Food Chemistry 159 (2014) 343–352

Contents lists available at ScienceDirect

Food Chemistry

journal homepage: www.elsevier.com/locate/foodchem

Analytical Methods

Immunochemical investigation of allergenic residues in experimental and commercially-available wines fined with egg white proteins

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ARTICLE INFO

Article history:
Received 4 March 2013
Received in revised form 13 October 2013
Accepted 7 March 2014
Available online 17 March 2014

Keywords:
Wine
Allergenic residues
Egg white proteins
Fining agent

ABSTRACT

Proteinaceous egg whites are widely used as a fining agent during the production of red wines. Residues of egg white in the final wine could present a risk for individuals allergic to eggs. This study investigated the presence of allergenic residues in both red and white wines fined with egg whites. Experimental and commercially available wines fined with egg whites, with or without subsequent bentonite fining, were studied. Unfined wines were used 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 a specifically developed/validated ELISA test and by immunoblotting. Both immunochemical tests used the same anti-total egg white protein antibody and were highly sensitive to the allergen. No egg white protein was detected in the wines studied in either immunochemical test, irrespective of the physicochemical characteristics of the wine, the type and dosage of the fining agent and the oenological process used. The risk of adverse reactions in egg-allergic individuals should therefore be considered negligible, but the exemption from labelling should be allowed only when the absence of residues is confirmed by analytical controls.

1. Introduction

Fining is one of the least expensive operations in wine production, but it has a major impact on wine quality. The aims of the fining process are to soften or reduce the astringency and/or bitterness of the wine; clarify and remove proteins capable of haze formation; and/or stabilise and reduce the colour by the adsorption and precipitation of polymeric phenolic compounds and tannins (Yokosuka & Singleton, 1995). Several proteinaceous fining agents are currently used, including gelatine, milk proteins, egg proteins, isinglass and, more recently, proteins derived from plants such as wheat and white lupin (Ribéreau-Gayon, Glories Maujean, & Dubourdieu, 2000). Water-soluble egg white (albumin or albumen) is the most commonly used fining agent for red wines. Its positively charged surface binds with negatively charged compounds such as tannins. The insolubility of the resulting aggregates allows them to be eliminated 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, which adsorbs proteins and helps to remove residual proteins from the wine (D'Amato, Kravchuk, Bachi, & Righetti, 2010).

Egg white contains several allergenic proteins such as ovalbumin, ovomucoid, ovotransferrin and lysozyme (Mine & Zhang, 2002).

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 when followed by racking and pre-bottling filtration processes. To date, however, there is limited evidence that commercially available wines are free from residues of proteinaceous fining agents. Studies that have evaluated wines for residual protein have produced conflicting results, perhaps reflecting different analytical methodologies as well as differences in manufacturing practice (Lifrani et al., 2009; Restani et al., 2012; Rolland, Apostolou, de Leon, Stockley, & O'Hehir, 2008; Weber, Steinhart, & Paschke, 2009). Although allergy

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http://dx.doi.org/10.1016/j.foodchem.2014.03.025
0308-8146/

to egg proteins is rare in adults (Asero et al., 2009; Clark et al., 2010; EFSA, 2007; Sampson, 2004), the presence of allergenic proteins in wine could cause an adverse reaction in sensitised individuals. As a consequence, to protect these consumers it is important to demonstrate the absence of egg protein residues in commercial treated wines.

European Union Directive 2003/89/EC (2003), last amended by Directive 2007/68/EC (2007), contains a list of allergenic substances (Annex III), including egg and egg derivatives, which must be declared on the label of foodstuffs. EC Directive 2005/26/EC (2005) 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 studies (Commission of the European Communities, 2007; Commission of the European Communities, 2009a). The inclusion of a statement such as "contains egg proteins" on the wine label can cause uncertainty amongst consumers (allergic or not) and, thereby, damage perception of the product. In order to maintain wine labelling esemption, it is crucial to provide evidences for ensuring that wines are free from egg protein residues.

Taking into consideration the different problems decribed above, the European Commission published the implementing regulation No. 579/2012 (European Commission, 2012) stating that "it is therefore necessary to establish detailed rules for labelling these beverages, including a mention of the substances referred to in Annex IIIa to Directive 2000/13/EC and used when making the beverages, if their presence can be detected in the final product....". This decision was based on scientific data from different studies, including those presented in this paper, where the presence of allergenic residues was evaluated in 14 experimental and 85 international, commercially-available wines (7 untreated and 78 wines fined with egg white). The allergenic residues were measured by a newly developed/validated ELISA test with enhanced limits of detection and quantification, and also by immunoblotting; both tests used antibodies specifically developed against the egg white fining agent.

2. Materials and methods

2.1. Wine samples

Experimental and commercially-available wines fined with egg proteins were included.

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 wines are listed in Table 1. A further group of 12 wines that had not been treated with egg albumin were included as negative controls (data not shown).

Commercially available bottled wines were supplied by several wine producers from five countries. 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 documented were included. Table 2 lists the 85 commercially available wines and shows their physicochemical characteristics. The fining agents used were as follows: 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 physicochemical characteristics that were evaluated in wine samples are listed below:

2.2.1. Alcoholic strength by volume (% vol.)

This is defined as the number of litres of ethanol contained in 100 litres of wine, measured at 20 °C, measured by distiling the wine volume by volume. The distillate was measured by electronic densitometry using a frequency oscillator (OIV., 2009).

2.2.2. Total alcoholic strength by volume

This is the potential alcohol concentration if all remaining sugars were to be fermented, calculated by adding potential alcoholic strength to the alcoholic strength by volume (Commission of the European Communities., 2009b). Potential alcoholic strength is defined as the number of volumes of pure alcohol at 20 °C produced by total fermentation of the sugars contained in 100 volumes of the product at that temperature and was 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 diluted to achieve the same concentration. Red wine was clarified with solutions of neutral lead acetate and calcium carbonate (Merck KgaA, Darmstadt, Germany). An alkaline solution of copper salts was heated and the copper titrated against the clarified/diluted wine in the presence of methylene blue as indicator (UIV internal method).

2.2.4. Total acidity

Wine total acidity was determined by acid–base potentiometric titration, using 0.1 mol/L NaOH, to pH 7 with an automatic titrator (OIV, 2009).

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 (OIV, 2009).

2.2.6. pH

The pH value of wine samples was determined by potentiometry using a calibrated pH-metre (OIV, 2009).

2.2.7. Ash content

Ash amount was measured by ignition of wine extract at 500–550 °C until complete combustion (oxidation) of organic material had been achieved. The residue was weighed using a balance sensitive to 0.1 mg (OIV, 2009).

2.2.8. Total dry extract and reduced extract

The total dry wine extract was calculated indirectly from the specific gravity of the alcohol-free wine after measuring the specific gravity (20 °C) of the wine and the water-alcohol mixture obtained by distillation of wine. The reduced extract was calculated as the difference between the total dry extract and the reducing sugars in excess of 1 g/L (OIV, 2009).

2.2.9. Total phenolic compounds

The total phenolic compounds were analysed using the Folin–Ciocalteau Method, with some modifications (OIV, 2009). Wine samples were diluted and then mixed with the Folin–Ciocalteau