IMMUNOCHEMICAL INVESTIGATION OF ALLERGENIC RESIDUES IN EXPERIMENTAL AND COMMERCIALLY-AVAILABLE WINES FINED WITH EGG WHITE PROTEINS

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Abstract

Proteinaceous egg whites are widely used as a fining agent during red winemaking. The presence of residues of egg white in the final wine could, however, represent a risk for egg allergic individuals. The aim of the study was to investigate the presence of allergenic residues in red and white wines fined with egg whites. Different experimental and commercially-available wines fined with egg whites, with or without subsequent bentonite fining, were included in this study. Unfined wines were examined as negative controls. The physicochemical characteristics of each wine were determined to assess their possible role in enhancing or hindering the elimination of allergenic residues from wine. The amount of egg white protein residues was investigated both by an ELISA test, specifically developed, and by immunoblotting. Both immunochemical tests used the same anti-total egg white protein antibody and showed high sensitivity to detect traces of allergen. No egg white protein was detected in the wines studied in both immunochemical tests, irrespective of the physicochemical characteristics of the wine, the type and dosage of the fining agent and the oenological processed used. Hence, the risk of adverse reactions in egg allergic individuals should be considered negligible.

***Key words**: wine, allergenic residues, egg white proteins, fining agent*

1. Introduction

Fining is one of the least expensive operations in wine production that has a major impact on wine quality. The aim of the fining process 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 stabilise and reduce the colour by the adsorption and precipitation of polymeric phenolic compounds and tannins.[1] Nowadays, a range of proteinaceous fining agents are used, including gelatine, milk proteins, egg proteins, isinglass and, more recently, proteins derived from plants such as wheat and white lupin.[2,3] Water-soluble egg white (albumin or albumen) is the most commonly used fining agent in red winemaking. It has a positively charged surface that binds with negatively charged compounds such as tannins. The high molecular weight of the resulting aggregates allows their mechanical elimination by racking and/or filtration prior to bottling or further maturation. A second fining agent may be used, such as the inorganic fining agent bentonite, that adsorbs proteins thus helping to remove residual proteinaceous fining agents from the wine.[4]

Egg white contains several allergenic proteins such as ovalbumin, ovomucoid, ovotransferrin and lysozyme.[5-7]

If fining agents are used and removed according to a good manufacturing practice, it can be assumed that these proteins are not present in the final wine product. Good manufacturing practice for fining is essentially defined as using the smallest amount of fining agent needed to achieve the desired result followed by racking and pre-bottling filtration processes (Organisation de la Vigne et du Vin 2012). To date there is limited evidence, however, that wines in the marketplace are free from residues of proteinaceous fining agents. In addition, the several studies that have evaluated wines for residual protein have had conflicting results perhaps partially reflecting different analytical methodologies as well as differences in manufacturing practice.[8-12] The presence of allergenic proteins in wine could cause an adverse reaction in sensitized individuals, although the prevalence of allergy to egg proteins is rare in

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adults. It is less rare, however, in children (*ca.* 0.6-2.6%), but generally resolves by six to seven years of age.[13-17]

The European Union adopted the Directive 2003/89/EC,[18] last amended by Directive 2007/68/EC, [19] which contains a list of allergenic substances (Annex III), including egg and egg derivatives that have to be declared on the label of foodstuffs. EC Directive 2005/26/EC[20] listed food ingredients that were provisionally excluded from the labelling requirement; inclusion of wine fining agents in this list was postponed until June 2012 to allow for further study since there was limited scientific data concerning their actual presence or absence in fined wines [19, 21]. It should be noted that the inclusion of a statement such as "contains egg proteins" on the wine label can contribute to the uncertainty of consumers (allergic or not), simultaneously damaging the "quality perception" of the product.

The present study was aimed to investigate the presence of allergenic residues in 14 experimental and 77 international, commercially-available wines fined with egg white by a newly developed ELISA test with improved limits of detection amd quantification, and by immunoblotting; both tests used antibodies specifically developed versus egg white fining agent.

2. Materials and methods

2.1. Wine samples

Experimental and commercially-available wines fined with egg proteins were included in the present work.

Experimental wines included red wines fined with 3 or 10 g/hL of egg white, both concentrations with the subsequent addition of 0, 10, 20 or 30 g/hL of bentonite. All wine samples were microfiltrated through a 3 μm-membrane pore size. The detailed characteristics of these 14 experimental wines are listed in Table 1. A further group of 12 wines which were not treated with egg albumin were included as negative controls (data not shown).

A panel of 84 commercially-available bottled wines supplied by several wine producers from five different countries were included in this study. Among them, 48 red wines and one white wine were supplied by Italian winemakers; seven of these samples were untreated wines and were used as negative controls. Other wines were from France (20 samples), Australia (12 samples), New Zealand (two samples) and Spain (two samples). Only wines where the oenological practices were known were included in this study. The detailed list of the 84 commercially-available wine samples studied, including their physicochemical characteristics, is presented in Table 2. The agents used during wine fining were the following: Albapur (Tecnofood, Italy); Albovo (Oliver Ogar, Italy); Albuclar (Vason Group, Italy); Albumin Dry (Enolife srl, Italy); Egg albumin (Dal Cin SpA, Sesto San Giovani, Italy); Albumin powder (Laffort Oenologie, France); Albumin powder (Lamothe Abiet, France); Blancoll (Esseco srl, Italy); Oviclair (La Littorale, France); Ovoclar (Pall Corporation, Italy); Ovoclaryl (Laffort, France); and Ovocol L (Martin Vialatte Oenology, France).

2.2. Physicochemical characteristics of wines

The following physicochemical characteristics were evaluated for the wine samples:

2.2.1. Alcoholic strength by volume (% vol.).

It is defined as the number of litres of ethanol contained in 100 litres of wine, measured at 20ºC. This method involves distilling wine volume by volume; the volumetric weight of the distillate is measured by electronic densitometry using a frequency oscillator. [22]

2.2.2. Total alcoholic strength by volume

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It is a calculation of the potential alcohol concentration if all remaining sugars were to be fermented. It is calculated by adding potential alcoholic strength to alcoholic strength by volume. [23]

Potential alcoholic strength by volume is defined as the number of volumes of pure alcohol at 20ºC produced by total fermentation of the sugars contained in 100 volumes of the product at that temperature and it is calculated by multiplying the concentration of reducing sugars (g/L) by 0.06.

2.2.3. Reducing sugars

To prepare samples with a sugar content ranging between 0.5 and 5 g/L, dry wine (sugar concentration < 5 g/L) was diluted 1:2 (v:v) with water; sweet wine was suitably diluted to reach the values reported above. Red wine was clarified with solutions of neutral lead acetate and calcium carbonate (Merck KgaA, Darmstadt, Germany). A specific quantity of an alkaline solution of copper salts is heated and the copper ions are titrated by the clarified/diluted wine, in the presence of methylene blue as indicator (UIV internal method, 2009).

2.2.4. Total acidity.

Wine total acidity was determined by acid-base potentiometric titration, using 0.1 N NaOH, to pH 7, with an automatic titrator. [22]

2.2.5. Volatile acidity.

To determinate the volatile acidity of wines, carbon dioxide was first removed from the wine sample. Volatile acids were then separated from wine by steam distillation and titrated using NaOH. The acidity of free and combined sulphur dioxide distilled under these conditions was substracted from the acidity of the distillate, after filtration by standard iodine solution. [25]

2.2.6. pH.

The pH value of wine samples was determined by potentiometry using a calibrated pH-meter. [26]

2.2.7. Ash content.

Ash amount was measured by ignition of wine extract at 500-550ºC until the complete combustion (oxidation) of organic material had been achieved. Then, the residue obtained after combustion was weighed using a balance having sensitivity of 0.1 mg.[27]

2.2.8. Total dry extract and reduced extract.

The dry total wine extract was indirectly calculated from the specific gravity of the alcohol-free wine, after measuring the specific gravity at 20ºC of the wine and of the water-

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alcohol mixture obtained by distillation of wine sample. The reduced extract was calculated as the difference between the total dry extract and the reducing sugars in excess of 1g/L.[28]

2.2.9. Total phenolic compounds.

The total phenolic compounds were analysed using the Folin-Ciocalteau Method, with some modifications.[29] Wine samples were diluted and then mixed with the Folin-Ciocalteau reagent, which oxidizes all the phenolic compounds, and sodium carbonate (Merck KgaA, Darmstadt, Germany). Afterwards, absorbance was measured at 760 nm.

2.2.10. Anthocyanins.

Total anthocyanins were determined according to Di Stefano and Cravero[30] with some modifications. Briefly, wine samples were diluted with an acidulated ethanol-water solution. The absorbance spectrum was then determined between 420 and 620 nm.

2.3. SDS-PAGE and Immunoblotting

Wine samples were analyzed by SDS-PAGE according to Ballabio et al.,[31] on a gel having the following characteristics:

Gradient running gel: 9-19% acrylamide; 0.08-0.17% bis-acrylamide; 0.36 M TRIS-HCl buffer pH 8.8; 35% glycerol; 0.1% SDS; 0.02% ammonium persulfate; and 0.15% N,N,N',N'-tetramethylenediamine (TEMED).

Stacking gel: 3.5% acrylamide; 0.09% bis-acrylamide; 0.125 M TRIS-HCl buffer pH 6.8; 0.1% SDS; 0.02% ammonium persulfate; and 0.15% (TEMED).

Running buffer: 25 mM TRIS, 0.19 M glycine and 0.1% SDS (w/v), pH 8.8.

Sample buffer: The composition of the *2x sample buffer* was: 0.25 M Tris-HCl buffer pH 6.8, 22.5 % glycerol, 2% SDS and 5% β-mercaptoethanol.

Purified egg proteins and fining agents were diluted in water at the final concentration of 4 mg/mL and then mixed with 2x sample buffer (1:1, v/v). Regarding wines, aliquots of 1mL of each wine sample were dried under nitrogen at room temperature until a dry extract was obtained. Afterwards, 200 μL of a solution containing water: 2x sample buffer (1:1, v/v) were added to the dry extract. Sample aliquots of 25 μL were loaded onto the gel. Prestained SDS-PAGE standard Broad Range (BioRad), containing proteins in the range 6,7-202,8 kDa, was used to control the electrophoretic run.

After the electrophoretic run, proteins were transferred onto a PVDF membrane (Millipore, Billerica, MA) by western blotting in a Trans-blot Electrophoretic Transfer Cell (Bio-Rad). The membranes were blocked with 1% gelatin and washed three times with 0.25% gelatin solution (150 mM NaCl, 5 mM TRIS, 0.05% Triton-X) to prevent non-specific adsorption of the immunological reagents. Afterwards, the membrane was immersed in 10 mL of 0.25% gelatin solution containing 10 μL of rabbit anti-total egg white protein IgG polyclonal antibodies. This antibody was specifically developed for this research using total egg proteins for immunization according to common protocols of sensitization. The antibody was characterized in order to ensure its capability to detect the different egg white allergens. Antigen-IgG complexes were detected by using 10 μL of goat anti-rabbit IgG antibodies labeled with peroxidase (Sigma Aldrich, Milan, Italy). The developing solution contained DAB (3,3' diaminobenzidine) Plus substrate and DAB Plus Chromogen (Sigma Aldrich).

2.4. *ELISA Test*

A sandwich ELISA kit (Euroclone SpA, Pero, Milano), specifically developed for the quantification of egg white proteins in wine was used.[32] It is a sandwich ELISA where the microplate is first coated with the specific anti-egg white protein antibody also used in immunoblotting; after incubation with the wine sample, a secondary anti-egg white protein antibody conjugated with horse radish peroxidase (HRP) is added to form a sandwich. To determine the detection limit (LOD) and the quantification limit (LOQ) the protocol described in the OIV "Compendium of international methods of analysis" E-AS1-10-LIMDET[33] was used. The limits were calculated according to the procedure "Determination on blank" using the data from 11 laboratories participating to a collaborative inter-laboratory study. For quantification, standard solutions contained increasing amounts of egg proteins and spiked wine samples containing egg white in the range 0-7 ppm were used. All standards and wines were diluted 1:5 (v/v) with the buffer supplied with the kit. Statistical analysis of the obtained results was performed according to UNI ISO 5725-2:2004 an to the OIV "Compendium of international methods of analysis" MA-EAS1-07-ETCOL; the repeatability and reproducibility of the ELISA method were determined.

3. Results

The present research examined 84 commercially-available bottled wines collected from five different countries and 14 experimental wines, fined by adding egg white proteins with or without subsequent bentonite fining. Untreated wines were evaluated in parallel as negative controls. Most of the samples were red wine since egg white proteins are generally only used to fine red wines. [2]

3.1. Physicochemical properties

Different physicochemical characteristics were studied in the wine samples in order to assess, in the case that allergenic residues were detected, their possible role in inhibiting or enhancing the elimination of allergens during the fining process. The results are presented in Table 1 (experimental wines) and Table 2 (commercially-available wines).

3.2. SDS-PAGE and silver staining

SDS-PAGE was assayed for its sensitivity using silver staining. [34] For these purposes, decreasing quantities of oenological egg white proteins were loaded onto the gel, and the detection limit was calculated, resulting to be 0.78 μg of oenological egg albumin (data not shown).

Afterwards, the method was applied to wine samples. To evaluate the possible presence of false-positive responses, unfined wines were also analysed by this technique. Some protein bands were present in all untreated wine samples, but they were associated with grapes (data not shown). The presence of these bands in the SDS-PAGE gels makes difficult the evaluation of the possible presence of egg white proteins when present in trace amounts. For this reason, SDS-PAGE has been used for the separation step but not for the quantification of allergenic residues.

3.3. Specificity of anti-egg white protein antibody

Since the quality of antibody is critical in immunochemical determinations, several antibodies specifically developed for this project were characterized by its binding capacity versus the main proteins contained in the fining agent (egg white proteins). Two of these antibodies were developed versus total egg white proteins and two versus the ovomucoid protein, since ELISA plates used for the detection of egg white allergens in food are usually coated with anti-ovomucoid antibodies. Figure 1 illustrates the specifity of the selected anti-total egg white protein antibody. It recognized all albumen proteins (ovotransferrin, ovomucoid, ovalbumin and lysozyme). Anti-ovomucoid antibodies (not shown) bound different egg white proteins but their affinities were lower and for this reason considered unsuitable for the aim of this project. The selected antibody was also used to coat the ELISA plates.

The binding capacity of the selected antibody was also verified with most fining agents, containing egg white proteins, present in the marketplace. The antibody recognized the egg white proteins in all fining agents evaluated and the differences in the bound affinity depended on the percentage of each egg white protein present in each product (not shown).

3.4. Immunobloting

The detection limit for egg white proteins in immunoblotting was determined by blotting decreasing quantities of oenological egg white proteins onto a PVDF membrane and incubating them with the anti-total egg white protein antibody selected. The lowest detectable amount of egg white protein in immunoblotting was 1.5 ng corresponding to 0.122 mg/L in the wine sample.

Once the limit of detection was calculated, the different wine samples were examined by immunoblotting. In order to check the possible presence of false-positive responses, unfined wines were also studied as negative controls. Figure 2 illustrates, as an example, the results of the immunoblotting performed on some wine samples. All experimental and commercial wines analyzed in the present work contained undetectable residues of egg white proteins, as listed in Tables 1 and 2. As expected, no egg white protein was found in the unfined wines.

3.5. ELISA test

The ELISA test used in the present work was specifically developed for this investigation to detect traces of egg white proteins in wine and was validated by a collaborative inter-laboratory study involving 11 laboratories. This method showed a reliable limit of detection of 0.056 mg/L in wine and a limit of quantification of 0.158 mg/L. The quality parameters of the method (reproducibility, repeatability and robustness) were in line with the criteria established by the Organisation Internationale de la Vigne et du Vin (OIV) in the Compendium of International Methods of Analysis.[35] None of the wine samples contained detectable amount of egg white proteins, regardless of the physicochemical properties of the wine, type and concentration of the fining agent used, as well as of the oenological practices employed on experimental and commercial wines, as shown in Tables 1 and 2. As expected, all unfined wines were free of egg white proteins (data not shown).

4. Discussion

Egg allergy is one of the most common food allergies in infancy and childhood, affecting 1–2% of young children.[36] Although its prevalence in adults is considerably lower[13-16, 37], the presence of egg white proteins in fined wines should be avoided to protect the most sensitized subjects. To our knowledge, no case of an allergic reaction after wine consumption due to the presence of residues of egg white protein has been reported and this is despite unlabelled egg white fined wines being in the marketplace in countries such as Canada, EU, USA and those of South East Asia which currently do not require allergen labelling for wine or have only recently inmplemented it This could be due to the actual absence of residues or to the consumers' and/or doctors' lack of awareness about the oenological practice of fining with egg white proteins.

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This study showed that no egg proteins were detected in any of the 77 commercially-available wines analysed (detection limit of 0.0564 mg/L), and that this was independent of the physicochemical characteristics of wines, despite the wide range of values for each parameter included. Specifically, this result was independent from:

- the type and dose of the agent used for fining: 0.075-100 g/hL of fining agent for experimental and 3-10 g/hL for commercialy-available wines; and
- the oenological practices applied: use or not of subsequent bentonite fining in both experimental and commercially-available wines.

Our findings are consistent with those reported by Rolland et al.[18] who did not find any residue of ovalbumin in 40 commercially-available Australian wines fined with egg white proteins. The same research group found no significant clinical response in a group of adult egg allergic subjects tested by a double-blind, placebo-controlled challenge with egg white protein fined wines.[8] It should be emphasized that although other ELISA tests previously reported [9, 11] show good quality characteristics, our method was specifically developed in wine samples in commercial form, in order to standardize its performances and to make it available for wine producers and laboratories involved in quality control.

There are only a few reports illustrating cases of allergy to wine, especially in Mediterranean countries, but these adverse reactions were associated with grape proteins[38-41] or to intolerances to acetaldehyde, biogenic amines such as tyramine or sulphur dioxide.[42-44]

The identification of the threshold safe for the most sensitive individuals is critical. Bindslev-Jensen, Briggs and Osterballe[45] defined a threshold value for egg of 8.6 mg that would protect 99% of egg allergic individuals. Moneret-Vautrin and Kanny[46] reported that 18% of egg allergic individuals can react to a concentration equal to or lower than 65 mg, while the threshold for egg white capable of triggering an allergic reaction in 1% of sensitized people was between 1 and 2 mg. Similarly, Morriset et al.[47] performed a double-blind, placebo-controlled food challenge with egg allergic individuals and reported that the lowest adverse effect level (LOAEL) for crude egg was 2 mg.

Taking into account these values, the limit of detection of the methods used in this study (0.0564 mg/L) and the limit of quantification (0.1578 mg/L) should be more than sufficient to protect egg allergic individuals. In fact, an egg allergic individual drinking 1 litre of wine at the limit of detection level would consume less than 0.06 mg of egg white. This ‘acute’ dose is conservative given it is relatively ‘difficult’ to drink 1 litre of wine at a single drinking occasion, and the risk of an allergic reaction from consuming wine should be considered negligible even in the most sensitized subjects.

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References

(1) Yokosuka, K.; Singleton, V. L. Interactive precipitation between phenolic fractions and peptides in wine-like model solution: turbidity, particle size and residual content as influenced by pH, temperature and peptide concentration. *Am. J. Enol. Vit.* **1995**, *46*, 329–338.

(2) Ribéreau-Gayon, P.; Glories, Y.; Maujean, A.; Dubourdieu, D. The chemistry of wine stabilization and treatments. In *Handbook of enology*; John Wiley & Sons: Chichester, UK, 2000, Vol. 2.

(3) Cosme, F.; Ricardo-da-Silva, J. M.; Laureano, O. Interactions between protein fining agents and proanthocyanidins in white wine. *Food Chem.* **2008**, *106*, 536–544.

(4) D'Amato, A.; Kravchuk, A.V.; Bachi, A.; Righetti, P. G. Noah's Nectar: the proteome content of a glass of red wine. *J. Proteomics*. **2010**, *73*, 2370–2377.

(5) Hoffman, D. R. Immunochemical identification of the allergens in egg white. *J. Allergy Clin. Immunol.* **1983**, *71*, 481–486.

(6) Bernhisel-Broadbent, J.; Dintzis, H. M.; Dintzis, R. Z.; Sampson H. A. Allergenicity and antigenicity of chicken egg ovomucoid (Gal d III) compared with ovoalbumin (Gal d I) in children with egg allergy and in mice. J. *Allergy Clin. Immunol.***1994***, 93*, 1047–1054.

(7) Mine, Y.; Zhang, J. W. Comparative studies on antigenicity and allergenicity of native and denatured egg white proteins. *J. Agric. Food Chem.* **2002**, *50*, 2679–2683.

(8) Rolland, J. M.; Apostolou, E.; Deckert, K.; de Leon, M. P.; Douglass, J. A.; Glaspole I.

N; Bailey, M.; Stockley, C. S.; O'Hehir, R. E. Potential Food Allergens in wine: Double-blind, placebo-controlled trial and basophil activation analysis. *Nutrition*. **2006**, *22*, 882–888.

(9) Rolland, J. M., Apostolou, E., de Leon, M. P., Stockley, C. S., & O'Hehir, R. E. Specific and sensitive enzyme-linked immunosorbent assays for analysis of residual allergenic food proteins in commecial bottled wine fined with egg white, milk, and non grape-derived tannins. *J. Agric. Food Chem.* **2008**, *56*, 349–356.

(10) 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*, 525-534.

(11) 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. *J. Agric. Food Chem.* **2009**, 57, 8399–8405.

(12) Restani, P.; Uberti, F.; Danzi, R.; Ballabio, C.; Pavanello, F.; Tarantino, C. Absence of allergenic residues in experimental and commercial wines fined with caseinates. *Food Chem.* (accepted).

(13) Sampson H. A. (2004). Update on food allergy. *J. Allergy Clin. Immunol.* **2004**, *113*, 805–19.

(14) EFSA. Opinion of the Scientific Panel on Dietetic Products, Nutrition and Allergies on a request from the Commission related to a notification from WFA and the AWRI on albumin (egg white) used in the manufacture of wine pursuant to Article 6, paragraph 11 of Directive 2000/13/EC- for permanent exemption from labelling. *The EFSA J.* **2007,** *566*, 1–7.

(15) Asero, R.; Antonicelli, L.; Arena, A.; Bommarito, L.; Caruso, B.; Crivellaro, M.; De Carli, M.; Della Torre, E.; Della Torre, F.; Heffler, E.; Lodi Rizzini, F.; Longo, R.;

Manzotti, G.; Marcotulli, M.; Melchiorre, A.; Minale, P.; Morandi, P.; Moreni, P.; Moschella, A.; Murzilli, F.; Nebiolo, F.; Poppa, M.; Randazzo, S.; Rossi, G.; Senna, G. E. EpidemAAITO: Feature of food allergy in Italian adults attending allergy clinics: a multi-centre study. *Clin. Exp. Allergy*. **2009**, *39*, 547–555.

(16) Asero, R.; Antonicelli, L.; Arena, A.; Bommarito, L.; Caruso, B.; Colombo, C.; Crivellaro, M.; De Carli, M.; Della Torre, E.; Della Torre, F.; Heffler, E.; Lodi Rizzini, F.; Longo, R.; Manzotti, G.; Marcotulli, M.; Melchiorre, A.; Minale, P.; Morandi, P.; Moreni, P.; Moschella, A.; Murzilli, F.; Nebiolo, F.; Poppa, M.; Randazzo, S.; Rossi, G.; Senna, G. E. Cases of food-induced anaphylaxis in Italian Adults: a multi centre study. *Int. Arch. Allergy Immunol*. **2009**, 150, 271–277.

(17) Clark, A. T.; Skypala, I.; Leech, S. C.; Ewan, P. W.; Dugué, P.; Brathwaite, N.; Huber, P. A.; Nasser, S. M. British Society for Allergy and Clinical Immunology guidelines for the management of egg allergy. *Clin. Exp. Allergy*. **2010**, *40*, 1116–1129.

(18) Commission of the European Communities. Directive 2003/89/EC of 10th of November 2003 amending Directive 2000/13/EC as regards indication of the ingredients present in foodstuffs. *Off. J. Eur. Union.* **2003,** *L 308*, 15–18.

(19) Commission of the European Communities. (2007) Directive 2007/68/EC of 27 November 2007 amending Annex IIIa of the Directive 2000/13/EC of the European Parliament and of the Council as regards certain food ingredients. *Off. J. Eur. Union.* **2007***, L 310*, 15–18.

(20) Commission of the European Communities. Directive 2005/26/EC of 21 March 2005 establishing a list of food ingredients or substances provisionally excluded from Annex IIIa of the Directive 2000/13/EC of the European Parliament and of the Council. *Off. J. Eur. Union.* **2005**, *L 75*, 33–44.

(21) Commission of the European Communities. Commission Regulation (EC) No 607/2009 of 14 July 2009 laying down certain detailed rules for the implementation of Council Regulation (EC) No 479/2008 as regards protected designations of origin and

geographical indications, traditional terms, labelling and presentation of certain wine sector products. *Off. J. Eur. Union*. **2009***, L 193*, 60–80.

(22) OIV MA-E-AS312-01-TALVOL 2009. Compendium of International Methods of Wine and Must Analysis.

(23) Commission of the European Communities. Total alcoholic strength by volume Reg. 491/09/EC All I + All III GU. **2009**, *L 154* 17/06/2009.

(24) OIV-ACITOT. OIV MA-E-AS313-01-ACITOT. Compendium of International Methods of Wine and Must Analysis. **2009**.

(25) OIV-ACIVOL. OIV MA-E-AS313-02-ACIVOL. Compendium of International Methods of Wine and Must Analysis. **2009**.

(26) OIV-pH. OIV MA-E-AS313-15-PH. **2009**.

(27) OIV-CENDRE. OIV MA-E-AS2-04-CENDRE. Compendium of International Methods of Wine and Must Analysis. **2009**.

(28) OIV-EXTSEC. OIV MA-E-AS2-03-EXTSEC. **2009**.

(29) OIV-INFOL. OIV MA-E-AS2-10-INFOL. **2009**.

(30) Di Stefano, R.; Cravero, M. C. I composti fenolici e la natura del colore dei vini rossi. *L'enotecnico*. **1989**, *10*, 81–87.

(31) Ballabio, C.; Bertino, E.; Coscia, A.; Fabris, C.; Fuggetta, D.; Molfino, S.; Testa, T.; Sgarella, M. C.; Sabatino, G.; Restani, P. Immunoglobulin-A profile in breast milk from mothers delivering full-term and pre-term infants. *Int. J. Immunopathol. Pharmacol.* **2007**, *20*, 119–128.

(32) Restani, P.; Uberti, F.; Ballabio, C.; Di Lorenzo, C.; Persico, A.; Tarantino, C. Development of analytical methods to detect egg allergens in wines according to the EU Directive on labelling. *Le Bulletin de l'OIV*. **2010**, *83*, 367–374.

(33) OIV-LIMDET. OIV E-AS1-10-LIMDET. Compendium of International Methods of Wine and Must Analysis. **2009**.

(34) Gromova, I.; Celis, J. E. Protein detection in gels by silver staining: a procedure compatible with mass-spectrometry. In *Cell Biology: A Laboratory Handbook;* Celis J. E., Carter N., Hunter T., Simons K., Small J. V., D. Shotton, Eds.; Academic Press: San Diego, California, 2006; pp. 219–225.

(35) OIV-ETCOL. OIV MA-EAS1-07-ETCOL. Compendium of International Methods of Wine and Must Analysis. **2009**.

(36) Heine, R. G.; Laske, N.; Hill, D. J. The diagnosis and management of egg allergy. *Curr. Allergy Asthma Rep*. **2006,** *6*, 145–152.

(37) Vierk, K. A.; Koehler, K. M.; Fein, S. B.; Street, D. A. Prevalence of self-reported food allergy in American adults and use of food labels. *J. Allergy Clin. Immunol.* **2007**, *119*, 1504−1510.

(38) Pastorello, E. A.; Farioli, L.; Pravettoni, V.; Ortolani, C.; Fortunato, D.; Giuffrida, M. G.; Garoffo, L. P.; Calamari, A. M.; Brenna, O.; Conti, A. Identification of grape and wine allergens as an endochitinase 4, a lipid-transfer protein, and a thaumatin. *J. Allergy Clin. Immunol.* **2003,** *111*, 350−359.

(39) Borghesan, F.; Basso, D.; Chieco Bianchi, F.; Favero, E.; Plebani, M. Allergy to wine. *Allergy*. **2004**, *59*, 1135–1136.

(40) Kalogeromitros, D. C.; Makris, M. P.; Gregoriou, S. G.; Katoulis, A. C.; Straurianeas, N. G. Sensitization to other foods in subjects with reported allergy to grapes. *Allergy Asthma Proc*. **2006**, *27*, 68–71.

(41) Schad, S. G.; Trcka, J.; Vieths, S.; Scheurer, S.; Conti, A.; Brocker, E. B.; Trautmann, A. Wine anaphylaxis in a German patient: IgE-mediated allergy against a lipid transfer protein of grapes. *Int. Arch. Allergy Immunol.* **2005**, *136*, 159− 164.

(42) Dahl, R.; Henriksen J. M.; Harving, H. Red wine asthma: a controlled challenge study. *J. Allergy Clin. Immunol.* **1986**, *78*, 1126–1129.

(43) Littlewood, J. T.; Gibb, C.; Glover, V.; Sandler, M.; Davies, P. T.; Rose F. C. Red wine as a cause of migraine. *The Lancet.* **1988,** *1*, 558–559.

(44) Alibrandi, B.; Parodi, A.; Varadlo, G. Purpura due to ethanol. *N. Engl. J. Med*. **1990**, *322*, 702.

(45) Bindslev-Jensen, C.; Briggs, D.; Osterballe, M. Can we determine a threshold level for allergenic foods by statistical analysis of published data in the literature?. *Allergy*. **2002**, *57*, 741–746.

(46) Moneret-Vautrin, D. A.; Kanny, G. Update on threshold doses of food allergens: implications for patients and the food industry. *Curr. Opin. Allergy Clin. Immunol.* **2004**, *4*, 215–219.

(47) Morisset, M.; Moneret-Vautrin D. A.; Kanny, G.; Guénard, L.; Beaudouin, E.; Flabbée, J.; Hatahet, R. Thresholds of clinical reactivity to milk, egg, peanut and sesame in immunoglobulin E-dependent allergies: evaluation by double-blind or single-blind placebo-controlled oral challenges. *Clin. Exp. Allergy*. **2003**, *33*, 1046–1051

Table 1. Physico-chemical characteristics and allergenic residues of experimental red wines

Sample	Oenological treatment	ASV (mL/100 mL)	TAS (mL/100 mL)	RS (g/L)	Specific gravity (g/L)	pH	TDE (g/L)	RE (g/L)	TA (g/L)	VA-SO_2 (g/L)	Ash (g/L)	TPC (mg/L)	TAC (mg/L)	ELISA	IMM
1A	-	16.99	17.12	2.2	0.99220	3.51	35.8	34.6	5.5	0.55	2.82	2190	182	Neg	Neg
2A	10 g/hL egg white	16.67	16.86	3.1	0.99325	3.53	37.4	35.3	5.6	0.60	2.90	2428	126	Neg	Neg
3A	10 g/hL egg white + 10 g/hL bentonite	17.01	17.17	2.7	0.99274	3.54	36.9	35.2	5.6	0.59	2.93	2236	126	Neg	Neg
4A	10 g/hL egg white + 20 g/hL bentonite	17.18	17.33	2.5	0.99242	3.53	36.6	35.1	5.6	0.61	2.83	2530	119	Neg	Neg
5A	10 g/hL egg white + 30 g/hL bentonite	16.83	17.02	3.1	0.99303	3.53	36.9	34.8	5.6	0.60	2.90	2571	120	Neg	Neg
6A	3 g/hL egg white + 10 g/hL bentonite	17.01	17.15	2.4	0.99276	3.51	37.0	35.6	5.6	0.61	2.90	2643	122	Neg	Neg
7A	3 g/hL egg white + 20 g/hL bentonite	16.99	17.15	2.6	0.99278	3.52	36.8	35.2	5.6	0.60	2.90	2402	125	Neg	Neg
8A	3 g/hL egg white + 30 g/hL bentonite	16.95	17.12	2.9	0.99281	3.53	36.8	34.9	5.5	0.61	2.92	2453	124	Neg	Neg
1V	-	13.03	13.12	1.5	0.99408	3.34	29.0	28.5	6.0	0.38	2.79	1672	270	Neg	Neg
2V	10 g/hL egg white	12.98	13.09	1.9	0.99413	3.32	29.1	28.2	6.4	0.69	2.80	1805	124	Neg	Neg
3V	10 g/hL egg white + 10 g/hL bentonite	13.10	13.21	1.9	0.99385	3.31	28.6	27.7	6.3	0.71	2.71	1824	120	Neg	Neg
4V	10 g/hL egg white + 20 g/hL bentonite	12.49	12.60	1.9	0.99472	3.32	29.0	28.1	6.1	0.55	2.76	1828	132	Neg	Neg
5V	10 g/hL egg white + 30 g/hL bentonite	12.63	12.73	1.6	0.99447	3.34	28.7	28.1	6.0	0.47	2.71	1897	132	Neg	Neg
6V	3 g/hL egg white + 10 g/hL bentonite	13.16	13.25	1.5	0.99375	3.32	28.5	28.0	5.9	0.45	2.76	1738	100	Neg	Neg
7V	3 g/hL egg white + 20 g/hL bentonite	13.15	13.22	1.2	0.99387	3.32	28.8	28.6	6.0	0.45	2.73	1834	124	Neg	Neg
8V	3 g/hL egg white + 30 g/hL bentonite	12.87	12.97	1.6	0.99422	3.33	28.9	28.3	6.0	0.50	2.75	1832	129	Neg	Neg

A: Amarone wine; V: Valpolicella wine; ASV: Alcoholic Strength by Volume; TAS: Total Alcoholic Strength; RS: Reducing Sugars; TDE, Total Dry Extract; RE, Reduced Extract; TA: Total Acidity; VA-SO2: Volatile Acidity SO2; TPC: Total Phenolic Compounds; TAC: Total Anthocyanins; IMM: Immunoblotting; Neg: negative.

Table 2. Physico-chemical characteristics and allergenic residues of commercial wines-part I-VI

Nº	Wine name	Origin	Fining agent	F. agent concentration (g/hL)	BEN	ASV (mL/100 mL)	TAS (mL/100 mL)	RS (g/L)	Specific gravity (g/L)	pH	TDE (g/L)	RE (g/L)	TA (g/L)	VA (g/L)	ASH (g/L)	TPC (mg/L)	TAC (mg/L)	ELISA	IMM
1	Chianti classico Riserva Fontale DOCG	Italy	No albumin	-	NO	14.05	14.11	1.0	0.99257	3.16	29.3	29.3	6.2	0.33	2.12	1731	229	Neg	Neg
2	Chianti classico Riserva - Vigneti La Selvanella DOCG 2006	Italy	No albumin	-	NO	13.39	13.45	1.0	0.99282	3.20	27.2	27.2	5.7	0.25	2.11	1630	306	Neg	Neg
3	Merlot delle Maestrelle (Santa Cristina) IGT2008	Italy	No albumin	-	NO	13.14	13.33	3.2	0.99460	3.43	30.1	27.9	5.4	0.37	2.61	1687	418	Neg	Neg
4	Il Bruciato – Bolgheri DOC 2007	Italy	No albumin	-	NO	14.32	14.40	1.3	0.99284	3.37	29.7	29.4	5.6	0.46	2.51	1944	259	Neg	Neg
5	Badia a Passignano – Chianti Classico DOCG 2007	Italy	No albumin		NO	14.23	14.29	1.0	0.99385	3.43	31.7	31.7	5.8	0.52	3.01	2193	368	Neg	Neg
6	Il Bruciato – Bolgheri DOC 2008	Italy	No albumin	-	NO	14.37	14.43	1.0	0.99311	3.42	30.1	30.1	5.6	0.47	2.78	2059	246	Neg	Neg
7	Badia a Passignano – Chianti Classico DOCG 2006	Italy	No albumin	-	NO	13.57	13.65	1.4	0.99273	3.38	27.1	26.7	5.7	0.49	2.39	2001	155	Neg	Neg
8	Rosso Toscano “i coltri” IGT 2007	Italy	Egg white	4.5	YES	13.31	13.42	1.8	0.99340	3.37	28.4	27.6	5.2	0.38	2.72	1636	284	Neg	Neg
9	Chianti DOCG 2008	Italy	Egg white	4	YES	12.69	12.81	2.0	0.99407	3.39	28.5	27.5	5.3	0.4	2.47	1881	415	Neg	Neg
10	Barbera d’Asti – Vigneti Castello del Poggio DOC 2006	Italy	Egg white	10	NO	13.10	13.54	7.4	0.99686	3.25	34.9	28.5	6.3	0.42	2.55	1712	165	Neg	Neg
11	Lambrusco Grasparossa di Castelvetro – Passione vino secco frizzante DOC	Italy	Ovoclar	10	YES	10.24	11.03	13.1	1.00057	2.11	33.6	21.5	7.1	0.20	3.84	1164	220	Neg	Neg
12	Taurasi DOCG 2000	Italy	Albuclar	10	NO	14.16	14.28	2.0	0.99383	3.32	32.2	31.2	7.1	0.57	2.28	2658	185	Neg	Neg
13	Chianti Classico 2006	Italy	Ovoclar	7	NO	13.39	13.48	1.5	0.99261	3.30	26.9	26.4	6.0	0.42	2.22	2322	121	Neg	Neg
14	Alturio - Refosco dal Ped. Roso DOC 2005	Italy	Blancoll	6	YES	13.60	13.75	2.5	0.99362	3.40	30.1	28.6	6.0	0.39	2.76	2535	455	Neg	Neg
15	Sangiovese maremma toscana IGT 2008	Italy	Ovoclar	8	YES	13.55	13.69	2.4	0.99382	3.43	29.6	28.2	5.8	0.44	2.53	1848	289	Neg	Neg

16	Oltrepò Pavese - Pinot nero Poggio Pelato DOC 2005	Italy	Blancoll	20	YES	13.49	13.57	1.3	0.99160	3.55	25.4	25.1	5.2	0.64	2.68	3013	66	Neg	Neg
17	Oltrepò pavese - Bonarda vivace DOC 2008	Italy	Blancoll	6	YES	11.84	12.55	11.9	0.99783	2.97	34.1	23.2	6.7	0.24	2.13	1947	363	Neg	Neg
18	Montepulciano d'Abruzzo DOC 2008	Italy	Blancoll	10	YES	12.42	12.83	6.8	0.99659	3.43	34.3	28.5	5.9	0.43	2.65	2004	320	Neg	Neg
19	Negroamaro Cabernet Sauvignon IGT 2007	Italy	Albapur	4	YES	13.43	13.97	9.0	0.99921	3.74	43.8	35.8	5.7	0.56	4.03	2764	348	Neg	Neg
20	Salice salentino – Masseria Trajone DOC 2005	Italy	Albapur	4	YES	13.14	13.75	10.2	0.99990	3.65	44.3	35.1	5.9	0.52	3.83	2780	275	Neg	Neg
21	Primitivo di Manduria - Epicuro DOC 2007	Italy	Albapur	4	YES	14.53	15.05	8.7	0.99875	3.85	46.0	38.3	5.6	0.59	4.22	2996	336	Neg	Neg
22	Aglianico IGT 2007	Italy	Albuclar	3	NO	12.69	13.13	7.4	0.99719	3.38	36.1	29.7	5.7	0.58	3.21	3239	356	Neg	Neg
23	Vino Rosso Primitivo IGT 2008	Italy	Albumin dry	10	YES	13.68	14.27	9.8	0.99962	3.61	45.4	36.6	5.5	0.59	3.46	2887	552	Neg	Neg
24	Primitivo di Manduria - Felline DOC 2006	Italy	Albumin dry	20	YES	14.77	15.12	5.8	0.99705	3.47	41.2	36.4	6.6	0.47	3.59	2941	540	Neg	Neg
25	Negroamaro del Salento - Pietraluna Torreguaceto IGT 2009	Italy	Albumin dry	20	YES	13.70	14.06	6.0	0.99792	3.33	40.3	35.3	7.3	0.42	3.28	2883	602	Neg	Neg
26	Vino Rosso del Salento – Alberello IGT 2007	Italy	Albumin dry	20	YES	13.32	13.64	5.4	0.99740	3.40	37.9	33.5	6.3	0.42	3.36	2687	559	Neg	Neg
27	Primitivo di Manduria – Archidamo Peruini DOC 2007	Italy	Albumin dry	20	YES	14.70	15.02	5.4	0.99699	3.42	41.3	36.9	6.8	0.38	3.41	3095	519	Neg	Neg
28	Valpolicella classico DOC 2007	Italy	Ovoclar	6	YES	13.33	13.60	4.5	0.99544	3.34	32.8	29.3	6.0	0.40	2.68	1777	241	Neg	Neg
29	Merlot Colli Berici DOC 2008	Italy	Ovoclar	4	YES	12.50	12.77	4.5	0.99552	3.39	31.1	27.6	5.6	0.33	2.86	1654	363	Neg	Neg
30	Montepulciano d'Abruzzo – Cerulli Spinozzi DOC 2008	Italy	Ovoclar	4	YES	13.23	13.51	4.7	0.99553	3.30	33.4	29.7	6.4	0.45	2.54	2197	461	Neg	Neg

31	Bardolino classico DOC 2009	Italy	Ovoclar	3	YES	12.47	12.74	4.5	0.99572	3.32	30.9	27.4	5.6	0.30	2.64	1536	469	Neg	Neg
32	Nero d'Avola Syrah – Feudo Sartanna IGT 2009	Italy	Ovoclar	3	YES	14.06	14.26	3.4	0.99501	3.42	33.3	30.9	5.7	0.45	2.83	2064	415	Neg	Neg
33	Bardolino classico DOC 2008	Italy	Ovoclar	4	YES	12.32	12.6	4.6	0.99576	3.25	30.9	27.3	5.5	0.30	2.66	1627	435	Neg	Neg
34	Regolo rosso veronese IGT 2006	Italy	Ovoclar	3	YES	13.84	14.26	7.0	0.99669	3.33	37.7	31.7	6.2	0.45	3.01	2255	324	Neg	Neg
35	Bardolino – Murari DOC 2008	Italy	Ovoclar	3	YES	12.2	12.49	4.9	0.99596	3.29	31.2	27.3	5.6	0.32	2.68	1450	400	Neg	Neg
36	Valpolicella classico superiore – Vigneti di Montegradella DOC 2006	Italy	Ovoclar	5	YES	13.59	13.95	6.0	0.99645	3.34	36.1	31.1	6.3	0.44	3.18	2141	280	Neg	Neg
37	Valpolicella superiore Ripasso - Vigneti di Valdimezzo DOC 2007	Italy	Ovoclar	8	YES	13.71	14.13	7.0	0.99701	3.35	37.4	31.4	6.1	0.42	3.23	2212	322	Neg	Neg
38	Amarone della Valpolicella DOC 2005	Italy	Ovoclar	8	YES	15.25	15.73	8.0	0.99657	3.39	41.2	34.2	6.2	0.50	3.29	2426	221	Neg	Neg
39	Aglianico – Vigne Sannite IGT 2008	Italy	Albuclar	5	YES	13.18	13.29	1.8	0.99388	3.38	29.8	29.0	6.1	0.59	2.45	2619	156	Neg	Neg
40	Refosco dal Peduncolo Rosso DOC 2007	Italy	Potassium Caseinate – Egg White - Lysozyme	20+20	NO	14.16	14.31	2.5	0.99526	3.46	35.7	34.2	6.1	0.59	3.62	2399	331	Neg	Neg
41	Merlot - Daunia Teanum Alta IGT 2008	Italy	Egg white	30	YES	14.98	15.06	1.3	0.99615	3.54	38	37.7	5.4	0.40	3.32	3170	386	Neg	Neg
42	Cabernet sauvignon Teanum Alta IGT 2008	Italy	Egg white	50	YES	13.54	13.68	2.3	0.99786	3.41	38.3	37.0	5.4	0.38	3.20	3474	491	Neg	Neg
43	Aglianico IGT 2008	Italy	Egg white	100	YES	14.3	14.4	1.7	0.99602	3.35	36.3	35.6	5.6	0.38	2.89	3337	344	Neg	Neg
44	Lareith Sudtirol Lagrein	Italy	Blancoll	4	YES 15 g/hL	13.27	13.39	2.0	0.99499	3.47	30.6	29.6	5.3	0.51	3.23	2956	644	Neg	Neg
45	Grobnerhof - Santa Maddalena Sudtirol DOC 2008	Italy	Vinpur Blancoll	1 2	YES 15 g/hL	13.31	13.45	2.4	0.99430	3.46	28.4	27.0	4.7	0.43	2.93	1847	368	Neg	Neg

46	Leuchtenburg Sudtirol - Kalterersee Lago di Caldaro DOC 2009	Italy	Vinpur Biancoll	1 3	YES 15 g/hL	13.12	13.26	2.4	0.99328	3.44	26.0	24.6	4.5	0.43	2.60	1911	293	Neg	Neg
47	Primitivo di Manduria DOC 2008	Italy	Albovo Oliver Ogar	6	NO	14.41	14.5	1.5	0.99450	3.29	32.9	32.4	5.9	0.43	2.72	1864	234	Neg	Neg
48	Taurasi - Vigna Quattro confini DOCG 2009	Italy	Egg white	1 g/ 5 hL	YES 3 g/hL	13.69	13.75	1.0	0.99237	3.16	26.7	26.7	6.8	0.42	1.95	1887	86	Neg	Neg
49	Greco di Tufo DOCG 2009	Italy	1%Egg white Oliver Star (caseinate)	1	YES	13.23	13.39	2.7	0.99102	3.20	21.4	19.7	6.1	0.27	1.44	259	-	Neg	Neg
50	Cuxac IGP 2009	France	Ovoclaryl	8	NO	14.03	14.09	1.0	0.99342	3.63	28.9	28.9	4.9	0.51	2.90	2125	564	Neg	Neg
51	Cabernet Franc/Mourvedre IGP 2009	France	Oviclair	10	NO	14.46	14.56	1.7	0.99382	3.58	31.3	30.6	5.1	0.53	2.76	2197	435	Neg	Neg
52	Petit Verdot IGP 2007	France	Oviclair	12	NO	13.88	14.02	2.4	0.99398	3.48	30.4	29.0	5.3	0.51	2.58	1928	269	Neg	Neg
53	Cabezac AOP 2007	France	Albumin poudre (Laffort)	6	NO	13.93	14.04	1.8	0.99343	3.52	28.7	27.9	5.2	0.43	2.34	2149	285	Neg	Neg
54	Carignan – Sicard Ignan AOP 2009	France	Albumin poudre (Laffort)	10	NO	14.16	14.37	3.5	0.99591	3.55	35.1	32.6	5.5	0.45	3.01	2358	593	Neg	Neg
55	La Cuvée Ghislain AOP 2004	France	Albumin poudre (Laffort)	5-6	NO	13.51	13.61	1.7	0.99264	3.48	26.9	26.2	4.8	0.55	2.57	2266	179	Neg	Neg
56	Caraguilhes AOP 2009	France	Albumin poudre (Laffort)	10	NO	13.75	13.87	2.0	0.99379	3.39	29.9	28.9	6.0	0.44	3.03	2999	221	Neg	Neg
57	Caraguilhes AOP 2007	France	Albumin poudre (Laffort)	10	NO	13.12	13.20	1.3	0.99444	3.55	28.8	28.5	5.1	0.46	2.55	1714	277	Neg	Neg
58	Merlot – Cabernet - Domaine du Vieux Parc IGP 2008	France	Oviclair	10	NO	13.91	13.97	1.0	0.99360	3.57	29.1	29.1	5.5	0.53	2.83	2179	386	Neg	Neg
59	Corbiéres AOP 2008	France	Oviclair	10	NO	13.67	13.81	2.4	0.99300	3.47	27.2	25.8	4.7	0.48	2.49	2415	498	Neg	Neg

60	Chateau du Grand Caumont AOP 2008	France	Oviclair	10	NO	12.61	12.73	2.0	0.99334	3.43	25.5	24.5	4.9	0.44	2.29	1783	354	Neg	Neg
61	Château du Grand Caumont AOP 2009	France	Oviclair	10	NO	12.76	12.86	1.6	0.99282	3.32	24.2	23.6	5.3	0.39	2.04	1491	222	Neg	Neg
62	Listrac - Cuvé 98 AOC 2008	France	Egg white (40 days)	66	NO	13.17	13.24	1.2	0.99326	3.42	26.7	26.5	5.3	0.45	2.51	2460	278	Neg	Neg
63	Moulis – Cuvé 56 cru bourgeois AOC 2008	France	Egg white (40 days)	66	NO	12.95	13.01	1.0	0.99334	3.42	26.0	26.0	5.2	0.42	2.36	2283	263	Neg	Neg
64	Malleret- Cru Bourgeois AOC 2009	France	Egg white (3 months)	40	NO	12.73	12.84	1.9	0.99496	3.58	29.7	28.8	5.0	0.51	3.18	2384	289	Neg	Neg
65	Corbière Cru Signé AOC 2009	France	Egg white	US	NO	13.73	13.82	1.5	0.99354	3.45	28.7	28.2	5.1	0.39	2.44	1868	421	Neg	Neg
66	Bois du roi AOC 2007	France	Egg white	US	NO	14.61	14.74	2.2	0.99330	3.72	30.9	29.7	5.4	0.94	2.58	1604	135	Neg	Neg
67	Montplaisir AOC 2007	France	Egg white	US	NO	14.2	14.39	3.1	0.99300	3.65	28.5	26.4	4.8	0.60	2.49	1290	99	Neg	Neg
68	Echantillon 9B	France	Egg white	Maximum dose	NO	10.84	12.39	25.9	1.00504	3.42	49.5	24.6	5.4	0.76	2.72	1580	167	Neg	Neg
69	Echantillon 8	France	Egg white	Maximum dose	NO	11.25	11.41	2.6	0.99507	3.44	25.8	24.2	5.0	0.45	2.74	1476	194	Neg	Neg
70	Grenache 58,13%; Shiraz 38,74% 2009	Australia	Egg White	3	Yes (37.00)	14.29	14.42	2.1	0.99371	3.28	30.7	29.6	5.8	0.43	2.24	1429	214	Neg	Neg
71	Cabernet-Sauvignon 2009	Australia	Egg White	4	Yes (1.20)	13.29	13.37	1.4	0.99525	3,05	31.2	30.8	6,4	0,38	1.91	2171	351	Neg	Neg
72	Merlot 2009	Australia	Egg White	5.9	Yes (0.90)	13.31	13.37	1.0	0.99454	3.08	28.9	28.9	6,2	0,34	1.53	1967	89	Neg	Neg
73	Shiraz 55,87%; Cabernet Sauvignon 29,90% 2008	Australia	Egg White	4	Yes (1.30)	13.39	13.52	2.1	0.99527	3,03	31.5	30.4	6,3	0,41	1.95	1730	264	Neg	Neg
74	Shiraz 2008	Australia	Egg White	4	Yes (0.18)	13.84	13.97	2.2	0.99493	3.26	32.3	31.1	6.0	0.49	2.65	1589	257	Neg	Neg

75	Pinot Noir 2010	Australia	Egg White	3.2	Yes (0.01)	12.95	13.03	1.4	0.99351	3.38	26.1	25.7	5.5	0.63	2.00	1605	181	Neg	Neg
76	Cabernet Sauvignon 2008	Australia	Egg White	5.3	Yes (4.50)	14.23	14.30	1.1	0.99363	3.23	30.5	30.4	6.2	0.44	2.16	2365	351	Neg	Neg
77	Cabernet Sauvignon 49,14%; Merlot 47,78% 2008	Australia	Egg White	4.1	Yes (0.72)	14.41	14.47	1.0	0.99396	3.21	31.4	31.4	6.5	0.43	2.25	2191	312	Neg	Neg
78	Shiraz 2008	Australia	Egg White	1,7	Yes (8.50)	13.66	14.00	5.7	0.99677	3.03	36.7	32.0	6.4	0.41	2.05	1876	364	Neg	Neg
79	Shiraz 2007	Australia	Egg White	3	Yes (2.20)	14.58	14.65	1.1	0.99440	3.23	33.6	33.5	6.4	0.51	3.00	1835	235	Neg	Neg
80	Pinot Noir 2009	Australia	-	-	Yes (0.02)	13.26	13.37	1.8	0.99461	3.16	29.4	28.6	6.4	0.60	1.70	2075	215	Neg	Neg
81	Cabernet Sauvignon 2008	Australia	Egg White	1	Yes (0.51)	14.15	14.21	1.0	0.99406	3.06	31.2	31.2	6.6	0.42	1.97	2408	360	Neg	Neg
82	Pinot Noir 2009	New Zealand	$CuSO_4.5H_2O$ 1.12 mg/L, Egg white 2.5 mg/L, Laffort Gecoll 2.42 mg/L	0.60	NO	14.85	14.98	2.1	0.99212	3.47	28.4	27.3	5.4	0.64	2.14	1402	152	Neg	Neg
83	Pinot Noir 2009	New Zealand	$CuSO_4.5H_2O$ 0.59 mg/L, Laffort Gecoll 0.2 mg/L	0.075	NO	14.60	14.71	1.9	0.99340	3.62	30.7	29.8	5.4	0.67	2.52	1412	152	Neg	Neg
84	Vino Navarra Reserva 2004	Spain	Albumin (Lamothe Abiet)	5	NO	13.64	13.73	1.5	0.99446	3.13	30.3	29.8	6.2	0.41	1.66	2296	129	Neg	Neg
85	Ysios Reserva 2005	Spain	Ovocol L (Martin vialatte)	46.6	NO	13.66	13.80	2.4	0.99389	3.27	29.3	27.9	5.3	0.47	2.21	1962	223	Neg	Neg

BEN: use of bentonite; ASV: Alcoholic Strength by Volume; TAS: Total Alcoholic Strength; RS: Reducing Sugars; TDE, Total Dry Extract; RE, Reduced Extract; TA: Total Acidity; VA: Volatile Acidity; TPC: Total Phenolic Compounds; TAC: Total Anthocyanins; IMM: Immunoblotting; Neg: negative.

Figure 1 - SDS-PAGE (A) and Immunoblotting (B) of two fining agents containing egg white proteins and purified fractions from hen egg white. The antibody used in immunoblotting was the anti total egg white proteins.

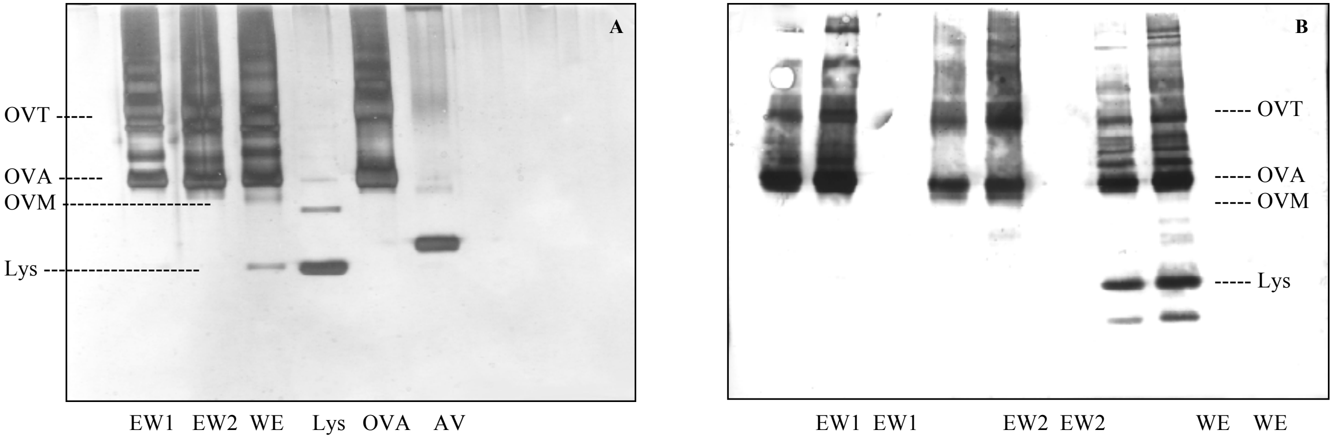


FIGURE 1

Legend

EW1 = commercial oenologic egg white n°1

EW2 = commercial oenologic egg white n°2

WE = whole egg

Lys = lysozyme

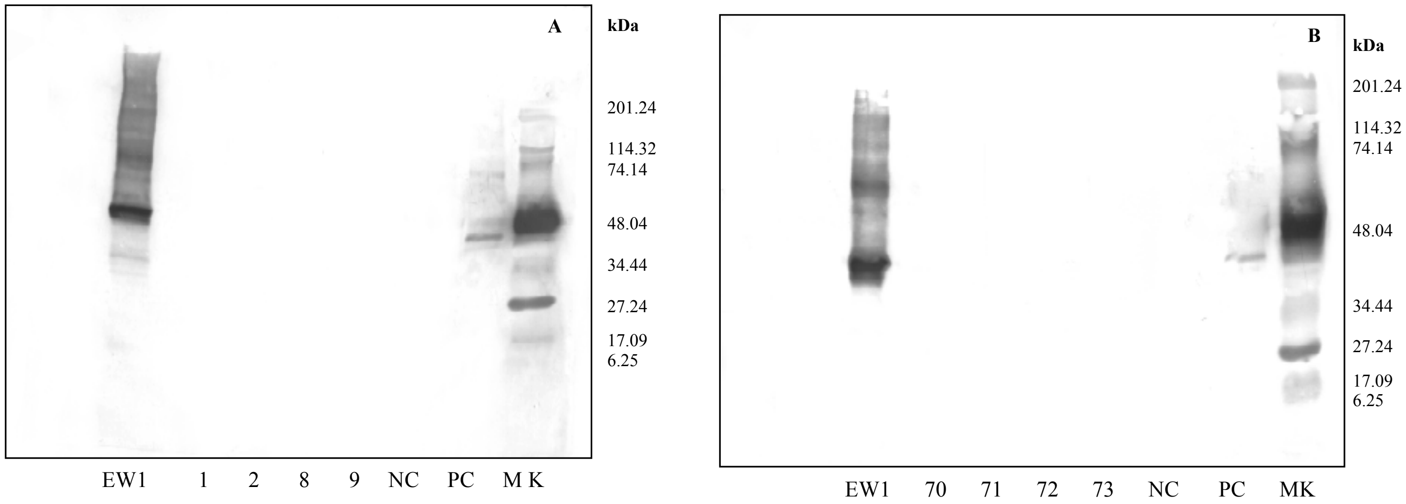
OVA = ovalbumin

AV = avidin

OVT = ovotransferrin

OVM = ovomucoid

Figure 2 - Immunoblotting of different commercial red wine samples obtained by incubating the PVDF membrane with the anti-total egg white protein antibody. (A) Italian red wine samples; (B) Australian red wine samples.



Legend

EW1 = commercial oenologic egg white n°1

1-2 = commercial Italian red wines fined with egg white proteins

8-9 = untreated commercial Italian red wines

70-73= commercial Australian red wines fined with egg white proteins

NC = negative control (unfined red wines)

PC = positive control (wine + 1ppm albumen)

MK = prestained SDS-PAGE standards