) Cereda, A., Kravchuk, AV., D'Amato, A Bachi, A., Righetti, PG. Proteomics of wine additives: Mining for the invisible via combinatorial peptide ligand libraries. J. Proteomics 2010; 73:1732-1739

The authors developed a highly sensitive proteomic analysis (with a lower detection limit of 1 µg/L of protein) and applied it to determine residual casein in commercially fined Italian white wines. They do not detail the results they found, other than to indicate that in one sample they found 50µg protein in a 750 mL bottle (67µg/L, or 0.067 mg/L), that they found “traces” of casein in almost all the samples they analyzed, down to 10µg total protein in a 750 mL bottle.

2) D'Amato, A., Kravchuk, AV., Bachi, A., Righetti, PG. Noah's nectar: The proteome content of a glass of red wine. J. Proteomics 2010; 73:2370-2377.

The authors applied their highly sensitive proteomic analysis (see (1) above) with a lower detection limit of 1 µg/L of protein to determine residual casein in commercially fined Italian red wines. In those wines that gave positive results, the highest level of casein found was 85 µg/L (or 0.085 mg/L). Results are only tabulated for a selection of the wines analyzed. The authors state, *“Probably, considering that minute levels of caseins found in most red wines (ranging from 45 to 85 µg/L), it is doubtful that such trace amounts might be sufficient to provoke severe allergic reactions.”*

3) Monaci, L., Losito, I., Palmisano, F., Visconti, A. Identification of allergenic milk proteins markers in fined white wines by capillary liquid chromatography–electrospray ionization- tandem mass spectrometry. J. Chromatography A, 1217 (2010) 4300-4305.

The authors developed a method based on capillary liquid chromatography combined with electrospray ionization-tandem mass spectrometry for the detection and identification of casein deriving peptides in fined white wine. They state that *“this MS based approach appears to be a useful tool for screening purposes as well as a confirmatory tool for the unequivocal identification of caseins in ELISA positive samples.”*

4) Lacorn, M., Gößwein, C., Immer, U. Determination of Residual Egg White Proteins in Red Wines during and after Fining. Technical Brief, Am. J. Enol. Vitic. 62:3 (2011)

The authors assessed the efficacy of a commercial ELISA test kit for determining residual egg proteins in fined wines. In summarizing the results of their “field study”, the authors state:

“The courses of concentrations for egg white protein in both red wines (Pinot noir and St. Laurent) revealed comparable results (Figure 1). Before fining, both wines had values below the LOQ of 0.5 mg/L. As expected, concentrations increased to values between 350 and 410 mg/L after adding the liquid egg white and remained at these high levels during stirring of the wine. These concentrations are close to the expected value of 381 mg/L and also show that even precipitates, which could be visually detected, are solubilized by the extraction procedure. After six hours of sedimentation, there is a clear difference of >200 mg/L between the two red wines.

This difference is not observable after 24 hr of sedimentation when egg protein concentrations are <40 mg/L. The first filtration over diatomaceous earth led to concentrations of 1.1 mg/L for Pinot noir and 9.4 mg/L for St. Laurent. After filtration over a 0.6 µm filter and bottling, the concentrations were below the LOQ for Pinot noir and 1.1 mg/L for St. Laurent. This was comparable to recently published results using highly sensitive ELISAs with detection limits of 4 µg/L for casein and 1 µg/L for egg white (Lifrani et al. 2009); that study also found detectable amounts at the beginning of the fining process but no residues after the final third filtration. Therefore, it could be speculated that a further filtration step in our study would lower the minimal residues of egg proteins in the St. Laurent below the LOQ as for the Pinot noir.”

5) Tolin, S., Pasini, G., Curioni, A., Arrigoni, G., Masi, A., Mainente, F., Simonato, B. Mass spectrometry detection of egg proteins in red wines treated with egg white. Food Control 23 (2012) 87-94.

The authors developed a tandem liquid chromatography-mass spectrometry analysis. However, they did not use the system quantitatively but only qualitatively. Though the paper does quote levels of egg protein in relation to wine, these seem to be the quantities of protein added in order to perform the fining process and not the residual levels in the treated and filtered wines.

Annex 5 -- The ability of the fining procedures elaborated in internationally agreed best practices to consistently achieve the desired outcomes

5.1 Analysis at the Point of Production

The information presented here comes from a commercial winery and was provided to the trade before 2012, after which commercial kits capable of detecting residues to 0.25ppm LOD became more available to companies wishing to demonstrate the effectiveness of their procedures by following FIVS Good Fining Practice Guidelines designed to mitigate residual levels of milk or egg proteins.

With growing awareness of the potential residue issues associated with the fining process, the company in question instituted internal procedures that are an integral part of its ISO/FSSC-22000 and internal laboratory ISO 17025 accreditations. These systems require that wine is tested for the presence of residual protein after fining and filtration steps, and that the treated wine is not permitted to proceed to further blending or bottling until a test result demonstrates a “none-detected” result for residual fining agent protein in that wine. In addition, data tracking systems and clearly defined staff responsibilities within the company ensure that this “hold pending a none-detected result” status is respected in the company cellar for treated wines. This system means that the wine is tested in a worst-case scenario, soon after the fining and filtration process but before possible subsequent blending and before final pre-bottling filtration steps. Both of these operations would tend to further reduce levels of any residual protein that is undetectable by the analyses conducted but may continue to coagulate and sediment from the wine over time. A specially designated Management Team within the company provides oversight to ensure that the aforementioned controls are effective.

The fining and filtration processes are typically conducted as follows. The milk protein or egg protein is added in-line during a pump-mix of the tank. The wine is mixed and then cross-flow filtered (nominal pore size of 0.2 µm) or pressure-leaf filtered using diatomaceous earth before a sample is taken for residual protein analysis.

The analyses are performed using commercially available ELISA test kits for milk and for egg proteins. The company laboratory conducted internal verification procedures with each kit to determine its performance within the laboratory environment. These tests included spiking wines with specified amounts of certified milk or egg protein standards obtained from the kit manufacturer, and then checking for recovery. Both kits were determined to be performing adequately for the intended purpose. Although the manufacturer of the kits indicates a detection sensitivity of 1 mg/L for egg protein and 2.5 mg/L for milk protein, these internal company verification procedures suggested that under the conditions of use within the company lab, the limit of detection (LOD) for the methods are 2.2 mg/L for the egg analysis and 2.7 mg/L for the milk method.

The company has provided information on the analyses that were performed during the previous 2 years¹⁰. In that time, a total of 560 samples were analyzed, on which 526 residual milk protein tests and 524 residual egg protein tests were performed. The tested wines were either produced and fined on site by the winery, or were obtained from other suppliers as part of normal business operations (samples of all wines obtained or being considered for purchase from other sources are tested for milk and egg protein residues as a routine practice and appropriate corrective actions are implemented where necessary).

¹⁰ This case study took place before 2012 and was made available to the trade to demonstrate the efficacy of its fining procedures over the preceding two years which it was then undertaking for routine due diligence purposes. The company that provided the information had been using the best commercially available kits at the time. These were not able to achieve the kind of limits of detection that the OIV subsequently specified, and commercial kits capable of detecting residues to 0.25 ppm LOD have become available since 2012.

The wines that were tested during this two-year period were red, white and blush wines of many different grape varieties. Of the 1050 tests conducted, all 1050 gave a “none-detected” result for residual milk or egg proteins.

The company also reported results from some wines that were fined with egg protein and immediately tested for residual protein without an intervening filtration step. Eight wines from this work initially showed trace levels of residual protein in a range from 2.5 mg/L to 16.0 mg/L. Each of the wines from which these samples were taken was then subjected to further processing (most often by the use of cross-flow filtration) and in each case **no residual protein** was detected on re-testing.

Finally, the company indicated that samples of 5 wines from external suppliers, being checked against specifications during the course of normal commercial operations, initially tested positive for egg proteins (the range of results being from 3.0 mg/L to 27.0 mg/L). All these wines were further processed by the suppliers, usually by filtration, and showed **no detectable residual fining agent protein** on retesting.

The experience of this company demonstrates that with the implementation of procedures such as those outlined in the Good Fining Practice Guidelines for Wine specified in this document, it is possible to treat wines with milk and egg-based fining agents in such a way that all treated wines consistently show no detectable protein residues using commercially available ELISA test kits.

5.2 Analysis on Importation

Analysis was carried out in the UK in 2015 on behalf of importers on over 1000 individual products fined with egg or milk derivatives. The range of wines analysed was wide, representing all major wine producing countries, varieties and styles.

Data showed clearly that no wine made following the ‘Good fining practice’ guidelines had tested positive for either egg or milk protein, see Section 3.

The information presented suggests that, although companies have due diligence procedures which reflect their individual businesses and supply chains, if good fining practices are followed, wines treated with egg and milk fining agents contain no detectable residues. As such routine allergen residue sampling procedures can be appropriately targeted, thus leading to a reduction in unnecessary and costly analysis.

Annex 6 -- Checklist - GMP Principles for Effective Allergen Management

Fundamentals	
	Have you conducted a risk assessment and constructed an “allergen map” representative of the manufacturing life cycle to identify where allergens are introduced and where exposure is likely?
	Can you develop a site specific Allergen Control Plan for each processing facility?
	Have you a process to regularly review and update the Allergen Control Plan?
Product Design	
	Is the use of a particular allergenic fining agent a technical necessity for the blend?
	Are different supplied forms available that provide equal efficacy at lower dosing?
Materials Storage & Handling	
	Do you have a materials receivable tagging and tracking process for use within your facility?
	Do you have handling procedures for allergenic materials to minimise unnecessary exposure?
	Do you have segregated and sealed storage away from non-allergenic winemaking materials?
Supplier Controls	
	Do you have supplier declarations of allergen status of all winemaking ingredients?
	Will your suppliers notify you if there is a change to the allergen status of any ingredients?
	Do you audit your suppliers on their allergen controls, sanitation procedures & validations?
	Do your suppliers provide ingredients in clearly marked, sealed containers?
Prevention of Cross-Contact	
	During production, is the lowest effective quantity of allergenic fining agent being used?
	During transfer and bottling, is there any changeover risk and need for product segregation and sanitation procedures?
	If wine is being reworked, is the allergenic status of additional ‘tip back’ wine or blended wine known?
Product Labelling	
	Do you use appropriate labelling for different national jurisdictions?
	Do you have a label approval process and version control process that validates declarations against manufacturing?
	Does your supply chain use inventory control and packaging staging control steps to ensure labels with the correct declarations are used?
Validated Allergen Cleaning Programs	
	Is your manufacturing plant designed for effective cleaning?
	Do you have Standard Operating Procedures for sanitations?
	Do you have Cleaning Validation & Verification Procedures?
	Are your procedures audited for compliance, and monitored for ongoing effectiveness?

Based on FARRP – Components of an effective Allergen Control Plan: A framework for Food Processors