Canadian Vintners Association

Association des Vignerons du Canada

GUIDANCE FOR THE FINING OF WINE AND THE LABELLING OF FINED WINES

July 2012

This document is intended as a guide only; legal requirements are contained in the Food and Drugs Act Regulations Amending the Food and Drug Regulations (1220 — Enhanced Labelling for Food Allergen and Gluten Sources and Added Sulphites). The information in this document should not be relied upon as legal advice or used as a substitute for legal advice.

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1 PURPOSE

This document details the regulatory background to 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 the use of these practices removes from the final wine product residual levels of egg, fish, milk proteins used as fining agents in winemaking.

This guidance is required to address situations where (based on the best available scientific information) eggs, fish and milk used as fining agents in the winemaking process are not present in the final product at levels which pose risk to consumers with food allergies.

2 REGULATORY BACKGROUND

In Canada, the *Food and Drugs Act* ("the Act") is the basis for the regulatory oversight of all substances used in food processing and manufacture. Under the Act, the *Food and Drug Regulations* ("the Regulations") require the labelling of food allergens, gluten sources and added sulphites that are present in food.

Under the new allergen labelling provisions announced February 4, 2011, standardized alcoholic beverages will not be required to provide a list of ingredients, but will require a "Contains:" statement to identify any common food allergens present in the product. When the statement "Contains:" appears on the label (either by choice or because it was triggered by the presence of food allergens in the product) this statement must be complete and must identify all common food allergens in the prepackaged product.

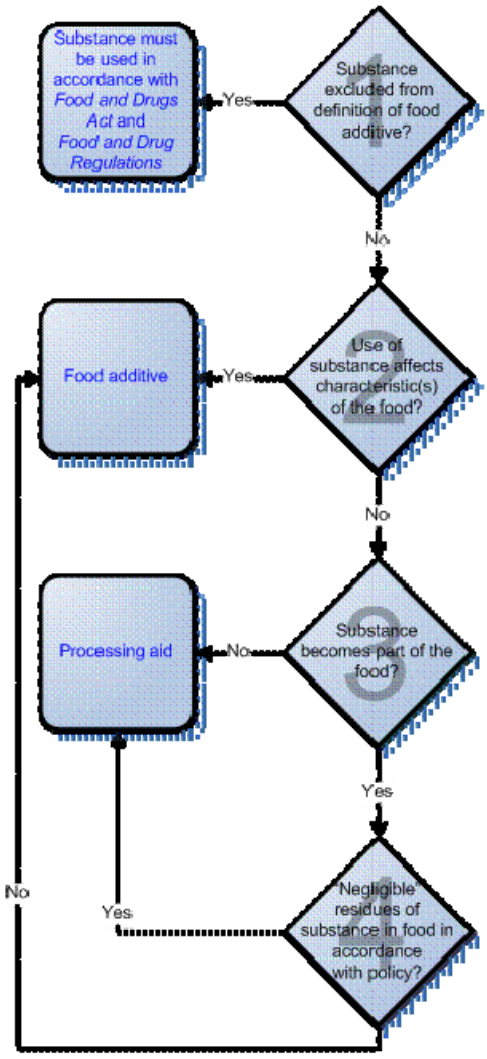
Health Canada has developed a position on the labelling requirements of vintage wines in preparation for the August 4, 2012 coming into force of the regulatory amendments to enhance the required labelling for food allergens, gluten sources and added sulphites. Health Canada's position is that the new allergen labelling regulations will continue to apply to all non-vintage wines and vintage wines with a year date of 2012 and later, but that vintage wines with a year date of 2011 and earlier will be exempt from the food allergen labelling regulations and can continue to be sold with their original labels.

2.1 Distinguishing between Food Additives and Processing Aids

2.1.1 Food Additive

"Food additive" is defined in section B.01.001 of the Regulations as *"any substance the use of which results, or may reasonably be expected to result, in it or its by-products becoming a part of or affecting the characteristics of a food."*

Fining agents are not automatically excluded from the Food and Drug Regulations as a "food additive". While the action of a fining agent in wine is consistent with that of a processing aid, whether it is regarded as such or as a food additive under the Regulations depends on the residue(s) of the agent (if any) in the finished product.



2.1.2 Processing Aid

A food processing aid is a substance that is used for a technical effect in food processing or manufacture, the use of which does not affect the intrinsic characteristics of the food and results in no or negligible residues of the substance or its by-products in or on the finished food.

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

2.1.3 Decision Tree for Distinguishing Between Food Additives and Processing Aids

Figure 1 shows a decision tree that can be used to distinguish between food additives and processing aids, based on the regulatory definition of “food additive”. The questions in the tree should be answered by following the principles outlined under "Principles for using the decision tree".
Figure 1 - Decision tree to distinguish between food additives and processing aids, based on the regulatory definition of “food additive”.

2.1.4 Principles for Using the Decision Tree in the Case of Wine Fining Agents

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

Principle1: Egg, fish and milk fining agents are defined as processing aids under the Regulations B.02.100 (Wine) but are not specifically excluded 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 the presence of the substance continues to have a technological function on the finished wine. Thus eggs, milk and fish products used as fining agents would be viewed as processing aids rather than additives provided there are negligible (if any) residues of the fining agent or its by-products 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) become part of the wine, which they do, unless it can be demonstrated that any residues of the substance in or on the finished wine are "negligible".

Question 4: Are residues of the substance in the wine "negligible" in accordance with this policy?

Principle 4: Negligible in the case of eggs, fish and milk fining agents used in winemaking means that there are no residues of public health significance in the finished wine, and that any residues that are present are at levels too low to exert a technical effect in or on the product. Health Canada has not specified a minimum threshold, but the data presented in Annexes 3-6 provide some scientific and technological guidance for consideration.

The Winemaker should determine, on a case-by-case basis, whether residues are, or are likely to be, negligible in quantity (and thus in public health significance) and in technical effect in the wine that will be offered for sale. Supporting evidence to assist in making this evaluation might include:

(1) The unit processes to which the product will be exposed after fining that will serve to reduce or eliminate residues of fining agents or their by-products from the wine (i.e., the filtration processes, blending steps, etc.);

(2) Data from analytical tests showing that the residue levels are at or below a low level (i.e., 1 mg/L); and/or

(3) Information from scientific literature and practical experience showing that residue levels are too low to have any technical effect in, or on the finished wine. In practice, this will always be the case at levels of 1 mg/L or lower for the fining agents in the purview of these Guidelines.

2.2 Labelling of Wine Fining Agents under Allergen Labelling Regulations

Wine which is fined in accordance with internationally agreed best practices (See Section 3) contains negligible (if any) amounts of residual fining proteins that can be detected using routine and readily available analytical methods sensitive at or below 1 mg/L. Therefore the fining agents are functioning as processing aids in the wine and will not be required to be indicated (i.e., eggs, milk or fish) in a “Contains:” statement on the label.

3 GOOD FINING PRACTICE GUIDELINES FOR WINE[1]

Fining is the winemaking process either before and/or after the fermentation process to remove unwanted insoluble particles and undissolved 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 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 indeed are designed to be entirely removed from the treated wine as part of the fining process.

The effectiveness of a given fining agent depends on the agent, its method of preparation and addition, the levels of addition, together with characteristics of the wine such as pH, metal content, temperature, presence of CO_2 and prior wine treatments.

In addition to the steps outlined below for good fining practices, winemakers should give attention to maintaining 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, in keeping with the normal requirements of traceability.

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

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

3. The laboratory trial runs should also duplicate, as far as possible, the treatment to be conducted in the cellar, giving attention to the batch of fining agent to be used, the method of its preparation and addition to the wine, and the temperature of the laboratory sample with respect to that of the bulk wine to be fined. Hydration protocols for protein fining agents should be consistent between laboratory and cellar.

4. A minimal volume of distilled, de-ionised or other suitably pure drinking water should be used in order to dissolve or disperse the fining agent without overly diluting the wine (applicable regulations must be observed).

5. The quantity of fining agent used should always be the smallest amount needed to achieve the desired result as stipulated by winemaker sensory and/or analytical evaluation, and in no case shall the amount used exceed any applicable regulatory limits.

6. Thorough and adequate mixing of the fining agent into the juice or wine should be ensured, and sufficient time should be allowed for the material to react prior to immediate racking and/or subsequent filtration.

7. Industry recognized best practice filtration methods (including passing the wine through a fine powder filtration process and/or pre-bottling filtration through a 0.65 µm or smaller filter, or performing treatments of equivalent effect) should be used to remove insoluble protein fining agents. Where the treated wine is simply racked off the lees remaining from the fining treatment, or where a less rigorous filtration or other technique for removal of the lees is applied, and it is desirable to confirm that there is

[1] Adapted from the FIVS "Good Fining Practice Guidelines for Wine" (2007, see Annex 2), taking account of information such as that presented in Annex 1, "Fining Agents - Technical Aspects".

only negligible (if any) residual fining agent, a laboratory test should be conducted to confirm this at some stage prior to bottling.

8. Routine, periodic monitoring of the fining process shall 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 in such a way as to negligible (if any) fining agent residues in the treated wine.

   Analysis shall always be conducted on fined wines that are intended to be bottled without filtration, to ensure that no residues of fining agents may be detected. Corrective action must be taken where the analysis of such wines indicates the presence of residual fining agents, or the product labels must reflect that presence in a "Contains:" statement.

9. Verification should be conducted at regular intervals, and should consist of a review designed to ensure that monitoring is occurring carefully and consistently, at a frequency that is adequate to give confidence that the fining processes are being conducted in such a way as to leave only negligible (if any) 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 (i.e., through false positive results).

4 FOOD SAFETY CONTROL MEASURES FOR WINE FINING[2]

A study has been conducted 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 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 risk to consumers with food allergies. This section of the document presents the method followed in this evaluation, together with the outcome.

4.1 VALIDATION STEPS

4.1.1 Pre-validation Tasks Undertaken

a. **Hazard:** The presence of residual allergenic protein in wines fined with milk, eggs and fish and their products.

b. **Food safety outcome required**: Negligible (if any) levels of residual fining agents (using routine and readily available test methods) in wines fined according to internationally agreed best practices (i.e., the FIVS "Good Fining Practice Guidelines for Wine" presented in Annex 1 or a derivative with at least equivalent provisions relating to filtration of the fined product).

c. **Control measure to be validated**: The fining and filtration processes applied to wines

4.1.2 Approach

Historical empirical data and recent scientific studies allow the control measure to be validated without the need for additional studies (see Annexes 3-6).

[2] Based on the Codex Alimentarius Commission " Guidelines For The Validation Of Food Safety Control Measures" - CAC/GL 69 - 2008

4.1.3 Parameters and Decision Criteria:

a. Parameters:

i. The amount of protein used to fine a wine should be determined to be the minimum amount required to produce the desired outcomes.

ii. Wines should be fined according to internationally agreed best practices.

b. Decision Criteria:

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

4.1.4 Relevant validation information and the need for further studies.

a. Historical and empirical evidence concerning the risks associated with consumption of fined wines by allergic individuals are summarized in Annex 3. They show that such risks are very small, based on literature reviews, emerging information on 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 4. They show that none of the 49 subjects in clinical trials 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.

c. Information to demonstrate that the proteinaceous wine fining agents casein and egg white used according to GMP in winemaking guarantee that there will only be residues of casein and ovalbumin below 0.07 and 0.002 mg/L (ppm), respectively, in the final wine product are presented in Annex 5. 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 has been reviewed to determine their pertinence to the internationally agreed best practices. The information is believed to be sufficient to validate the control measure without the need for further studies.

4.1.5 Analysis of results

a. Data acquired on the ability of the fining procedures elaborated in internationally agreed best practices to consistently achieve the desired outcomes. The results of this analysis are presented in Annex 6.

4.1.6 Documents and decisions supporting the validation of the control measure

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 negligible (if any) residual levels of protein from fish, eggs or milk food allergens in the finished wine product.

b. These data can be used to establish a program of monitoring for residual fining agents in treated wines.

4.2 MONITORING STEPS

Routine, periodic monitoring of the fining process shall 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 in such a way as to leave negligible (if any) fining agent residues in the treated wine.

Analysis should always be conducted on fined wines that are intended to be bottled without filtration, to ensure that no residues of fining agents may be detected. Corrective action must be taken where the analysis of such wines indicates the presence of residual fining agents, or the product labels must reflect that presence in a "Contains:" statement.

4.3 VERIFICATION STEPS

Verification should be conducted at regular intervals, and should consist of a review designed to ensure that monitoring is occurring carefully and consistently, at a frequency that is adequate to give confidence that the fining processes are being conducted in such a way as to leave negligible (if any) fining agent residues in the treated wine. 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 (i.e., through false positive results).

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 wines[3], 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 agent 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 risk to consumers with food allergies (see Annexes 3 to 6). In such circumstances an individual consuming 2 glasses of wine (284 mL) would ingest 0.284 mg of potentially allergenic protein.

[3] 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
White Wine	Isinglass	10-25[4] 20-50[4] 6-10[5]	Good clarity. Intensifies yellow colour. Light flakes, bulky, settles slowly
	Milk, Casein, sodium and potassium caseinate.	50-500[8]	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[8]	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 little of the protein remains in the wine after the fining/ clarification, as the presence of relatively large amounts of residual fining agent will 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. Casein is often

[4] Ribéreau-Gayon et al. (2000).
[4] Wine Australia (2008)
[5] New Zealand Winegrowers (2008)
[8] Results of new studies to evaluate the potential allergenicity of wine made using proteinaceous processing aids (OIV 2010)
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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 is 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 -- FIVS Good Fining Practice Guidelines for Wine[9]

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 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 indeed are designed to be entirely removed from the treated wine as part of the fining process.

The effectiveness of a given fining agent depends on the agent, its method of preparation and addition, 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 steps outlined below for good fining practices, winemakers should give attention to maintaining 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, in keeping with the normal requirements of traceability.

1. Fining agents should 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.
2. It is recommended that laboratory scale trial runs be conducted prior to treatment of wine in the cellar.
3. The laboratory trial runs should also duplicate as far as possible the treatment to be conducted in the cellar, giving attention to the batch of fining agent to be used, the method of its preparation and addition to the wine, and the temperature of the laboratory sample with respect to that of the bulk wine to be fined. Hydration protocols for protein fining agents should be consistent between laboratory and cellar.
4. A minimal volume of distilled, de-ionised or other suitably pure water should be used in order to dissolve or disperse the fining agent without overly diluting the wine (applicable regulations must be observed).
5. The quantity of fining agent used should always be the smallest amount needed to achieve the desired result as stipulated by winemaker sensory and/or analytical evaluation, and in no case should the amount used exceed any applicable regulatory limits.
6. Thorough and adequate mixing of the fining agent into the juice or wine should be ensured, and sufficient time should be allowed for the material to react prior to immediate racking and/or subsequent filtration.
7. Industry recognized best practice filtration methods (including passing the wine through a fine powder filtration process and/or pre-bottling filtration through a 0.65 µm or smaller filter, or performing treatments of equivalent effect) should be used to remove insoluble protein fining agents. Where the treated wine is simply racked off the lees remaining from the fining treatment, or where a less rigorous filtration or other technique for removal of the lees is applied, and it is desirable to confirm the absence of detectable residual fining agent, a laboratory test should be conducted to confirm this at some stage prior to bottling.

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[9] FIVS is a worldwide organization for all sectors of the alcohol beverage industry, including wine, beer, and spirits. Its members include producers, distributors, importers, exporters, and trade associations. FIVS is a non-governmental organization (NGO) that gathers and disseminates information related to activities of interest to its members and advocates consensus positions to international organizations. Founded in July 1951, FIVS has its headquarters in Paris, France. Best Fining Practices approved March 7, 2007

Annex 3 -- 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 identifies 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 proteinacous 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 sulfur dioxide, usually the threshold dose is considered to be 10 mg/L in sensitive individuals, which reflects existing Australian and international legislation (Vally and Thompson 2001). It has been clinically demonstrated, however, that sulfur 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 aids 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 includes 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 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).

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These observations are further supported by date 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 4 -- Summary of scientific data documenting the risks associated with consumption of fined wines by allergic individuals

Research groups in Australia, France and Germany 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 include 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 FEV_1) 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 reactions – one 'laryngeal/pharyngeal discomfit' 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 5a -- 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 programs 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 Enology 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 0.7 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 BfRof 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 guarantee that there will 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 20090; (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 5b -- 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 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 6 -- The ability of the fining procedures elaborated in internationally agreed best practices to consistently achieve the desired outcomes

The information presented here comes from a foreign commercial winery and was made available to Canadian Vintners' Association to demonstrate the effectiveness in commercial practice of the fining procedures contained in the Good Fining Practice Guidelines for Wine that are designed to mitigate residual levels of milk or egg proteins that might arise from the use of milk-based or egg-based fining agents in wine.

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 years. 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).

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.

Annex 7 -- CFIA Food Safety Action Plan Report 2009-2010 Targeted Surveys on Allergens

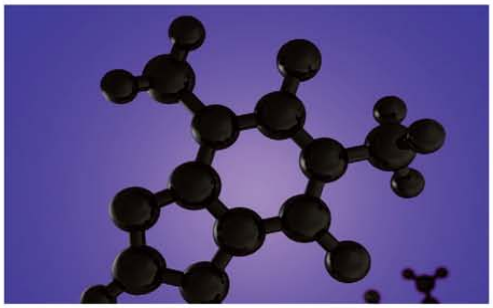
Canadian Food Inspection Agency | Agence canadienne d'inspection des aliments

Food Safety Action Plan

REPORT

2009-2010 Targeted Surveys

Allergens



Fining Agents in Wine

TS-ALLERGEN-09/10

Canada

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1 Executive Summary

The Food Safety Action Plan (FSAP) aims to modernize and enhance Canada's food safety system. As part of the FSAP enhanced surveillance initiative, targeted surveys are used to evaluate various foods for specific hazards.

The main objectives of this targeted survey were to:

- Provide baseline surveillance data for allergens in the fining agents used in wine production
- Determine if the traces of allergens observed in wines would potentially pose a risk to the allergic population in Canada

Fining agents are used to remove fine particles naturally present in wine. This improves the clarity, as well as, enhances the palatability of unfinished wine prior to filtration and bottling. Commonly used fining agents are milk protein, Isinglass (fish protein), and egg white. As a result of using these products there is the potential for allergenic proteins to be left behind, which could result in a reaction of a sensitive individual.

One hundred samples of wine were collected at retail. The one hundred samples consisted of forty nine red wine samples, one rosé and fifty white wine samples (of which one was a sparkling wine). Samples of the wine were analysed for milk (casein and beta-lactoglobulin) and egg allergens using a proven method validated in multiple matrices. We did not analyze for fish, an allergen found in Isinglass, as there are no commercially available methods for this component. None of the samples analysed had detectable levels of the milk or egg allergens. At the present time, allergens including sulphites are exempted from declaration on wine labels under the *Food and Drugs Act and Regulations (FDA&R)*. No milk or egg allergens were detected in the wine samples collected for this targeted survey. Therefore, the samples are considered to be compliant with current regulations.

2 Introduction

2.1 The Food Safety Action Plan

The Food Safety Action Plan (FSAP) is a five-year project (2008-2013) led by the Canadian Food Inspection Agency (CFIA) and is a part of the Food and Consumer Safety Action Plan (FCSAP), a joint federal initiative with Health Canada, the Public Health Agency of Canada and the Canadian Institutes of Health Research. The FCSAP encompasses a series of initiatives to modernize and strengthen Canada's safety system for food, health and consumer products and to better support the collective responsibilities that government, industry and consumers have for product safety. The four main priorities identified for the FSAP were imported food ingredients, fresh produce, mycotoxins in cereals and undeclared allergens.

Within FSAP, the CFIA gained increased ability to monitor potential food risks and to prevent unsafe food products from entering the Canadian marketplace. The CFIA fulfils this mandate through an enhanced surveillance initiative which includes targeted surveys. The CFIA works on these targeted surveys with input from other federal partners (e.g., Health Canada) and Provincial and Territorial (P/T) representatives.

2.2 Targeted surveys

Targeted surveys are pilot surveys used to test various foods for specific hazards and are a complementary approach to the CFIA's regular programs and inspection activities. The surveys are designed to answer specific questions about specific hazards in a given food. Generally, they test for the occurrence and magnitude of defined hazards in targeted foods, often with the testing focusing on a specific segment of the population (i.e., consumers with an allergy or intolerance). Surveys may be developed using a number of factors such as policies and/or regulations, existing data from food safety investigations, inspections, and other regular agency activities.

This targeted survey focused on the presence of two undeclared allergens, milk and egg proteins in wines available at retail. The information gathered will identify if these commodities require follow up with industry in order to provide further guidance, education and monitoring for the presence of allergens when they are not expected or declared. This survey will also inform the allergic consumer about the present and potential levels of milk and egg protein in the wine they may be consuming.

2.3 Acts and Regulations

The *Food and Drug Act* (F&DA) is the legal authority that governs the sale of food in Canada. The *Canadian Food Inspection Agency Act* stipulates that the CFIA is responsible for enforcing restrictions on the production, sale, composition and content of foods and food products as outlined in the *Food and Drugs Act & Regulations* (FDAR).

If a pre-packaged food product displays a list of ingredients without disclosing potential allergens this may result in an unsafe product for allergic consumers. Failure to declare allergenic components may be contrary to Subsection 5(1) of the F&DA. These products may therefore be subject to regulatory measures taken by the CFIA, which can include a product recall.

Health Canada has enhanced labelling regulatory amendments to the *Food and Drugs Regulations* (FDR) for the nine priority allergens, gluten and sulphites in pre-packaged food sold in Canada. On February 16th, 2011 Health Canada Published Amendments to the Food Allergen Labelling Regulations in *Canada Gazette*, Part II (CGII). The amendments require that food allergen and gluten sources be declared on the labels of pre-packaged foods, having a list of ingredients, whenever the protein, modified protein or protein fractions of the food allergen or gluten source are added to the product. The amendments also require the labelling of added sulphites. In addition to requirements around gluten labelling, mustard seed is proposed for addition to the list of priority.

Due to the complexity of the changes and the shelf-life of foods, Health Canada is allowing manufacturers 18 months to implement the new allergen labelling regulations. Health Canada continues to encourage industry to declare priority allergens, gluten sources and added sulphites on pre-packaged food labels to provide Canadians with the information necessary to make informed food choices. Canada's new food allergen labelling regulations will come into force on August 4, 2012. Further information on these proposed regulations can be found on the Health Canada website[1].

3 Allergen Survey

3.1 Rationale

The presence of an undeclared allergen or gluten in a food for sensitive individuals can be life threatening or contribute to chronic health issues. Current estimates indicate that food allergies affect as many as 6% of young children and 3% to 4% of adults[2]. Celiac

[1] Health Canada. *Health Canada's Modifications to Regulatory Project 1220- Enhanced Labelling for Food Allergens, Gluten Sources and Added Sulphites* [online]. 2010. Accessed October 27, 2010, http://www.hc-sc.gc.ca/fn-an/label-etiquet/allergen/proj1220-modifications-eng.php.
[2] Health Canada. *Food Allergies and Intolerances* [online]. 2010. Accessed October 27, 2010, http://www.hc-sc.gc.ca/fn-an/securit/allerg/index-eng.php.

disease has been recognized as a common chronic disease affecting 1 in every 100-200 people[3]. In Canada, eight main allergens (known as priority allergens) have been identified by Health Canada as responsible for causing the majority of allergic reactions[4]. These allergens are: milk, eggs, peanut, sesame seeds, tree nuts, soy, wheat and seafood. Sulphites have also been recognized as having the potential to produce serious symptoms similar to an allergen in sensitive individuals. There is no cure for a food allergy, and the most important strategy for a person with a food allergy, or a person choosing food for an individual with a food allergy, is avoidance. Allergens and gluten sources should be appropriately labelled to ensure consumers have complete, accurate information when choosing food products.

This survey was designed to sample wines for milk and egg allergens found in commonly used fining agents which are used to clarify and increase the palatability of the final product. The main objective is to determine if the allergenic proteins from eggs and milk still remain in the finished wine after the fining process is completed. The information gathered will be an indicator of potential food safety concerns relating to undeclared milk and egg allergens in the wines tested.

3.2 Hazard Allergic Proteins

Fining agents are introduced prior to filtration and are used to improve clarity, odour, colour, flavour and physical stability of wine. They do so by attracting positively and negatively charged particles in unclear wine. Once completed the fining agents and the captured compounds settle to the bottom and form a precipitate.[5] Commonly used fining agents in wine production are milk protein, Isinglass (fish protein), and egg white. As a result of the precipitate formed by these agents, there is the potential for allergenic proteins to be left behind after filtration. Any of the allergenic proteins remaining in the wine could result in a possible reaction in a sensitive individual.

When a food allergen is consumed it can trigger a reaction of the immune system in sensitive individuals. The immune system in a sensitive individual produces antibodies, called immunoglobulin E (IgE) in response to the presence of allergenic proteins in the body. When the immune system is re-exposed to the allergenic protein the IgE antibodies and other defence chemicals are released causing allergic reactions that can vary by type, severity and rates of development. Symptoms of an allergic reaction can include hives, swelling, trouble breathing, weakness, cramps, vomiting, drop in blood pressure, shock, loss of consciousness and even death[6].

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[3] Health Canada. *Celiac Disease, The Gluten Connection* [online]. 2010. Accessed October 27, 2010, http://www.hc-sc.gc.ca/fn-an/alt_formats/hpfb-dgpsa/pdf/securit/gluten_conn-lien_gluten-eng.pdf.
[4] Health Canada. *Food Allergies and Intolerances* [online]. 2010. Accessed October 27, 2010, http://www.hc-sc.gc.ca/fn-an/securit/allerg/index-eng.php
[5] Bill Collings. *Fining and Fining Agents* [online]. 2002. Accessed October 27, 2010, http://www.bcawa.ca/winemaking/fining.htm
[6] Health Canada. *Food Allergies and Intolerances* [online]. 2010. Accessed October 27, 2010, http://www.hc-sc.gc.ca/fn-an/securit/allerg/index-eng.php

3.3 Sample Distribution

The survey targeted a variety of wines, domestic and imported. A total of 100 samples of red wine, white wine, sparkling white wine and rose were collected nationally. The distribution of sample by type and commodity is listed in Table 1. Wines were collected at provincial liquor stores across the country.

Table 1 Sample Distribution

Country	Number of Samples			
	Red Wine	White Wine	Sparkling White Wine	Rosé
Argentina	4	4	-	-
Australia	4	4	-	-
Canada	24	24	1	1
Chile	4	4	-	-
France	5	5	-	-
Italy	4	4	-	-
United States	4	4	-	-
Total	**49**	**49**	**1**	**1**

4 Methodology

The samples were tested for beta-lactoglobulin (milk protein), casein (milk protein) and egg. There was no knowledge of the type of fining agents used during the production of the wines tested. Food allergen proteins were detected and measured by CFIA laboratories using ELISA-based accredited methodology. Fish was not included in this targeted survey due to the fact there are no commercially available kits to analyze for fish protein.

The methods and limits of detection were as follows:

- Beta-Lactoglobulin, ELISA Systems, Beta-Lactoglobulin Residue, ESMRDBLG-48, limit of detection 0.10 ppm beta-lactoglobulin
- Casein, ELISA Systems, Casein Residue, ESCASPRD-48, limit of detection 0.52 ppm casein
- Egg, Neogen Veratox Quantitative Egg Assay, 8450, limit of detection 2.5 ppm egg

4.1 Limitations

A total of 100 samples were collected and analysed in the 2009-2010 fining agents in wine targeted survey. In comparison to the total number of products available, 100 samples represents a small fraction of wine types available to consumers. This data is

meant to provide a snapshot of the targeted commodities and has the potential to highlight commodities that warrant further investigation. Also, this survey does not examine year-to-year trends, impact of product shelf-life or cost of the commodity on the open market.

5 Results and Discussion

5.1 Allergen Analysis

Although other fining agents may contain allergens, such as Isinglass (fish protein), this survey focuses on the potential traces of milk and egg protein. Fish was not included in this survey as Health Canada and CFIA have not completed an evaluation of available methods of analysis for the detection of fish protein. Health Canada and CFIA are in the process of evaluating additional methods of analysis which may include an analysis kit for the detection of fish protein. Other fining agents used, such as betonite clay, silicon dioxide and carbon, do not contain allergenic proteins that would be considered a potential hazard to the allergic population.

The 100 samples underwent all three analysis types in order to determine if there were any residual milk and/or egg proteins as a result of using fining agents. All analysis results were below the limit of detection.

There have been very few studies on the level of possible allergenicity of alcoholic beverages as a result of using fining agents containing allergenic proteins. It is widely assumed that the majority of the allergenic proteins, from fining agents, likely to illicit an allergic reaction in sensitive individuals would be removed from the finished wines prior to bottling.[7] One study conducted showed that measurable levels of allergenic protein were as a result of spiking with five times the normal dose used and that residual allergenic proteins from using commercial levels of fining agents were not likely.[8] The results of this survey support these observations.

6 Conclusion

A total of 100 wine samples available at retail were collected. Red, rosé and white wines from eight countries, a total of 49 red, 49 white, 1 rosé and 1 white sparkling wine were collected and analysed for the presence of casein, beta-lactoglobulin and egg protein. The results of the analysis showed that within the 100 samples tested no residual allergenic proteins remained as a result of the fining process.

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[7] 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. Chrom. A* 2010; 1217:4300-4305.

[8] 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; 55:3127-3133.

One hundred samples collected were compliant with labelling requirements according to the F&DA with respect to labelling allergens. This survey was limited in the number of samples collected, however, it met the objective of gathering baseline information on the occurrence of undeclared priority allergens in wine as a result of the fining process. Based on the results, no gaps in food safety for allergens in wines were identified.