SCIENTIFIC OPINION

Scientific Opinion related to a notification from the International Organisation of Vine and Wine (OIV) on casein/caseinate/milk products to be used in the manufacture of wine as clarification processing aids pursuant to Article 6, paragraph 11 of Directive 2000/13/EC – for permanent exemption from labelling

EFSA Panel on Dietetic Products, Nutrition and Allergies (NDA)

European Food Safety Authority (EFSA), Parma, Italy

ABSTRACT

Following a request from the European Commission, the Panel on Dietetic Products, Nutrition and Allergies (NDA) was asked to deliver a scientific opinion related to a notification from the International Organisation of Vine and Wine on casein/caseinate/milk products to be used in the manufacture of wine as clarification processing aids pursuant to Article 6, paragraph 11 of Directive 2000/13/EC – for permanent exemption from labelling. In earlier assessments, the Panel concluded that wines fined with casein/caseinate/milk products may trigger adverse reactions in susceptible individuals under the conditions of use stated by the applicants based on the limited information provided on the characterisation of the fining agents, on the limitations of the methods used to quantify casein and other milk allergens in wine, and on the clinical studies presented, which were inconclusive. This application refers to new analytical methods developed for the detection of milk allergens in the fining agent and the detection of casein in wine. There were no changes in the wine manufacturing process and no new clinical studies were provided. Taking into account the information provided on the characterisation of the fining agents regarding their content of milk proteins other than casein, the lack of standardisation of the wine manufacturing process, and that no new clinical data have been provided in the present application, the Panel concludes that wines fined with casein/caseinate/milk products may trigger adverse reactions in susceptible individuals under the proposed conditions of use. © European Food Safety Authority, 2011

KEY WORDS

Wine, fining agents, milk products, casein, food allergy.

1 On request from the European Commission, Question No EFSA-Q-2010-01015, adopted on 15 September 2011.
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3 Acknowledgement: The Panel wishes to thank the members of the Working Group on Allergy: Pagona Lagiou, Martinus Løvik, Rosangela Marchelli, Martin Stern, Stephan Strobel, Hendrik van Loveren, Jean Michel Wal for the preparatory work on this scientific opinion.


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SUMMARY

Following a request from the European Commission, the Panel on Dietetic Products, Nutrition and Allergies (NDA) was asked to deliver a scientific opinion related to a notification from the International Organisation of Vine and Wine on casein/caseinate/milk products to be used in the manufacture of wine as clarification processing aids pursuant to Article 6, paragraph 11 of Directive 2000/13/EC – for permanent exemption from labelling.

Taking into account that the prevalence of allergic reactions to milk proteins, and particularly to casein, among the general population of adults has been reported to be around 0.5-1.0 %, it is appropriate for the Panel to assess the likelihood of adverse reactions in allergic individuals consuming products where casein/caseinate/milk products have been used during the manufacturing process.

Three applications submitted for permanent exemption from labelling were the basis for earlier EFSA assessments of casein/caseinate/milk products as fining agents in the production of wine. The Panel concluded that wines fined with casein/caseinate/milk products may trigger adverse reactions in susceptible individuals under the conditions of use stated by the applicants. The conclusion was based on the limited information provided on the characterisation of the fining agents, on the limitations of the methods used to quantify casein and other milk allergens in wine, and on the limited information provided in relation to the clinical studies presented, which were inconclusive.

This application contains new information and data mainly with regard to analytical methods developed for the detection of milk allergens in the fining agent and the detection of casein in wine. There were no changes in the wine manufacturing process and no new clinical studies were provided.

Commercial preparations of milk-based fining agents sold as “casein/caseinate” may contain proteinaceous materials in addition to casein. Milk allergens other than casein were found in every milk-based fining product examined. The Panel notes that milk-based fining agents have not been sufficiently characterised in the application regarding their content of milk proteins other than casein.

Cow’s milk-based fining preparations are used in particular for the treatment of astringency and for the clarification of white and rosé wines, but are also sometimes used with red wines. The Panel notes that the recommended amounts of casein/potassium caseinate/milk products to be added to wine as fining agents, as well as the manufacturing process and steps recommended to decrease residual allergens in wine, are very variable, and that according to the applicant no changes in the manufacturing process have been introduced since the last application.

Two reports on the detection of casein in wine were provided. The Panel notes that, in the new analytical studies provided by the applicant, casein was detected in trace amounts (<2 mg/L) in two (out of 32) experimental wines without bentonite treatment and in three (out of 61) commercial wines with unknown treatment, and that the results from an Italian study which showed no detectable amounts of casein in any of the 63 commercial wines tested cannot be extrapolated to a random sample of commercial wines due to the consistent use of non-mandatory manufacturing practices (microfiltration, bentonite treatment) which may have affected proteinaceous fining agent residues in the wine. The Panel also notes that these studies do not adequately address the presence in wine of other milk allergens contained in caseinates for which the ability (and limit of detection) of the analytical methods used have not been reported, and that no new analytical data on wines fined with milk products other than “casein/caseinate” have been provided. The Panel considers that although the sensitivity and performance of the methods used for the detection of casein in wine have improved relative to previous applications, the studies provided by the applicant do not provide information about the levels of residual casein or other milk allergens which may be found in commercial wines fined with casein/caseinate/milk products under the proposed conditions of use.
Taking into account the information provided on the characterisation of the fining agents regarding their content of milk proteins other than casein, the lack of standardisation of the wine manufacturing process, and that no new clinical data has been provided in the present application, the Panel considers that the improvement on the methods used for the detection of casein in wine and the new analytical studies provided are insufficient to change its previous conclusion about the likelihood of adverse reactions triggered in susceptible individuals by the consumption of wines fined with casein/caseinate/milk products.

The Panel concludes that wines fined with casein/caseinate/milk products may trigger adverse reactions in susceptible individuals under the conditions of use proposed by the applicant.
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BACKGROUND AS PROVIDED BY THE EUROPEAN COMMISSION

Article 6, paragraph 11 of Directive 2000/13/EC\(^4\) establishes the cases and conditions for amending Annex IIIa to that Directive, which includes a list of food ingredients or substances known as likely to trigger allergic reactions in sensitive individuals. It also sets up a procedure for exempting from labelling, under certain conditions, derivatives of these ingredients.

Pursuant to the procedure referred to above, a list of ingredients or substances derived from ingredients listed in Annex IIIa has been adopted by the Commission and is included in the Annex to Commission Directive 2007/68/EC\(^5\) of 27 November 2007, amending Annex IIIa to Directive 2000/13/EC of the European Parliament and of the Council as regards certain food ingredients. Applicants who are seeking the exclusion of a given product from Annex IIIa have to submit a request, completed with the results of relevant scientific studies.

Therefore, in the context of the permanent labelling exemption procedure, the European Food Safety Authority is asked to provide scientific opinions on submissions in accordance with the present terms of reference.

TERMS OF REFERENCE AS PROVIDED BY THE EUROPEAN COMMISSION

In accordance with Article 29(1)(a) of Regulation (EC) N° 178/2002, the European Commission requests the European Food Safety Authority to evaluate the scientific data submitted by the International Organisation of Vine and Wine (OIV) in the framework of the procedure laid down in Article 6, paragraph 11 of Directive 2000/13/EC. On the basis of that evaluation, EFSA is requested to issue an opinion on the information provided, and in particular to consider the likelihood of adverse reactions triggered in susceptible individuals by the consumption of the following ingredients/substances used under the conditions specified by the applicant: casein/caseinate/milk products to be used in the manufacture of wine as clarification processing aids.

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ASSESSMENT

Taking into account that the prevalence of allergic reactions to milk proteins and particularly to casein among the general population of adults has been reported to be around 0.5-1.0 % (EFSA, 2004), it is appropriate for the Panel to assess the likelihood of adverse reactions in allergic individuals consuming products where casein/caseinate/milk products have been used during the manufacturing process.

Applications submitted by the Winemakers’ Federation of Australia (WFA) and the Australian Wine Research Institute (AWRI), and by the Deutscher Weinbauerverband (DWV) and the Office National Interprofessionnel des Fruits, des Légumes, des Vins et de l’Horticulture (VINIFLHOR), to the European Commission pursuant to Article 6, Paragraph 11 of Directive 2000/13/EC as amended by Directive 2003/89/EC for permanent exemption from labelling were the basis for earlier assessments of casein/caseinate/milk products as fining agents in the production of wine by the Panel on Dietetic Products, Nutrition and Allergies (NDA) (EFSA, 2007a, 2007b, 2007c). The Panel concluded that wines fined with casein/caseinate/milk products may trigger adverse reactions in susceptible individuals under the conditions of use stated by the applicants. The conclusion was based on the limited information provided on the characterisation of the fining agents, on the limitations of the methods used to quantify casein and other milk allergens in wine, and on the limited information provided in relation to the clinical studies presented, which were inconclusive.

The present opinion is based on a dossier from the International Organisation of Vine and Wine (OIV), with an application for permanent exemption. This application contains new information and data mainly with regard to analytical methods developed for the detection of milk allergens in the fining agent and the detection of casein in wine. There were no changes in the wine manufacturing process and no new clinical studies were provided.

1. Characterisation of the fining agent

In previous submissions (EFSA, 2007a, 2007b, 2007c), milk-based fining agents sold as “casein/caseinate” and used as fining agents were characterised by the applicant using SDS-PAGE electrophoresis and, in parallel with Coomassie blue staining, with western blotting using the sera of milk allergic patients and of non-allergic controls. Different protein bands were detected. Sera from allergic patients were incompletely characterised. Tests were also performed using sera of rabbits or mice immunized with casein. Purified rabbit IgG recognized a total of 16 bands, most but not all corresponding to Coomassie blue staining. Human IgE recognized six bands at 22, 54, 67, 74, 130 and 155 kDa. This implied that bands of high molecular weight unrelated to pure caseins (20-30 kDa) may have been related to aggregates or other immunoreactive proteins in the fining material. Thus, “casein/caseinate” which were used as fining agents in wine were a mixture of several proteins and may have contained residual amounts of other proteins (e.g. whey proteins), including allergens as impurities.

In the present application, product sheets of milk-based fining agents sold as “casein/caseinate” as well as SDS-PAGE and immunoblotting studies have been submitted. Product sheets generally provide no information with regard to protein purity. Commercial preparations of the fining agents sold as “casein/caseinate” may contain added organic (e.g. gelatine, and egg white proteins) and non-organic (e.g. E558 bentonite or similar clay, E1202 polyvinylpyrrolidone (PVPP)) materials other than pure casein. The applicant claims that the commercial preparations of the fining agent used in the manufacturing of wine comply with the OIV and International Oenology Codex standards.

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Twelve different milk-based fining preparations were investigated by SDS-PAGE and immunoblotting as described by Weber et al. (2009). Milk allergens other than casein were found in every fining product. The applicant reported that in one milk-derived fining agent no casein was detected, but rather β-lactoglobulin. Weber et al. (2009) pointed out the importance of using antibodies capable of detecting all milk allergens (such as α-lactalbumin, β-lactoglobulin and bovine serum albumin), as well as their fragments, in the assessment of residual milk allergens in fined wine. The Panel agrees with this statement and notes that the application does not provide quantification of milk proteins other than casein finally present in the commercial fining preparations sold as “casein/caseinate”, and therefore possibly also in the wine.

The Panel notes that milk-based fining agents have not been sufficiently characterised in the application regarding their content of milk proteins other than casein.

2. Conditions of use

Cow’s milk-based fining preparations are used in particular for the treatment of astringency and for the clarification of white and rosé wines, but are also sometimes used with red wines.

In previous submissions (EFSA, 2007a, 2007b, 2007c), applicants described the winemaking process for different wines (red, white and rosé wines) that were produced in different regions. The winemaking process could vary according to the region, vintage and colour of the wine, as well as the skill of the winemaker. Fining is decided upon after evaluation of each particular batch of wine, as stated also in the present application. However, wine manufacturing was claimed to follow the professional guidance documents, namely Référentiel des pratiques oenologiques intégrées (ITV, 2001) and Guide des bonnes pratiques hygiéniques filière vins (ONIVINS, 2000). The applicant states that the conditions of processing were similar in France and in Germany. The amount of casein added to wine usually ranges from 100 to 1000 mg/L, the amount of potassium caseinate from 200 to 1200 mg/L, and the amount of skimmed milk powder from 20 to 200 mg/L (EFSA, 2007c).

In the present application, the criteria and guidance documents set by the OIV regarding materials and procedures to be used in wine production were provided. The applicant states that the amount of casein added to wine usually ranges from 100 to 500 mg/L for white wines and from 50 to 250 mg/L for red wines. Potassium caseinate (100 to 500 mg/L), skimmed milk (approximately 15-30 mL/L, corresponding to 450-900 mg/L casein and 225-450 mg/L albumins), or powdered skimmed milk (approximately 530 mg/L, corresponding to about 140 mg/L casein) may alternatively be used. A typical dose to reduce bitterness and browning may be higher, between 500 and 1000 mg/L casein or potassium caseinate. Specifications for casein were set in 2003 (OIV, 2003). Reference is made to the guidance document regarding casein issued by the applicant in 2009 (OIV, 2009), and to a book from 2000 (Ribéreau-Gayon et al., 2000).

The principle of wine fining is to mechanically remove insoluble and colloidal substances from wine. This aims to clarify and stabilize the wine, and to preserve and improve its flavour and taste. Casein adsorbs phenolic compounds, particularly tannins, which may affect wine colour and taste. Casein coagulates and precipitates in the acidic environment of must and wine, and is usually removed by sedimentation, filtration and/or centrifugation. In previous submissions (EFSA, 2007a, 2007b, 2007c), the applicants stated that filtration may not be applied to certain wines, including some European wines. According to the literature provided, in some cases fining may be performed after filtration. A wide variety of the filtration materials and methods that may be used are described in the application. The applicant declared that no changes have been introduced in the manufacturing process with respect to previous applications.

The Panel notes that the recommended amounts of casein/potassium caseinate/milk products to be added to wine as fining agents, as well as the manufacturing process and steps recommended to
decrease residual allergens in wine, are very variable, and that according to the applicant no changes in the manufacturing process have been introduced since the last application.

3. Analysis of residual allergens in wine

In a previous submission (EFSA, 2007c), no residual casein was detected with a competitive ELISA in five German wines fined with casein at concentrations of 60-300 or 100-500 mg/L depending on the wine. A wine experimentally fined with casein (500 or 1000 mg/L) in France was found positive (above 4 µg/L) with a sandwich ELISA. Four hundred commercial French wines were also analysed for the presence of casein using this test. Thirteen were found positive for casein, including three organic wines, most of which were red wines (10 out of 13) (Lifrani et al., 2009).

Two reports were provided in the present application, one from Germany (Paschke et al., 2010, unpublished) and one from Italy (Restani, 2010, unpublished).

In the German report, one white wine was treated with different fining aids including casein (Paschke et al., 2010, unpublished). Casein was used at the maximum recommended dose (400 mg/L, OIV, 2009) and at double the maximum recommended dose (800 mg/L) as a worst case scenario. The wine thereafter passed through pasteurization and filtering processes. Three different pasteurization and filtering processes were assessed: flash-pasteurization (72°C for 2 seconds), fine and sterile filtration using pad filters (“Seitz” K-100 and EK, respectively), and filtration with diatomaceous earth (Kieselgur). Casein residues in the wine were investigated with a newly developed indirect ELISA (Weber et al., 2009). The limit of detection (LOD) claimed in the application for this ELISA was 0.07 mg/L; the limit of quantification (LOQ) was 0.09 mg/L. No residues of casein were detected in the wine, neither in the wine treated with the lower concentration of the fining agent nor in the wine treated with the higher. In the wine untreated post-finishing (400 mg/L) and the wine treated with flash pasteurisation, 0.07 mg/L and 10.43 mg/L of casein were found, respectively. The investigators concluded that when the wine was filtered after fining under the conditions of use described in this study, residual levels of casein in wine were <0.07 mg/L both with the lower and the higher dose of fining agent. The Panel notes that only one experimental wine was tested.

Further, in the German report, 16 Australian commercially available white wines not allergen labelled (n=5) or labelled with “may contain milk” (n=11, of which three were also labelled for egg) (Paschke et al., 2010, unpublished) were tested for casein residues with the same ELISA as above (Weber et al., 2009). No casein residues were detected in any of these wines. The Panel notes that no information was provided on how these wines were selected for analysis. The Panel also notes that no information was provided about the manufacturing process (including doses of fining agent used) of these wines. The Panel therefore considers that this study provides information of only very limited value for use in the context of the present application.

Finally, Weber et al. (2009) reported results from the testing of 32 experimental and 61 commercial white wines with the new ELISA. Two of 32 experimental wines fined with 300 mg/L caseinate and not treated with bentonite were found to contain detectable traces of casein (>0.2 mg/L but <2 mg/L), whereas no casein was detected when the wines had been additionally treated with bentonite or had been fined with a lower dose (i.e. 60 mg/L) of casein, with or without bentonite treatment. Further, among 61 commercial wines, three wines (one French, one German and one Italian) revealed casein traces (estimated up to 0.4 mg/L). The authors concluded that allergic reactions due to the consumption of casein-treated wines cannot be excluded. The Panel notes that the lowest dose of casein capable of triggering an allergic reaction in a sensitive individual is highly uncertain, but that reactions have been described to a dose of 300 mg of low-fat milk powder, which corresponds to approximately 105 mg of milk protein or 90 mg of casein (Lam et al., 2008), and that some case reports suggest significantly lower doses as capable of triggering reactions in highly sensitive individuals.
The report of an Italian study (Restani, 2010, unpublished) was also provided. Two experimental white wines and 63 commercial wines fined with “casein”, with or without bentonite treatment, (creating 16 different samples) were studied. Of these, 59 were white wines, two were rosé and two were red wines. Experimental wines were fined with “casein/caseinate” at doses of 200 and 500 mg/L, whereas commercial wines were treated with “casein/caseinate” at doses from 20 mg/L to 300 mg/L except for three samples (two used 350 and one 400 mg/L). All samples were microfiltered, and five of the six wines not treated with bentonite used tangential plus three-step microfiltration. The Panel notes that microfiltration and bentonite treatment are not mandatory steps in wine processing as recommended by the applicant (OIV, 2009), and that these may significantly affect the amount of residual fining agents in wine (Weber et al., 2009). Commercial wines were fined with 13 different commercial fining agents, most of which contained potassium caseinate (ranging from 65 to 100 % of the product by weight), two also contained bentonite, and one also contained montmorillonites and α-cellulose.

The most important physico-chemical characteristics of the wine samples were determined in order to assess their role in hindering or promoting the elimination of allergenic residues. Three different methods were used to detect caseinates in wine samples: ELISA test, SDS-PAGE with silver staining (Gromova and Celis, 2006) and immunoblotting with specific anti-caseinate antibodies. The ELISA test was specifically developed for the wine fining project, and was validated in a ring test of 12 samples (three wines, spiked or not spiked) between 11 laboratories (Università degli Studi di Milano et al., 2011, unpublished). LODs for ELISA, SDS-PAGE with silver staining, and immunoblotting were found to be 0.28, 0.43 and 0.98 mg/L, respectively and LOQs were 0.76, 0.43 and 0.98 mg/L, respectively. All wine samples were found to be free of detectable allergenic residues. The Panel notes that although the sensitivity and performance of the methods used for the detection of casein in wine have improved relative to previous applications, the ability of the antibodies used to detect milk allergens other than casein fractions was not reported.

In summary, the Panel notes that in the new analytical studies provided by the applicant, casein was detected in trace amounts (<2 mg/L) in two (out of 32) experimental wines without bentonite treatment and in three (out of 61) commercial wines with unknown treatment (Weber et al., 2009), and that the results from an Italian study which showed no detectable amounts of casein in any of the 63 commercial wines tested cannot be extrapolated to a random sample of commercial wines due to the consistent use of non-mandatory manufacturing practices (microfiltration, bentonite treatment) which may have affected proteinaceous fining agent residues in the wine. The Panel also notes that these studies do not adequately address the presence in wine of other milk allergens contained in caseinates, for which the ability (and limit of detection) of the analytical methods used have not been reported, and that no new analytical data on wines fined with milk products other than “casein/caseinate” have been provided.

The Panel considers that although the sensitivity and performance of the methods used for the detection of casein fractions in wine have improved relative to previous applications, the studies provided by the applicant do not provide information about the levels of residual casein or other milk allergens which may be found in commercial wines fined with casein/caseinate/milk products under the proposed conditions of use.

4. Estimated level of exposure

The applicant indicates that 26 % of French wines are fined with casein and 15 % with casein mixed with bentonite or PVPP. In Germany, 20 % of wines are fined with casein. Another source cited indicates that, globally, 30 % of wines are treated with casein.

In a previous application, the applicant estimated the wine consumption in France to be around 60 L per person per year. In Germany the average consumption of wine was about 23 L per person in 2005. These and other published data on higher wine intakes (Weber et al., 2009) do not reflect variability
between individuals and geographical areas, nor variations for a given individual over time. The Panel notes that intake on a single occasion is more relevant regarding food allergic reactions than average daily or yearly intake. Further, the Panel notes that no generally applicable threshold levels of intake have been defined for food allergens (EFSA, 2004).

No estimate of exposure to casein residues or other milk allergens through the consumption of fined wines was provided by the applicant.

5. **Evidence of non-allergenicity**

5.1. **History of non-allergenicity of the product**

The applicant provided a general review on food allergens and particularly on milk allergens. Regarding milk products used as processing aids in this application and regarding the specific finished products that are concerned, reference to historical reviews was provided. The applicant stated that in the medical literature there is no indication of an allergenic potential of wine that has been fined with milk products, but no justification for this statement was given. The strategy and the sources used for the literature search were not described. The Panel notes that reactions to wine, including allergic reactions, are well documented (Armentia, 2008; Vally and Thompson, 2003). Since consumers and health professionals may be unaware that milk and milk-derived products are used in the winemaking process, allergic reactions to casein and other milk proteins have generally not been considered following allergic reactions to wine and therefore under-reporting of reactions caused by milk after ingestion of wines may have occurred.

5.2. **Animal studies**

Animal studies which were provided in previous applications were considered inconclusive by the Panel (EFSA, 2007c). No new animal studies were provided.

5.3. **Clinical studies**

Clinical studies which were provided in previous applications were considered inconclusive by the Panel with respect to the likelihood of adverse reactions triggered in susceptible individuals by the consumption of wines fined with casein/caseinate/milk products (EFSA, 2007c). No new clinical studies were provided with the present application.

**CONCLUSIONS**

Taking into account the information provided on the characterisation of the fining agents regarding their content of milk proteins other than casein, the lack of standardisation of the wine manufacturing process, and that no new clinical data have been provided in the present application, the Panel considers that the improvement on the methods used for the detection of casein in wine and the new analytical studies provided are insufficient to change its previous conclusion about the likelihood of adverse reactions triggered in susceptible individuals by the consumption of wines fined with casein/caseinate/milk products.

The Panel concludes that wines fined with casein/caseinate/milk products may trigger adverse reactions in susceptible individuals under the conditions of use proposed by the applicant.
DOCUMENTATION PROVIDED TO EFSA


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**GLOSSARY / ABBREVIATIONS**

<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Description</th>
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<tbody>
<tr>
<td>AWRI</td>
<td>Australian Wine Research Institute</td>
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<tr>
<td>DWV</td>
<td>Deutscher Weinbauverband</td>
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<tr>
<td>ELISA</td>
<td>Enzyme-Linked Immunosorbent Assay</td>
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<td>LOD</td>
<td>Limit of detection</td>
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<td>LOQ</td>
<td>Limit of quantification</td>
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<td>OIV</td>
<td>International Organisation of Vine and Wine</td>
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<tr>
<td>PVPP</td>
<td>Polyvinylpyrrolidone</td>
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<tr>
<td>SDS-PAGE</td>
<td>Sodium Dodecyl Sulfate Polyacrylamide Gel Electrophoresis</td>
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<tr>
<td>WFA</td>
<td>Winemakers’ Federation of Australia</td>
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