# Grape seed extract: the first protein-based fining agent endogenous to grapes

D. GAZZOLA[1,2], S. VINCENZI[1,2], M. MARANGON[3], G. PASINI[1] and A. CURIONI[1,2]

[1] Department of Agronomy, Food, Natural Resources, Animals and Environment (DAFNAE), University of Padova, 16 – 35020 Legnaro, Padova, Italy; [2] Centro Interdipartimentale per la Ricerca in Viticoltura ed Enologia (CIRVE), University of Padova, 14 – 31015 Conegliano, Treviso, Italy; [3] Plumpton College, Lewes, East Sussex BN7 3AE, England
Corresponding author: Dr Simone Vincenzi, email simone.vincenzi@unipd.it

## Abstract

**Background and Aims:** There is a growing interest in finding alternative wine fining agents to replace potentially allergenic animal-derived and plant-derived proteins. In this context, the potential use of grape-derived fining agents would be beneficial as they would not introduce additional, potentially allergenic products to the finished wine. In this project, grape seed flour, a by-product of the grape oil seed industry, has been used to prepare a grape seed extract (GSE) for testing as a novel wine fining agent.

**Methods and Results:** The fining performance of GSE was compared with that of patatin, pea proteins, polyvinylpolypyrrolidone and potassium caseinate in a white and in a rosé wine, and of ovalbumin and gelatin in a red wine. Reduction of turbidity, effect on wine colour, the concentration of phenolic substances, browning potential and wine sensory attributes were determined. Grape seed extract was effective in decreasing white wine turbidity when compared to potassium caseinate. In red wine, GSE removed some anthocyanin and proanthocyanins, while wine colour was only slightly affected. The greatest GSE effect was observed on the sensory properties of the treated wines, as it strongly reduced the vegetal notes in the rosé wine and improved the overall taste of the red wine as a result of the reduction in both acidity and astringency.

**Conclusions:** Grape seed extract can be considered a valid allergen-free alternative to the most common wine fining agents.

**Significance of the Study:** Grape seed extract is the first effective fining agent endogenous to grapes, thus not attracting the legal restrictions concerning the presence of foreign substances.

***Keywords**: astringency, fining, grape seed, sensory analysis, wine*

## Introduction

Fining plays a key role in the winemaking process, being applied to grape juice or wine before, during and/or after the alcoholic fermentation. Fining has several aims: (i) to remove unwanted insoluble particles, improving clarity and filterability (Marchal et al. 2002a,b); (ii) to modulate the phenolic composition of wines, obtaining colour stability by reducing phenol oxidation (Cosme et al. 2012, Gonzàlez-Neves et al. 2014, Granato et al. 2014); and (iii) to improve wine sensory attributes by reducing astringency and off-flavours (Mira et al. 2006, Gambuti et al. 2012). A common fining approach uses protein-based processing products, whose addition mainly results in the removal of protein-reactive phenolic substances. The most popular animal-derived fining agents include porcine gelatin (since bovine gelatin was abandoned as a precaution following the spread of Bovine Spongiform Encephalopathy), egg albumin (derived from egg whites) and casein (milk protein) (Tschiersch et al. 2010). Egg and milk proteins are well-known food allergens, therefore posing a risk to allergic consumers if residues remain in the wine after the fining treatment (Tolin et al. 2012, Deckwart et al. 2014). To manage this risk, EU regulation No. 579/2012 of 29 June 2012 (European Commission 2012) was introduced and made it compulsory that potentially allergenic fining agents have to be declared on the wine label if they are found in wine at a concentration greater than 0.25 mg/L.

As an alternative to those from animal sources, plant proteins derived from wheat and peas have been authorised for use in winemaking by the Organisation Internationale de la Vigne et du Vin (OIV) OENO 28/2004 resolution (Organisation Internationale de la Vigne et du Vin 2004). No commercial products containing wheat proteins, however, are currently on the market because of the risk of adverse reactions to gluten (European Commission 2007), while several products containing pea proteins are commercially available. More recently, potato protein (patatin) was proposed as a grape juice/wine fining agent because of its low allergen risk (Gambuti et al. 2012, 2016) and its use has been approved by amending the OIV OENO 28/2004 resolution in June 2013 (Organisation Internationale de la Vigne et du Vin 2013). Although rare, however, allergy to potato has been reported (Lee et al. 2006).

The list of allergenic materials to be declared on the label present in Annex III of Directive 2007/68/EC (European Commission 2007) is far from definitive and will be regularly reviewed and updated according to new scientific evidence, so it is possible that materials currently allowed as deemed not causing allergic reactions might be included in the list.

A solution to this problem would be to use fining agents sourced from grape, wine or yeast materials (Organisation Internationale de la Vigne et du Vin 2011). No label declaration would be required following this approach, since no foreign substances would be introduced to the wine. Accordingly, several

doi: 10.1111/ajgw.12268
© 2017 Australian Society of Viticulture and Oenology Inc.

authors have proposed yeast derivatives as wine fining aids, an approach that yielded promising results as these products were able to decrease turbidity, reduce astringency and improve stability of the treated wines (Charpentier et al. 2006, Iturmendi et al. 2010, Fernandes et al. 2015, Lochbuhler et al. 2015,).

More recently, a material endogenous to grape extracted from grape seeds (GSE) showed promising results in reducing the astringency of red wines (Vincenzi et al. 2013). In this work, Vincenzi and colleagues successfully extracted GSE from grape seed flour, a by-product of the grape seed oil industry. Because the extraction of grape seed components naturally occurs during winemaking (Yokotsuka and Singleton 1996), the application of GSE as a fining agent would certainly not require its declaration in the label.

The aim of this work was to evaluate the potential for GSE as an alternative to other wine fining aids commonly used in winemaking. For this purpose, the effect of GSE on turbidity, chromatic characteristics, oxidative stability, reduction of the concentration of phenolic substances and sensory properties were tested on white, rosé and red wines and compared with fining agents of synthetic [polyvinylpolypyrrolidone (PVPP)], animal (caseinate, ovalbumin and gelatin) and plant (patatin and pea proteins) origin.

## Materials and methods

### *Wines*

Three unfined wines (vintage 2013) from the Veneto region of Italy were used for the fining experiments: a white wine (cv. Chardonnay), a rosé wine (cv. Raboso Piave) and a red wine (cv. Raboso Piave). The analytical characteristics of these wines are reported in Table 1.

**Table 1.** Analytical characteristics of the wines used in the experiments.

| Wine attribute | Chardonnay | Rabosos rosé | Raboso red |
|---|---|---|---|
| Alcohol concentration (%) | 12.2 | 11.4 | 12.0 |
| pH | 3.30 | 3.30 | 3.50 |
| Total acidity (g/L tartaric acid) | 6.2 | 9.7 | 7.6 |
| Free $SO_2$(mg/L) | 11.0 | 5.0 | 16.0 |
| Total $SO_2$(mg/L) | 74.0 | 55.0 | 25.7 |
| NTU (before fining) | 782 ± 31 | 1484 ± 8 | 24 ± 2 |

NTU, nephelometric turbidity unit.

### *Fining experiments*

Wines were filled into 500 mL bottles, where each fining agent was added at room temperature. The fining performance of the GSE was compared with that of patatin (Vegecoll, LaffortOenologie, Bordeaux, France), pea proteins (Fitoproteina P, Enologica Vason, Verona, Italy) PVPP (ArtEnology, Treviso, Italy), potassium (K) caseinate (Caseospeed, EVER, Venezia, Italy), ovalbumin (Albumina d ˈuovo spray, EVER) and gelatin (Oro Polvere, EVER). Fining aids were prepared by diluting them with distilled water as follows: GSE 1:2 (v/v) starting from a stock at 150 g/L (75 g/L dry matter; 33 g/L of protein); patatin, pea proteins, K-caseinate and gelatin 1:20 (w/v); PVPP and albumin 1:10 (w/v). Each fining aid was then added to the wines at three rates reported as low (L), medium (M) and high (H), according to the rates recommended by the manufacturer of each product (Table 2). For each wine, a sample containing no fining agent was used as the Control. All fining experiments were carried out in duplicate, at 12°C and extended for a specific time for each wine.

At the end of the fining treatment, wine samples were racked off their fining lees and filtered under vacuum on GF/A 1.5 μm filters (VWR International SAS, Leuven, Belgium). An aliquot (50 mL) of wine was further filtered through a 0.45 μm cellulose acetate filter (Sartorius, Goettingen, Germany) for chemical analysis, while the remaining wine was bottled in 500 mL glass bottles sealed with crown caps and bidules and stored at 4°C until sensory analysis.

### *Preparation of GSE*

Grape seed extract was prepared from the dry residue remaining after the extraction of grape seed oil (Oleificio Medio Piave, Fontanelle, Italy). The dry residue was resuspended in an aqueous solution (1:10 w/v) buffered at pH 10.5 with 10 mol/L NaOH (Merck KGaA, Darmstadt, Germany), maintaining the pH constant throughout the 3 h extraction time. After extraction, the suspension was decanted for 3 days before the liquid phase was collected and acidified to pH 3.0 with 6 mol/L HCl. The resulting acid-precipitated material was recovered by centrifugation (3000 *g*, 10 min, room temperature) and dissolved in 0.01 N NaOH. This solution, containing 15% (w/v) of dry matter, was stored at −20°C.

### *Sodium dodecyl sulfate – polyacrylamide gel electrophoresis*

Fining agents were subjected to sodium dodecyl sulfate – polyacrylamide gel electrophoresis (SDS–PAGE) analysis according to Gazzola et al. (2014). Gels were stained with 0.08% colloidal Coomassie G-250 (w/v) and then de-stained

**Table 2.** Rates of addition of the fining agents.

| Fining agent | Chardonnay and Raboso rosé | | | | | | Raboso red | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Dosage (g/hL) | | | Protein (g/hL) | | | Dosage (g/hL) | | | Protein (g/hL) | | |
| | L | M | H | L | M | H | L | M | H | L | M | H |
| GSE | 5 | 10 | 20 | 2.2 | 4.4 | 8.7 | 5 | 10 | 20 | 2.2 | 4.4 | 8.7 |
| Patatin | 5 | 10 | 20 | 3.5 | 6.9 | 13.8 | 5 | 10 | 15 | 3.5 | 6.9 | 10.4 |
| Pea proteins | 3 | 6 | 10 | 2.0 | 3.9 | 6.6 | 3 | 6 | 10 | 2.0 | 3.9 | 6.6 |
| PVPP | 10 | 40 | 70 | — | — | — | — | — | — | — | — | — |
| K-caseinate | 20 | 35 | 50 | 13.1 | 23.0 | 32.8 | — | — | — | — | — | — |
| Albumin | — | — | — | — | — | — | 5 | 10 | 15 | 4.1 | 8.2 | 12.3 |
| Gelatin | — | — | — | — | — | — | 3 | 5 | 10 | 2.6 | 4.3 | 8.5 |

GSE, grape seed extract; H, high; K-caseinate, potassium caseinate; L, low; M, medium; PVPP, polyvinylpolypyrrolidone.

© 2017 Australian Society of Viticulture and Oenology Inc.

with water for 24 h. Stocks of each fining agent were prepared at 2 mg/mL.

*Protein concentration of fining agents*
Total nitrogen in GSE, patatin, pea proteins and K-caseinate was determined after sample mineralisation with a HACH Digesdahl apparatus (HACH Company, Loveland, CO, USA). Ammonia was quantified with the Nessler's reagent (Fluka, Buchs, Switzerland) (Vogel and Svehla 1979), and protein concentration was computed as ammonia × 6.25.

*Turbidity of wines*
Wine turbidity was assessed using a HI 83749 Turbidity & Bentocheck Meter (Hanna Instrument, Szeged, Hungary) and was measured before the fining treatment and post-fining after the wine was racked off the lees. Turbidity was expressed in nephelometric turbidity units (NTU).

*Wine analysis*
**Chromatic characteristics.** The absorbance of wine samples was measured at 420 (cv. Chardonnay) and 520 nm (cv. Raboso Piave, rosé and red wines) with an Ultrospec 2100 pro UV–VIS spectrophotometer (Amersham Biosciences, Uppsala, Sweden), using 10 (420 nm) and 1 mm (520 nm) path length quartz cells.

**Phenolic substances.** The concentration of phenolic substances was estimated by the Folin–Ciocalteu assay (Singleton and Rossi 1965). An aliquot of the diluted sample (200 μL) was added to 1 mL of 1:10 diluted Folin–Ciocalteu reagent (Titolchimica, Rovigo, Italy) after which 0.80 mL of 7.5% (w/v) sodium carbonate was added. After 30 min of incubation at 40°C, the absorbance at 765 nm was measured. Gallic acid (12.5–200 mg/L) was used as the standard for the calibration curve.

**Anthocyanin.** The concentration of anthocyanin was determined using the $SO_2$-bleaching method (Ribéreau-Gayon and Stonestreet 1965). Each sample (500 μL) was added to 500 μL of ethanol acidified with 0.1% HCl and 10 mL of 2% HCl solution. Then, 2.5 mL of this solution was added to 1 mL of distilled water in one tube (t1). In another tube (t2), 2.5 mL of the same solution was mixed with 1 mL of 20% w/v $K_2S_2O_5$ solution. After 15 min in the dark at room temperature, the absorbance at 520 nm was measured. The concentration of anthocyanin was calculated using the equation:

$$\text{Anthocyanin (mg/L)} = 875 \times (\text{Abs t1} - \text{Abs t2}).$$

**Proanthocyanidin.** Proanthocyanidin was determined with the butanol-hydrochloric acid method (Bate-Smith 1975). Six millilitres of an acidic solution of ferrous sulfate [75 mg of $Fe_2(SO_4)_3$ dissolved in 500 mL of 1:1 *n*-butanol:HCl] were added to 2 mL of each sample previously diluted with ethanol. After mixing, half of the mixture was transferred into another tube (t2) at room temperature, while the other half was incubated at 100°C for 30 min (t1). Absorbance at 550 nm was measured against a blank. The standard calibration curve (range 6.25–100 mg/L) was prepared with purified proanthocyanidin extracted from grape skins (cv. Manzoni Bianco). From the difference in absorbance between t1 and t2, compared with the calibration curve, the concentration of proanthocyanidin in the samples was calculated.

**Browning potential.** The predisposition of white wine samples towards browning was determined by the POM-Test (Müller-Späth 1992) which was slightly modified. Briefly, 2 mL of wine were heated at 60°C for 1 h after the addition of 10 μL of a 3% hydrogen peroxide solution. The absorbance at 420 nm against distilled water was measured before and after oxidation. The amount of browning produced was estimated on the basis of the increase in absorbance at 420 nm, as follows:

$$\%\ \text{increase}\ H_2O_2 = [(\text{A420}\ H_2O_2 - \text{A420}\ H_2O)/\text{A420}\ H_2O)] \times 100.$$

For rosé and red wine samples, in which the decrease in absorbance at 520 nm by addition of $H_2O_2$ is due to the oxidation of anthocyanin (Celotti et al. 2006), an adaptation of the previous method was used. In these cases, samples were heated at 65°C for 1 h. The browning produced was estimated on the basis of the decrease of the absorbance at 520 nm, as follows:

$$\%\ \text{decrease}\ H_2O_2 = [(\text{A520}\ H_2O - \text{A520}\ H_2O_2)/\text{A520}\ H_2O)] \times 100.$$

*Sensory analysis*
The wines with optimal dosage of each fining agent (as determined after a first preliminary screening step) were submitted to sensory analysis. A panel of ten trained judges evaluated the visual, aroma and taste properties of the samples in comparison to those of the untreated wine. Twelve attributes were selected: visual (colour intensity), aroma (intensity, floral, fruity, vegetal and aroma defects) and taste (body, acidity, bitterness, astringency, flavour persistence and flavour defects). These attributes were quantified with a ten-point intensity scale. Samples were presented to the panel in tasting glasses marked with codes and in a randomised order. All evaluations were conducted according to standardised procedures (International Organization for Standardization 2011).

*Statistical analysis*
Each fining experiment was performed twice with independently prepared samples, whereas the analyses were performed at least in triplicate. Data were submitted to two-way ANOVA to examine the main effects 'fining agent' (GSE, patatin, pea proteins, PVPP, K-caseinate, albumin and gelatin) and 'dosage' (L, M, and H) on the parameters considered. Means were compared by the Fisher least significant difference (LSD) comparison test ($P \leq 0.05$).

Within each fining agent, a one-way ANOVA was applied to examine differences among the three dosages, followed by the Fisher LSD comparison test ($P \leq 0.05$). For sensory data, the ANOVA and media separation by the Fisher LSD

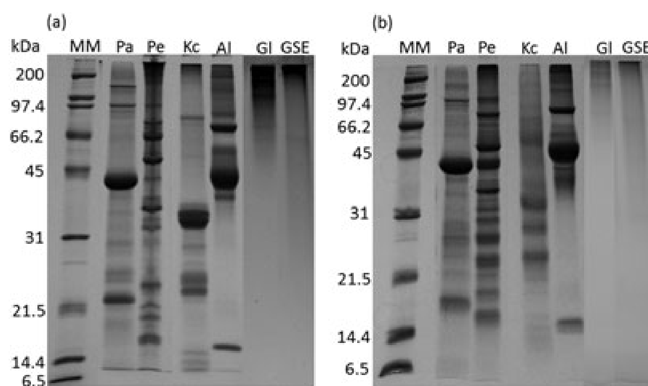


**Figure 1.** Analysis of the proteins used for wine fining under (a) non-reducing and (b) reducing conditions by sodium dodecyl sulfate-polyacrylamide gel electrophoresis. Pa, patatin; Pe, pea proteins; Kc, K-caseinate; Al, albumin; Gl, gelatin; GSE, grape seed extract. MM, molecular mass standard proteins. MM in kDa is shown on the left.

© 2017 Australian Society of Viticulture and Oenology Inc.

comparison test ($P \leq 0.05$) were applied. Data were analysed with COSTAT STATISTICS software version 6.4 (COHORT software, Pacific Grove, CA, USA).

## Results

### *Analysis of fining agents*

**SDS-PAGE analysis.** The protein composition of GSE compared with that of five commercial protein fining agents, including potato protein (patatin), pea proteins, K-caseinate, egg albumin and gelatin, was determined by SDS-PAGE under non-reducing and reducing conditions (Figure 1). As reported by others (Tschiersch et al. 2010, Gambuti et al. 2012, Lomolino et al. 2015), patatin showed a major band at 40 kDa, which did not change its apparent molecular mass (MM) when reduced by 2-mercaptoethanol (2-ME). Other bands at ≈120, 97.4 and between 20 and 30 kDa were observed. The lowest MM protein band (22 kDa) moved tentatively to 17 kDa under reducing conditions. The pea proteins showed a broad range band distribution from 97.4 to 14.4 kDa. Under non-reducing conditions, K-casein presented two intense individual bands located at 32 and 34 kDa, probably corresponding to α-casein and β-casein, respectively, (Gambuti et al. 2012, Fleminger et al. 2013). Egg albumin presented the typical bands at 68, 43, and 15 kDa (Marchal et al. 2002a, Cosme et al. 2007, Gambuti et al. 2012). As already reported by others (Cosme et al. 2007, Gambuti et al. 2012, Simonato et al. 2013), gelatin showed poorly resolved protein bands with an MM, in this case, higher than 50 kDa (under non-reducing conditions), most of them being located at the top of the gel, in particular above 116 kDa. Analysis of GSE by SDS-PAGE revealed only a smear, with some stained material blocked at the top of the gel, and this occurred under both reducing and non-reducing conditions. Both under non-reducing and reducing conditions, GSE revealed a band slightly noticeable at 65 kDa, consistent with a grape seed storage protein 11S (Gazzola et al. 2014). A clean-up procedure to improve the electrophoretic analysis by applying a preliminary step of protein precipitation to remove phenolic substances (Vincenzi et al. 2005) was unsuccessful (data not shown). This could be due to a pre-existing strong complexation of GSE proteins and phenolic substances in the material used for the extraction, which contains, in addition to proteins, a large amount of phenolic substances (Vincenzi et al. 2013). Furthermore, the removal of phenolic substances by de-hulling the grape seeds before protein extraction, allowed the grape seed proteins to be characterised by electrophoretic analysis, which gave clearly resolved bands (Gazzola et al. 2014).

**Protein concentration of fining agents.** Nitrogen analysis showed GSE to contain 43.6 ± 2.6% protein ($N \times 6.25$), a value which is higher than that measured previously for a similar extract (Vincenzi et al. 2013). Such a difference may be due to the scaled-up extraction process prolonged for 3 h applied here, which probably allowed for a higher recovery of protein. It could not be excluded that the nature of grape seeds has also affected the protein yield. In general, the total protein concentration of the commercial fining agents tested was higher than that of GSE, with pea proteins and K-caseinate containing similar protein values of 65.6 ± 5.7 and 65.6 ± 1.2%, respectively. The protein concentration of patatin was 69.0 ± 1.4%, a value in accordance with that reported by Gambuti et al. (2012). Finally, the total protein concentration of the gelatin and egg albumin samples was indicated by the manufacturer (EVER) as >85 ($N \times 5.5$) and ≈82%, respectively.

**Table 3.** Effect of seven fining agents at three levels of addition on the turbidity and percentage of variation in turbidity of the Chardonnay, Raboso rosé and Raboso red wines.

| Fining agent | NTU | | |
|---|---|---|---|
| | L | M | H |
| **Chardonnay** | | | |
| Control | 17.3 ± 0.2 | — | — |
| GSE | 9.2 ± 0.7 (−46.9) | 7.0 ± 1.1 (−59.8) | 5.7 ± 0.0 (−67.0) |
| Patatin | 13.7 ± 3.7 (−21.0) | 5.5 ± 0.1 (−68.1) | 2.5 ± 0.6 (−85.5) |
| Pea proteins | 16.4 ± 0.3 (−5.6) | 16.0 ± 0.9 (−7.6) | 15.4 ± 1.8 (−11.1) |
| PVPP | 16.2 ± 0.5 (−6.5) | 16.0 ± 0.5 (−7.9) | 19.2 ± 0.1 (+9.9) |
| K-caseinate | 14.3 ± 0.1 (−17.4) | 14.1 ± 0.3 (−18.9) | 12.5 ± 0.7 (−27.8) |
| **Raboso rosé** | | | |
| Control | 19.6 ± 2.3 | — | — |
| GSE | 14.8 ± 2.8 (−24.5) | 11.4 ± 3.3 (−41.8) | 9.8 ± 3.3 (−49.9) |
| Patatin | 4.4 ± 2.6 (−77.3) | 2.2 ± 0.6 (−89.0) | 2.2 ± 0.3 (−88.6) |
| Pea proteins | 12.0 ± 1.1 (−38.8) | 7.5 ± 1.4 (−61.7) | 7.9 ± 0.2 (−59.8) |
| PVPP | 12.4 ± 1.2 (−37.0) | 5.4 ± 1.0 (−72.6) | 7.2 ± 0.9 (−63.5) |
| K-caseinate | 3.9 ± 1.1 (−79.9) | 4.5 ± 1.4 (−76.8) | 5.1 ± 0.9 (−73.8) |
| **Raboso red** | | | |
| Control | 15.8 ± 0.1 | — | — |
| GSE | 14.2 ± 1.4 (−10.0) | 13.4 ± 0.4 (−15.3) | 10.9 ± 0.1 (−31.1) |
| Patatin | 16.3 ± 0.2 (+3.1) | 10.9 ± 0.5 (−31.1) | 8.6 ± 0.9 (−45.5) |
| Pea proteins | 16.3 ± 0.3 (+3.1) | 17.8 ± 0.3 (+11.2) | 19.3 ± 0.4 (+18.1) |
| Albumin | 8.9 ± 0.1 (−43.8) | 6.5 ± 0.4 (−58.8) | 5.0 ± 0.8 (−68.5) |
| Gelatin | 16.6 ± 0.6 (+4.8) | 15.6 ± 1.8 (−1.4) | 13.3 ± 2.6 (−16.3) |

NTU values are the mean (± SD) of four analyses (from two independent fining treatments). Values in brackets are the variation (%) from the control. GSE, grape seed extract; H, high; L, low; K-caseinate, potassium caseinate; M, medium; PVPP, polyvinylpolypyrrolidone.

© 2017 Australian Society of Viticulture and Oenology Inc.

### *Effect of fining agents on wine turbidity and composition*

**Turbidity.** The turbidity of wines before fining was 782 ± 31 NTU for Chardonnay, 1484 ± 8 NTU for Raboso rosé and 24 ± 2 NTU for Raboso red. The turbidity of the three wines allowed to settle spontaneously (i.e. without protein addition, Control samples) was compared with that of the same wines treated with the fining agents at three levels of addition (Table 3). The time of the treatments (specified afterwards for each case) was that needed for wines to reach a turbidity lower than 20 NTU, which is a value allowing an efficient wine filtration (for more details, the settling kinetics are reported in Table S1 in the Supporting information).

When added to the Chardonnay wine, GSE was one of the most effective clarifying agents. After 240 h of settling, GSE reduced the turbidity of the original wine, with the reduction increasing with the dosage. In particular, the highest GSE dosage (20 g/hL) reduced the wine turbidity by 67%, whereas the M (10 g/hL) and L (5 g/hL) doses decreased the NTU by ≈60 and ≈47%, respectively. Patatin provided the greatest reduction in turbidity (85%) when added at 20 g/hL; however, at the lowest addition rate (5 g/hL), patatin was less efficient than GSE, reducing wine turbidity by 21%. The other three fining agents tested in Chardonnay were clearly less efficient than GSE and patatin at all doses tested (Table 3).

The Raboso rosé wine had the highest initial turbidity among the wines tested; however, only 48 h were necessary to achieve a turbidity of <20 NTU in the treated wine. Turbidity was reduced by about 50% by the GSE treatment at 20 g/hL, whereas the L and M GSE doses were less effective reducing turbidity by 24 and 42%, respectively, indicating that the clarifying action was proportional to the dose added. All other fining agents tested, however, reduced wine turbidity better than GSE, independently of the dosage tested. In particular, patatin showed the best clarifying action (NTU reduction of 89% at the M dose of 10 g/hL). K-caseinate was most effective (≈80% NTU reduction) at the lowest dose (20 g/hL), whereas the pea proteins and the PVPP were most effective at the M dose (6 and 40 g/hL, respectively), reducing the turbidity by 62 and 73%, respectively (Table 3).

Clarification (<20 NTU) of the Raboso red wine was completed after 120 h of settling. The most effective fining agent was egg albumin, reducing wine turbidity to a low value at all doses tested. At L (5 g/hL), M (10 g/hL) and H (20 g/hL) doses, GSE reduced the turbidity by only 10, ≈15 and ≈31%, respectively. This latter value was achieved by using patatin at 10 g/hL. Patatin at 15 g/hL almost halved the NTU of the wine, but at 5 g/hL was ineffective. In contrast, the pea proteins were not able to clarify the wine tested, but rather appeared to increase the turbidity, while gelatin decreased the wine turbidity only at the highest dose, proving to be not as effective as the other treatments (Table 3).

**Chromatic characteristics.** The variation of wine colour intensity due to fining was determined by spectrophotometry (Table 4). In general, the variation in colour intensity (measured at 420 nm) in Chardonnay wine was low and data analysis indicated the variation depended on both the type of fining agent and the dosage. Grape seed extract did not reduce the colour intensity of the white wine at all dosages tested, whereas K-caseinate (at 35 and 50 g/hL) and PVPP (at 70 g/hL) reduced the absorbance significantly at 420 nm; these results agree with those of Cosme et al. (2012). This is not unexpected as caseinates are commonly used in winemaking to reduce the colour

**Table 4.** Effect of seven fining agents at three levels of addition on the chromatic characteristics of the Chardonnay, Raboso rosé and Raboso red wines.

| Fining agent | L | M | H | Mean |
|---|---|---|---|---|
| **Chardonnay A420 nm** | | | | |
| Control | 0.072 ± 0.002a | — | — | 0.072 ± 0.002A |
| GSE | 0.071 ± 0.002a | 0.071 ± 0.002a | 0.070 ± 0.002a | 0.070 ± 0.002A |
| Patatin | 0.069 ± 0.005ab | 0.067 ± 0.003ab | 0.064 ± 0.002b | 0.066 ± 0.004B |
| Pea proteins | 0.071 ± 0.003a | 0.068 ± 0.002ab | 0.066 ± 0.003b | 0.068 ± 0.003AB |
| PVPP | 0.071 ± 0.006a | 0.066 ± 0.005ab | 0.063 ± 0.004b | 0.067 ± 0.006B |
| K-caseinate | 0.068 ± 0.006a | 0.065 ± 0.005b | 0.064 ± 0.005b | 0.065 ± 0.005B |
| Mean | 0.070 ± 0.004A | 0.068 ± 0.004AB | 0.066 ± 0.004B | — |
| **Raboso rosé A 520 nm** | | | | |
| Control | 0.518 ± 0.001a | — | — | 0.518 ± 0.001A |
| GSE | 0.503 ± 0.001c | 0.507 ± 0.001b | 0.501 ± 0.000c | 0.504 ± 0.003B |
| Patatin | 0.496 ± 0.001b | 0.491 ± 0.001c | 0.480 ± 0.001d | 0.489 ± 0.007C |
| Pea proteins | 0.514 ± 0.000b | 0.504 ± 0.000c | 0.496 ± 0.001d | 0.505 ± 0.008B |
| PVPP | 0.505 ± 0.001b | 0.462 ± 0.001c | 0.433 ± 0.000d | 0.466 ± 0.032D |
| K-caseinate | 0.479 ± 0.000b | 0.459 ± 0.001c | 0.441 ± 0.000d | 0.460 ± 0.017E |
| Mean | 0.502 ± 0.013A | 0.490 ± 0.023B | 0.478 ± 0.033C | — |
| **Raboso red A 520 nm** | | | | |
| Control | 5.085 ± 0.021a | — | — | 5.085 ± 0.021A |
| GSE | 4.935 ± 0.035b | 4.875 ± 0.007b | 4.855 ± 0.049b | 4.888 ± 0.046E |
| Patatin | 5.015 ± 0.007b | 5.010 ± 0.028bc | 4.955 ± 0.021c | 4.993 ± 0.034B |
| Pea proteins | 5.005 ± 0.021b | 4.970 ± 0.000b | 4.850 ± 0.028c | 4.942 ± 0.074C |
| Albumin | 4.850 ± 0.014b | 4.875 ± 0.021b | 4.855 ± 0.007b | 4.860 ± 0.017F |
| Gelatin | 4.965 ± 0.007b | 4.925 ± 0.007c | 4.855 ± 0.007d | 4.915 ± 0.050D |
| Mean | 4.976 ± 0.078A | 4.957 ± 0.079B | 4.909 ± 0.093C | — |

Values are the mean (± SD) of four analyses (from two independent fining treatments). Small letters denote differences among dosages within the same fining agent. Capital letters denote differences among fining agents/dosages (LSD, $P \le 0.05$). GSE, grape seed extract; H, high; L, low; K-caseinate, potassium caseinate; M, medium; PVPP, polyvinylpolypyrrolidone.

© 2017 Australian Society of Viticulture and Oenology Inc.

of white wines. Similarly, patatin also reduced the colour of the Chardonnay wine but was significant only when used at 20 g/hL. With the rosé and red Raboso wines variation in the optical density at 520 nm was measured as an indication of changes in red coloration. Data analysis showed that all the fining agents tested decreased the red colour of the original wines in a dose-dependent manner.

For the rosé wine, GSE and the pea proteins had a similar minor effect on colour proving to be more suitable for this wine where a drastic reduction in colour should be avoided. Patatin also had a low impact, followed by PVPP, whereas K-caseinate was the treatment resulting in the greatest colour removal. For all treatments except GSE, the colour reduction was proportional to the dosage used (Table 4).

In Raboso red wine, all fining agents, at all doses, reduced the absorbance at 520 nm compared with that of the untreated wine. Grape seed extract removed part of the red colour, as did the animal proteins, with egg albumin inducing the greatest reduction in colour. Patatin was the least effective in removing colour, especially at the L dosage (5 g/hL), followed by pea proteins. The low effect of patatin on colour removal confirms the results reported for the red wine Aglianico (Gambuti et al. 2012) (Table 4).

**Phenolic substances.** In Chardonnay, GSE and patatin did not affect significantly the amount of phenolic substances measured at all addition rates when compared with that of the untreated samples. In contrast, pea proteins and K-caseinate significantly removed phenolic substances, but only at M (6 g/hL) and at H (50 g/hL) doses, respectively; PVPP was effective in removing phenolic substances at all doses, confirming its capacity to act as an adsorbent for phenolic substances (Cosme et al. 2012) (Table 5).

The addition of GSE and pea proteins similarly did not decrease significantly the concentration of phenolic substances in Raboso rosé wine, independently of the dosage. In contrast, patatin and PVPP (at M and H doses) and caseinate (at all doses) reduced significantly the concentration of phenolic substances of the rosé wine, and PVPP confirmed its ability in binding phenolic substances (Cosme et al. 2012) and removed 133 mg/L of phenolic substances compared with the untreated sample at the H dose.

Fining of Raboso red wine, a wine typically rich in phenolic substances (De Rosso et al. 2010), showed that, regardless of the dosage used, GSE resulted in a significant reduction of the concentration of phenolic substances, removing an average of 65 mg/L of phenolic substances from the Control wine, a value similar to that removed by addition of egg albumin (−94 mg/L), gelatin (−66 mg/L) and patatin (−46 mg/L) (Table 5).

**Wine oxidisability.** The POM-test was applied to Chardonnay samples to determine whether the fining agents were able to eliminate part of the phenolic substances prone to oxidative browning. In this test, the oxidation of phenolic substances in the presence of hydrogen peroxide led to an increase in the absorbance at 420 nm, which is proportional to the presence of oxidisable phenolic substances involved in colour variation. The Chardonnay wine treated with the H dose (20 g/hL) of GSE was significantly less prone to oxidation than the Control wine, as were the samples treated with PVPP and K-caseinate, which decreased significantly Chardonnay oxidisability at H (70 g/hL) and L (20 g/hL) doses, respectively. The index of

**Table 5.** Effect of seven fining agents at three levels of addition on the concentration of phenolic substances of the Chardonnay, Raboso rosé and Raboso red wines.

| | Phenolic substances (mg/L) | | | |
|---|---|---|---|---|
| **Fining agent** | **L** | **M** | **H** | **Mean** |
| **Chardonnay** | | | | |
| Control | 246.55 ± 1.09a | — | — | 246.55 ± 1.09A |
| GSE | 243.06 ± 6.22a | 245.80 ± 1.05a | 243.17 ± 5.16a | 243.71 ± 4.95A |
| Patatin | 244.26 ± 5.20a | 243.29 ± 10.35a | 242.47 ± 2.57a | 243.39 ± 6.11A |
| Pea proteins | 237.13 ± 3.17a | 228.23 ± 11.59b | 245.30 ± 2.61a | 235.94 ± 10.14B |
| PVPP | 230.74 ± 4.19b | 222.23 ± 2.24c | 207.92 ± 4.07d | 219.81 ± 10.18C |
| K-caseinate | 242.43 ± 4.90ab | 244.72 ± 3.00a | 238.34 ± 7.73b | 242.20 ± 5.56A |
| Mean | 240.42 ± 6.76A | 236.55 ± 11.94B | 235.47 ± 15.28B | — |
| **Raboso rosé** | | | | |
| Control | 536.36 ± 52.14a | — | — | 536.36 ± 52.14A |
| GSE | 518.43 ± 35.04a | 515.46 ± 37.73a | 513.01 ± 40.00a | 515.50 ± 35.58AB |
| Patatin | 504.20 ± 17.88ab | 494.85 ± 23.97b | 478.11 ± 24.96b | 491.15 ± 24.38BC |
| Pea proteins | 517.87 ± 19.76a | 513.79 ± 37.96a | 504.82 ± 43.10a | 511.66 ± 33.82AB |
| PVPP | 504.47 ± 45.97ab | 465.31 ± 54.58b | 403.67 ± 40.89c | 460.17 ± 61.77D |
| K-caseinate | 489.22 ± 25.76b | 479.10 ± 28.28b | 457.66 ± 30.39b | 474.84 ± 29.92CD |
| Mean | 511.37 ± 36.51A | 497.02 ± 45.30AB | 480.88 ± 56.21B | — |
| **Raboso red** | | | | |
| Control | 3006.10 ± 43.45a | — | — | 3006.10 ± 43.45A |
| GSE | 2972.06 ± 77.15ab | 2925.65 ± 69.48b | 2912.48 ± 6.50b | 2940.58 ± 67.67CD |
| Patatin | 2996.17 ± 55.09a | 2964.26 ± 51.61ab | 2928.62 ± 52.73b | 2959.58 ± 57.94BC |
| Pea proteins | 3002.86 ± 58.90a | 2987.66 ± 17.02a | 2964.56 ± 22.50a | 2984.75 ± 40.96AB |
| Albumin | 2942.07 ± 66.23ab | 2937.30 ± 36.05ab | 2860.00 ± 122.47b | 2911.91 ± 89.42D |
| Gelatin | 2898.30 ± 69.92c | 2997.87 ± 62.37ab | 2933.56 ± 45.61bc | 2939.67 ± 68.58CD |
| Mean | 2969.74 ± 70.09A | 2967.23 ± 56.44A | 2935.40 ± 74.95B | — |

Values are the mean (± SD) of eight analyses (from two independent fining treatments). Small letters denote differences among dosages within the same fining agent. Capital letters denote differences among fining agents/dosages (LSD, $P \leq 0.05$). GSE, grape seed extract; H, high; L, low; K-caseinate, potassium caseinate; M, medium; PVPP, polyvinylpolypyrrolidone.

© 2017 Australian Society of Viticulture and Oenology Inc.

anthocyanin oxidisability (Celotti et al. 2006) was used to study the potential for oxidation of the anthocyanin fraction of the Raboso red wine and is reported as a reduction of the absorbance at 520 nm. None of the clarifying agents used prevented anthocyanin oxidation, although patatin appeared to slightly reduce it (Table 6). The anthocyanin oxidisability could not be applied to Raboso rosé because of the specific composition of the phenolic substances of this wine. Both classes of oxidisable molecules (anthocyanin and flavonoids) interfered with each other in the measurement.

**Proanthocyanidin.** Raboso Piave is a native deep-coloured red grape cultivar from the Veneto region, and the resulting wine is typically characterised by a high level of astringency and acidity. As a result, winemakers commonly fine the wine to modify its tannic profile, thus improving its drinkability.

All the fining treatments used on average decreased the concentration of proanthocyanidin of Raboso Piave, with gelatin showing the greatest effect in tannin removal already at L (3 g/hL) dose. Albumin also significantly reduced the concentration of proanthocyanidin at the lowest addition rate (Table 7). Grape seed extract at dose M (10 g/hL) was effective in removing proanthocyanidin, and its efficacy did not increase at the higher rates, indicating that the medium dosage was sufficient to obtain a significant reduction of the proanthocyanidin concentration for the wine tested. Patatin appeared to be efficient already at L (5 g/hL) dose, behaving as egg albumin and gelatin (Table 7).

**Anthocyanin.** In Raboso rosé wine, the only fining treatment causing a significant reduction in anthocyanin was PVPP, which removed a modest amount of this phenolic fraction at M (40 g/hL) and H (70 g/hL) doses.

After the fining of Raboso red, the anthocyanin concentration was generally reduced by the fining agents compared with that of the Control, except for GSE at the L (5 g/hL) and H (20 g/hL) doses, patatin at the L (5 g/hL) dose, pea proteins at all doses and gelatin at the H (10 g/hL) dose. Egg albumin, especially at the M (10 g/hL) dose, and patatin at the M (10 g/hL) and H (15 g/hL) doses were the most effective in removing anthocyanin. In particular, the role of patatin fining in removing a significant amount of anthocyanin from red wines, without interfering with the colour estimated by the A520, has already been demonstrated (Gambuti et al. 2012), and these findings are in agreement with results shown here (Table 8). Furthermore, for the fining agents considered, no dose-dependent effect in anthocyanin removal was observed, with the intermediate dose being the one inducing the highest reduction of anthocyanin (Table 8).

*Sensory analysis*

The sensory data for the Chardonnay wine showed that for almost all the descriptors, the sample treated with GSE at the H dose (20 g/hL) was no different from that of the Control, nor from the wine fined with patatin, pea proteins, PVPP and K-caseinate. The wine treated with GSE, however, showed significantly less aroma and flavour defects compared with that of the Control, while it did not differ from any of the other fined samples. The taste of wine treated with GSE was the only wine that differed significantly from the Control, showing to be the least defective (Table 9).

The Raboso rosé wine treated with GSE at the M dose (10 g/hL) differed from the Control, significantly decreasing the olfactory attribute of vegetal, as did patatin. In contrast,

**Table 6.** Effect of seven fining agents at three levels of addition on the oxidisability of phenolic substances of the Chardonnay, Raboso rosé and Raboso red wines.

| | Oxidisability of phenolic substances (%) | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | Chardonnay | | | | | Raboso red | | | | |
| | Untreated | L | M | H | Mean | Untreated | L | M | H | Mean |
| Control | 17.67 ± 0.72a | — | — | — | 17.67 ± 0.72B | 47.86 ± 6.80a | — | — | — | 47.86 ± 6.80AB |
| GSE | — | 12.51 ± 4.02ab | 11.89 ± 3.56ab | 10.10 ± 4.87b | 11.63 ± 3.80D | — | 49.55 ± 7.63a | 50.05 ± 6.21a | 50.01 ± 5.47a | 49.87 ± 5.88AB |
| Patatin | 17.67 ± 0.72b | 20.73 ± 1.13ab | 22.10 ± 1.98a | 22.40 ± 3.94a | 21.74 ± 2.50A | — | 46.41 ± 4.06a | 45.84 ± 3.35a | 48.16 ± 3.91a | 46.80 ± 3.58B |
| Pea proteins | — | 12.82 ± 1.54b | 15.11 ± 3.01ab | 17.62 ± 1.83a | 15.19 ± 2.87C | — | 47.10 ± 2.21a | 47.54 ± 4.04a | 47.87 ± 2.64a | 47.50 ± 2.79AB |
| PVPP | — | 13.33 ± 0.10ab | 11.59 ± 3.49ab | 9.87 ± 4.81b | 11.38 ± 3.49D | — | — | — | — | — |
| K-caseinate | — | 10.19 ± 3.19b | 10.89 ± 0.99b | 10.55 ± 1.63b | 10.54 ± 1.96D | — | — | — | — | — |
| Albumin | — | — | — | — | — | — | 49.55 ± 3.12a | 49.05 ± 3.83a | 49.98 ± 4.88a | 49.52 ± 3.65AB |
| Gelatin | — | — | — | — | — | — | 51.37 ± 5.52a | 50.83 ± 6.62a | 51.30 ± 5.46a | 51.16 ± 5.33A |
| Mean | — | 14.51 ± 4.36A | 14.90 ± 4.73A | 15.01 ± 5.73A | — | — | 48.67 ± 4.86A | 48.56 ± 4.92A | 49.26 ± 4.52A | — |

Values are the mean (± SD) of four analyses (from two independent fining treatments). Small letters indicate a difference among dosages within the same fining agent and capital letters indicate a difference among fining agents/dosages (LSD, $P \leq 0.05$). GSE, grape seed extract; H, high; L, low; LSD, least significant difference; K-caseinate, potassium caseinate; M, medium; PVPP, polyvinylpolypyrrolidone; SD, standard deviation.

© 2017 Australian Society of Viticulture and Oenology Inc.

**Table 7.** Effect of seven fining agents at three levels of addition on the concentration of proanthocyanidin in Raboso red wine.

| Fining agent | Proanthocyanidin (mg/L) | | | | |
|---|---|---|---|---|---|
| | Untreated | L | M | H | Mean |
| Control | 3.70 ± 0.19a | — | — | — | 3.70 ± 0.19A |
| GSE | — | 3.58 ± 0.08ab | 3.55 ± 0.16b | 3.48 ± 0.05b | 3.54 ± 0.12B |
| Patatin | — | 3.57 ± 0.06b | 3.52 ± 0.06b | 3.49 ± 0.05b | 3.53 ± 0.06B |
| Pea proteins | — | 3.60 ± 0.09ab | 3.50 ± 0.08bc | 3.45 ± 0.06c | 3.51 ± 0.10B |
| PVPP | — | — | — | — | — |
| K-caseinate | — | — | — | — | — |
| Albumin | — | 3.55 ± 0.12b | 3.51 ± 0.06b | 3.47 ± 0.06b | 3.51 ± 0.09B |
| Gelatin | — | 3.43 ± 0.16b | 3.43 ± 0.05b | 3.38 ± 0.10b | 3.41 ± 0.12C |
| Mean | — | 3.57 ± 0.14A | 3.54 ± 0.14AB | 3.50 ± 0.14B | — |

Values are the mean (± SD) of eight analyses (from two independent fining treatments). Small letters indicate a difference among dosages within the same fining agent and capital letters indicate a difference among fining agents/dosages (LSD, $P \leq 0.05$). GSE, grape seed extract; H, high; K-caseinate, potassium caseinate; L, low; LSD, least significant difference; M, medium; PVPP, polyvinylpolypyrrolidone; SD, standard deviation.

the samples treated with the pea proteins, PVPP and K caseinate failed to modify the vegetal characteristics of the wine. Grape seed extract greatly improved the taste by significantly reducing both the perception of acidity (as did PVPP) and phenolic bitterness. In this latter case, GSE was the only fining agent able to lower significantly the bitter taste (Table 9).

The optimal dosage of GSE for the Raboso wine was 5 g/hL (L). The treatment with GSE enhanced significantly the perception of wine body, not differing from the sample treated with the patatin. Treatment with GSE reduced both acidity (as did gelatin and pea proteins) and astringency (as did albumin and pea proteins), compared with that of the Control (Table 9). It is noteworthy that GSE action on astringency was obtained with a dosage three times lower than albumin, confirming its efficacy (Vincenzi et al. 2013).

## Discussion

The problem of the mandatory declaration of allergenic residues found in protein-fined wines has raised concerns among winemakers in the last few years. This is mainly due to the possible negative reaction of consumers when facing a wine label that reports the use of unexpected substances. The issue can be tackled in two ways. The first is to continue using those fining agents that must be declared (at the moment, egg and milk proteins), adopting good practices to avoid the presence of their residues in the wine according to the OIV Guidelines (Resolution OIV-OENO-SECSAN 520–2014) (Organisation Internationale de la Vigne et du Vin 2014). Despite being feasible, this approach, however, would result in additional costs for producers including those for immunological analyses (Organisation Internationale de la Vigne et du Vin 2012) needed to assure that fining residues are present in amounts lower than the thresholds set by the regulation. The second possibility is to totally avoid the use of egg and milk proteins, replacing them with materials with similar fining performance. Many proteins, mainly of plant origin, have been proposed for this purpose, as, for example, patatin (Gambuti et al. 2012). It should be noted, however, that almost all plant proteins can have some allergenic potential and the inclusion of new materials in the list of the allergens to be declared cannot be excluded in the future. In this context, the phenomenon of allergenic cross-reactivity should also be considered. For example, legume proteins are well-known cross-reacting allergens (Verma et al. 2013), and it is surprising that pea proteins are not to be declared on the labels when peanuts, lupin or soybean are present in the list of allergenic foods of the Directive 2007/68/EC (European Commission 2007).

Therefore, if proteins need to be used for fining wine, a rational approach to overcome the aforementioned problems should focus on the use of proteins that are naturally found in grapes, yeast or wine. In this way, the possible presence in fined wines of ‘foreign’ protein material would be completely eliminated and, consequently, the problem of allergenic risk (and its labelling) clearly bypassed.

A possible source of grape proteins suitable for this task are grape seeds, and these proteins have been recently characterised (Gazzola et al. 2014). Grape seeds are normally discarded as a waste or, more rarely, used for the extraction of the grape seed oil. In this latter case, the dried residue of the oil extraction is also considered an industrial waste, although it contains a large amount of phenolic substances, polysaccharides and proteins (Vincenzi et al. 2013).

Based on this knowledge, a series of experiments has explored the possibility of GSE being used as a novel wine fining agent. Previous results have clearly indicated that GSE is able to potentially remove astringent compounds from tannin solutions and from red wines, thus reducing astringency (Vincenzi et al. 2013), and this effect has been confirmed here. It is likely that this ability of GSE is due to its protein fraction, which would bind reactive tannin in wine, although an interference of seed polysaccharides in the salivary protein–tannin interaction may also be implicated. Furthermore, some polysaccharides, such as RGIIs and PRAGs, have the potential to bind wine tannin, which in this way would not interact with salivary proteins (Vidal et al. 2004, Rinaldi et al. 2012).

According to OIV resolution OENO 28/2004 (Organisation Internationale de la Vigne et du Vin 2004), plant proteins for wine fining should be mainly made up of proteins (total nitrogen must be more than 65% in protein) but can also contain carbohydrates (fibre, starch and sugars), fats and minerals. With GSE, the amount of protein is lower than that required for fining materials of plant origin. The protein extraction yield from grape seeds was always low in early studies, ranging from 8.44 (Igartuburu et al. 1991) to 25.9% (Fazio et al. 1983), and this can be due to the high content of seed proanthocyanidin that can bind the proteins, thus limiting their extraction (Fazio et al. 1983).

Especially in the white wine studied here, however, GSE was effective in wine fining suggesting the possibility that it

© 2017 Australian Society of Viticulture and Oenology Inc.

**Table 8.** Effect of seven fining agents at three levels of addition on the concentration anthocyanin in Raboso rosé and Raboso red wines.

| Fining agent | Anthocyanin (mg/L) | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | Raboso rosé | | | | | Raboso red | | | | |
| | Untreated | L | M | H | Mean | Untreated | L | M | H | Mean |
| Control | 58.31 ± 7.65a | — | — | — | 58.31 ± 7.65A | 373.93 ± 9.60a | — | — | — | 373.93 ± 9.60A |
| GSE | — | 56.00 ± 3.35a | 57.75 ± 6.04a | 57.25 ± 5.67a | 57.00 ± 4.95A | — | 368.48 ± 2.56ab | 359.92 ± 2.07b | 365.90 ± 2.07ab | 365.14 ± 4.28BC |
| Patatin | — | 57.00 ± 3.18a | 54.95 ± 10.57a | 56.70 ± 5.36a | 56.31 ± 6.30AB | — | 367.21 ± 3.28a | 345.63 ± 0.76b | 352.90 ± 7.11b | 355.27 ± 10.29D |
| Pea proteins | — | 57.69 ± 5.20a | 59.13 ± 6.22a | 56.13 ± 6.69a | 57.65 ± 5.89A | 373.93 ± 9.60ab | 364.29 ± 6.19b | 365.60 ± 2.16b | 379.53 ± 0.93a | 368.59 ± 7.64B |
| PVPP | — | 56.38 ± 3.92ab | 51.25 ± 7.04b | 51.06 ± 6.30b | 52.90 ± 6.15B | — | — | — | — | — |
| K-caseinate | — | 54.95 ± 3.41a | 58.00 ± 4.94a | 56.00 ± 4.82a | 56.49 ± 4.46AB | — | — | — | — | — |
| Albumin | — | — | — | — | — | — | 347.38 ± 1.24BC | 337.90 ± 9.01c | 356.78 ± 0.93b | 346.32 ± 8.69E |
| Gelatin | — | — | — | — | — | — | 364.11 ± 3.74b | 354.70 ± 2.33c | 366.33 ± 0.91ab | 361.30 ± 5.84C |
| Mean | — | 56.81 ± 1.03A | 56.65 ± 3.17A | 55.87 ± 2.49A | — | — | 363.63 ± 9.64A | 356.21 ± 12.87B | 365.61 ± 10.11A | — |

Values are the mean (± SD) of six analyses (from two independent fining treatments). Small letters indicate a difference among dosages within the same fining agent and capital letters indicate a difference among fining agents/dosages (LSD, $P \leq 0.05$). GSE, grape seed extract; H, high; K-caseinate, potassium caseinate; L, low; LSD, least significant difference; M, medium; PVPP, polyvinylpolypyrrolidone; SD, standard deviation.

**Table 9.** Sensory analysis of the Chardonnay, Raboso rosé and Raboso red wines treated with seven fining agents at the optimal dosage.

| | Aroma defects | Flavour defects | — |
|---|---|---|---|
| **Chardonnay** | | | |
| Control | 7.0 ± 0.0a | 6.0 ± 0.0a | — |
| GSE H | 5.6 ± 1.2b | 5.3 ± 0.5b | — |
| Patatin M | 5.7 ± 0.7b | 5.6 ± 1.0ab | — |
| Pea proteins M | 6.5 ± 0.8ab | 6.1 ± 0.2a | — |
| PVPP L | 6.3 ± 1.3ab | 6.0 ± 0.3a | — |
| K-caseinate L | 6.0 ± 1.4b | 5.8 ± 0.8ab | — |
| | **Vegetable** | **Acidity** | **Bitterness** |
| **Raboso rosé** | | | |
| Control | 5.0 ± 0.0ab | 9.0 ± 0.0a | 5.0 ± 0.0a |
| GSE M | 3.6 ± 1.0d | 7.3 ± 1.2c | 3.6 ± 1.6b |
| Patatin L | 3.9 ± 1.1cd | 8.1 ± 1.3abc | 4.4 ± 1.3ab |
| Pea proteins L | 5.5 ± 1.2a | 8.1 ± 1.2abc | 4.7 ± 1.5ab |
| PVPP H | 4.9 ± 1.4abc | 7.9 ± 1.3bc | 4.5 ± 1.1ab |
| K-caseinate L | 4.1 ± 1.0bcd | 8.7 ± 0.9ab | 3.9 ± 1.3ab |
| | **Body** | **Acidity** | **Astringency** |
| **Raboso red** | | | |
| Control | 4.5 ± 0.0b | 6.0 ± 0.0a | 5.5 ± 0.0a |
| GSE L | 5.1 ± 0.7a | 5.6 ± 0.4bc | 4.9 ± 0.5bc |
| Patatin L | 5.1 ± 0.4a | 5.7 ± 0.5abc | 5.0 ± 0.7abc |
| Pea proteins M | 4.4 ± 0.8b | 5.5 ± 0.4c | 4.4 ± 0.8c |
| Albumin H | 4.7 ± 0.6ab | 5.9 ± 0.3ab | 4.9 ± 0.8bc |
| Gelatin H | 4.8 ± 0.8ab | 5.6 ± 0.6BC | 5.1 ± 0.6ab |

Values are the mean (± SD) of ten scores (ten panellists). Different letters indicate a significant difference among fining agents (LSD, $P \leq 0.05$). A ten-point scale was used. Only the descriptors showing significant differences between the Control and treated wines are shown. GSE, grape seed extract; H, high; K-caseinate, potassium caseinate; L, low; LSD, least significant difference; M, medium; SD, standard deviation.

be used as an alternative to K-caseinate, even at a lower dose. In particular, unlike PVPP and K-caseinate, GSE did not show any detrimental effect on white wine colour but improved its stability to browning similarly to K-caseinate at the same dose and to PVPP at a dose 3.5 times higher. Therefore, because K-caseinate is involved in the problem of allergenic fining agents and PVPP is a synthetic additive that is not allowed in organic winemaking, GSE could replace those aids to efficiently remove oxidised phenolic substances and those susceptible to oxidation, without negatively affecting the sensory characteristics of the wine.

In the rosé wine, the fining agents tested were all more efficient than GSE in reducing wine turbidity. Similarly to what observed for the Chardonnay, however, GSE maintained the wine colour unchanged, unlike the other fining agents. This can be particularly important for rosé wines, whose colour generally should be preserved as an important factor of quality. Moreover, the treatment with GSE improved the rosé sensory properties, drastically reducing the vegetal notes and enhancing its palatability.

In the red wine, GSE was not particularly effective in decreasing wine turbidity; however, it outperformed gelatin, whose main applications are to decrease wine turbidity and to

© 2017 Australian Society of Viticulture and Oenology Inc.

reduce wine astringency. Moreover, at least in Raboso red wine, which has a high concentration of phenolic substances (De Rosso 2010), GSE reduced significantly this quantity and especially that of proanthocyanidin, at least in a manner similar to ovalbumin and gelatin. Finally, GSE definitely improved the wine taste by reducing both acidity and astringency, also at low doses, confirming its ability to improve the mouthfeel of red wines (Vincenzi et al. 2013).

## Conclusion

The grape seed extract here described has to be considered a source of grape-endogenous proteins. For this reason, wines treated with this new fining agent have to be considered allergen-free, satisfying also the demand for more 'organic-friendly' wines. In addition, given its performances in different wine styles assessed in this study, this extract represents a good and viable alternative to the most common fining agents of both animal and plant origin. The presence in the wines of residual proteins from GSE has not been assessed and cannot be excluded. This could be probably performed by MS analysis or ELISA methods. This appears to be a minor problem, however, because grape seed proteins do not represent a health risk for wine consumers, and their presence in trace quantity can virtually occur in most of the wines. Despite GSE having been proved to be effective in three different wine styles, its use will need to be further characterised by investigating its effect in different cultivars and other wine styles.

Despite the low amount of protein used in the GSE fined wines (Table 2) when compared with other fining agents, GSE has shown desirable effects on wine turbidity, chromatic characteristics, oxidative stability, reduction in phenolic substances and sensory properties. These findings suggest that the GSE composition in general, and of its protein profile in particular, is quite efficient when compared with other agents trialled.

Alone or in combination with inorganic fining aids, GSE definitively represents a new material for wine fining, and its extraction from a by-product could create new opportunities and have a positive economic impact for the wine industry.

## References


Bate-Smith, E.C. (1975) Phytochemistry of proanthocyanidins. Phytochemistry **14**, 1107–1113.

Celotti, E., Franceschi, D. and Ferrarini, R. (2006) The analytical evaluation of wine oxidability. Australian & New Zealand Grapegrower & Winemaker **505**, 47–52.

Charpentier, C., Caillet, M.M. and Feuillat, M. (2006) Essais de collage de moûts blancs et de vins rouges avec un extrait protéique levurien: comparaison avec les colles traditionnelles. Revue des Oenologues et des Techniques Vitivinicoles et Oenologiques: Magazine Trimestriel d 'Information Professionnelle **33**, 47–50.

Cosme, F., Ricardo-da-Silva, J. and Laureano, O. (2007) Protein fining agents: characterization and red wine fining assays. Italian Journal of Food Science **19**, 39–56.

Cosme, F., Capão, I., Filipe-Ribeiro, L., Bennett, R. and Mendes-Faia, A. (2012) Evaluating potential alternatives to potassium caseinate for white wine fining: effects on physicochemical and sensory characteristics. Food Science and Technology **46**, 382–387.

Deckwart, M., Carstens, C., Webber-Witt, M., Schäfer, V., Eichhorn, L., Schröter, F., Fischer, M., Brockow, K., Christmann, M. and Paschke-Kratzin, A. (2014) Impact of wine manufacturing practice on the occurrence of fining agents with allergenic potential. Food Additives & Contaminants: Part A **31**, 1805–1817.

De Rosso, M., Panighel, A., Carraro, R., Padoan, E., Favaro, A., Dalla Vedova, A. and Flamini, R. (2010) Chemical characterization and enological potential of Raboso varieties by study of secondary grape metabolites. Journal of Agricultural and Food Chemistry **58**, 11364–11371.

European Commission (2007) Commission Directive 2007/68/EC of 27 November 2007 amending Annex IIIa to Directive 2001/13/EC of the European Parliament and of the Council as regards certain food ingredients. Official Journal of the European Union L **310**, 11–14.

European Commission (2012) Commission Implementing Regulation (EU) No 579/2012 of 29 June 2012 amending Regulation (EC) No. 607/2009 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. Official Journal of the European Union L **171**, 4–7.

Fazio, G., Gattuso, A., Cilluffo, V. and Arcoleo, G. (1983) Preparazione e caratterizzazione di materiali proteici estratti da farine di vinaccioli. Rivista della Societa Italiana di Scienza dell'Alimentazione **6**, 496–478.

Fernandes, J.P., Neto, R., Centeno, F., De Fátima Teixeira, M. and Gomes, A.C. (2015) Unveiling the potential of novel yeast protein extracts in white wines clarification and stabilization. Frontiers in Chemistry **3**, 20.

Fleminger, G., Ragones, H., Merin, U., Silanikove, N. and Leitner, G. (2013) Low molecular mass peptides generated by hydrolysis of casein impair rennet coagulation of milk. International Dairy Journal **30**, 74–78.

Gambuti, A., Rinaldi, A. and Moio, L. (2012) Use of patatin, a protein extracted from potato, as alternative to animal proteins in fining of red wine. European Food Research and Technology **235**, 753–765.

Gambuti, A., Rinaldi, A., Romano, R., Manzo, N. and Moio, L. (2016) Performance of a protein extracted from potatoes for fining of white musts. Food Chemistry **190**, 1–1192.

Gazzola, D., Vincenzi, S., Gastaldon, L., Tolin, S., Pasini, G. and Curioni, A. (2014) The proteins of the grape (*Vitis vinifera* L.) seed endosperm: fractionation and identification of the major components. Food Chemistry **155**, 132–139.

González-Neves, G., Favre, G. and Gil, G. (2014) Effect of fining on the colour and pigment composition of young red wines. Food Chemistry **157**, 385–392.

Granato, T.M., Nasi, A., Ferranti, P., Iametti, S. and Bonomi, F. (2014) Fining white wine with plant proteins: effects of fining on proanthocyanidins and aroma components. European Food Research and Technology **238**, 265–274.

Igartuburu, J.M., Del Río, R.M., Massanet, G.M., Montiel, J.A., Pando, E. and Luis, F.R. (1991) Study of agricultural by-products. Extractability and amino acid composition of grapeseed (*Vitis vinifera*) proteins. Journal of the Science of Food and Agriculture **54**, 489–493.

International Organization for Standardization (2011) ISO 3972. Sensory analysis–methodology – method of investigating sensitivity of taste (International Organization for Standardization: Geneva, Switzerland) https://www.iso.org/obp/ui/#iso:std:iso:3972:ed-3:v1:en.

Iturmendi, N., Durán, D. and Marín-Arroyo, M. (2010) Fining of red wines with gluten or yeast extract protein. International Journal of Food Science & Technology **45**, 200–207.

Lee, S.K., Ye, Y.M., Yoon, S.H., Lee, B.O., Kim, S.H. and Park, H. S. (2006) Evaluation of the sensitization rates and identification of IgE-binding components in wild and genetically modified potatoes in patients with allergic disorders. Clinical and Molecular Allergy **4**, 1–10.

Lochbühler, B., Manteau, S., Morge, C., Caillet, M., Charpentier, C., Schnell, S., Grossmann, M. and Rauhut, D. (2015) Yeast protein extracts: an alternative fining agent for red wines. European Food Research and Technology **240**, 689–699.

Lomolino, G., Vincenzi, S., Gazzola, D., Crapisi, A. and Curioni, A. (2015) Foaming properties of potato (*Solanum tuberosum*) proteins: a study by the gas sparging method. Colloids and Surfaces A: Physicochemical and Engineering Aspects **475**, 75–83.

Marchal, R., Marchal-Delahaut, L., Lallement, A. and Jeandet, P. (2002a) Wheat gluten used as a clarifying agent of red wines. Journal of Agricultural and Food Chemistry **50**, 177–184.

Marchal, R., Marchal-Delahaut, L., Michels, F., Parmentier, M., Lallement, A. and Jeandet, P. (2002b) Use of wheat gluten as clarifying agent of musts and white wines. American Journal of Enology and Viticulture **53**, 308–314.

Mira, H., Leite, P., Ricardo-Da-Silva, J. and Curvelo-Garcia, A. (2006) Plant proteins in wine fining: influence on chemical and sensory characteristics. Bulletin de l'OIV **79**, 277–296.

Müller-Späth, H. (1992) Der POM-test. Der Deutsche Weinbau **23**, 1099–1100.


© 2017 Australian Society of Viticulture and Oenology Inc.

Organisation Internationale de la Vigne et du Vin (2004) OENO resolution 28/2004. CODEX - Matieres proteiques d'origine vegetale (Organisation Internationale de la Vigne et du Vin: Paris, France) Available online: http://www.oiv.int/public/medias/620/oeno-28-2004-fr.pdf

Organisation Internationale de la Vigne et du Vin (2011) OENO resolution 417/2011. Fining using yeast protein extracts (Organisation Internationale de la Vigne et du Vin: Paris, France) Available online: http://www.oiv.int/public/medias/3490/e-code-ii-3214.pdf.

Organisation Internationale de la Vigne et du Vin(2014) OENO resolution 520-2014. Code of good fining practices for wine to be applied in the use of proteinaceous wine fining agents with allergenic potential (casein and egg white) (Organisation Internationale de la Vigne et du Vin: Paris, France) Available online: http://www.oiv.int/public/medias/1645/oiv-oeno-secsan-520-2014-en.pdf

Ribéreau-Gayon, P. and Stonestreet, E. (1965) Le dosage des anthocyanes dens le vin rouge. Bulletin de la Société Chimique de France **9**, 2649–2652.

Rinaldi, A., Gambuti, A. and Moio, L. (2012) Precipitation of salivary proteins after the interaction with wine: the effect of ethanol, pH, fructose, and mannoproteins. Journal of Food Science **77**, 485–490.

Simonato, B., Mainente, F., Selvatico, E., Violoni, M. and Pasini, G. (2013) Assessment of the fining efficiency of zeins extracted from commercial corn gluten and sensory analysis of the treated wine. Food Science and Technology **54**, 549–556.

Singleton, V. and Rossi, J.A. (1965) Colorimetry of total phenolics with phosphomolybdic-phosphotungstic acid reagents. American Journal of Enology and Viticulture **16**, 144–158.

Tolin, S., Pasini, G., Simonato, B., Mainente, F. and Arrigoni, G. (2012) Analysis of commercial wines by LC–MS/MS reveals the presence of residual milk and egg white allergens. Food Control **28**, 321–326.

Tschiersch, C., Nikfardjam, M.P., Schmidt, O. and Schwack, W. (2010) Degree of hydrolysis of some vegetable proteins used as fining agents and its influence on polyphenol removal from red wine. European Food Research and Technology **231**, 65–74.

Verma, A.K., Kumar, S., Das, M. and Dwivedi, P.D. (2013) A comprehensive review of legume allergy. Clinical Reviews in Allergy & Immunology **45**, 30–46.

Vidal, S., Francis, I.L., Williams, P.J., Kwiatkowski, M., Gawel, R., Cheynier, V. and Waters, E.J. (2004) The mouth-feel properties of polysaccharides and anthocyanins in a wine like medium. Food Chemistry **85**, 519–525.

Vincenzi, S., Dinnella, C., Recchia, A., Monteleone, E., Gazzola, D., Pasini, G. and Curioni, A. (2013) Grape seed proteins: a new fining agent for astringency reduction in red wine. Australian Journal of Grape and Wine Research **19**, 153–160.

Vincenzi, S., Mosconi, S., Zoccatelli, G., Dalla Pellegrina, C., Veneri, G., Chignola, R., Peruffo, A., Curioni, A. and Rizzi, C. (2005) Development of a new procedure for protein recovery and quantification in wine. American Journal of Enology and Viticulture **56**, 182–187.

Vogel, A. and Svehla, G. (1979) Vogel's textbook of macro and semimicro qualitative inorganic analysis, 5th edn (Longman: London, England).

Yokotsuka, K. and Singleton, V.L. (1996) Grape seed nitrogenous components and possible contribution to wines. American Journal of Enology and Viticulture **47**, 268–278.

*Manuscript received:* 10 March 2016
*Revised manuscript received:* 04 October 2016
*Accepted:* 17 October 2016

## Supporting information

Additional Supporting Information may be found in the online version of this article at the publisher's web-site: http://onlinelibrary.wiley.com/doi/10.1111/ajgw.12268/abstract

**Table S1.** Effect of seven fining agents at three levels of addition on the turbidity during settling of the chardonnay, raboso rosé and raboso red wines.

© 2017 Australian Society of Viticulture and Oenology Inc.