# Alternative fining of Sangiovese wine: effect on phenolic substances and sensory characteristics

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## Abstract

**Background and Aims:** In recent years, alternative fining practices have increasingly been adopted by the wine industry. The use of plant proteins, as alternatives to traditional fining agents of animal origin for clarification, meets the expectations of a safe product. The effect of alternative fining practices was studied in Sangiovese wine.

**Methods and Results:** Four wines of variable phenolic composition were fined with a protein of vegetable origin (patatin) and one of animal origin (gelatin) at 5 and10 g/hL, and the influence on phenolic substances (colour parameters, CIElab coordinates, polymeric pigments, anthocyanins, flavans reactive to vanillin, tannins reactive to bovine serum albumin and saliva) and sensory characteristics (astringency, bitterness, astringency subqualities, odour, and aroma) compared. Colour showed that the efficiency of fining depends on: dose for high tannin wine and protein type for wines with a low concentration of anthocyanins. Astringency decreased in high tannin wine and bitterness in low anthocyanin wine following fining. Fined wines resulted in improved astringency subqualities and aroma descriptors.

**Conclusions:** The efficacy of traditional and alternative fining practices is influenced by the composition of wine phenolic substances.

**Significance of the Study:** Due to the potential allergenicity of animal proteins, plant proteins represent a safe and healthy alternative to the traditional fining of Sangiovese wine.

***Keywords:*** *astringency, colour, fining, plant protein, Sangiovese wine*

## Introduction

The presence of different colloidal substances in wine can cause precipitates to form, making the wine unstable even in the bottle. In order to prevent this phenomenon, clarification is necessary. This involves adding a proteinaceous substance to the wine, which binds the unstable colloidal substances responsible for turbidity and/or instability, causing flocculation and settling, and allowing recovery of stable and clarified wine (Ribéreau-Gayon et al. 2006). The clarification process also results in a decrease in the phenolic substances responsible for astringency and bitterness (Maury et al. 2001) and can affect other sensory characteristics such as aroma (Moio et al. 2004). Among the organic agents available for clarification, animal proteins have been the most commonly used substances. Problems, however, related to the bovine spongiform encephalopathy epidemy raised concerns about the possible transmission to humans, through ingestion of products of animal origin. Therefore, alternative fining practices have increasingly been adopted by the wine industry, as producers and consumers alike have become more attentive and sensitive to health and food safety. The use of proteins of plant origin, as alternatives to traditional clarification agents derived from animal protein, reduces the likelihood of allergenicity in sensitive individuals (Peñas et al. 2015). It would also satisfy the needs of vegetarian and vegan wine consumers. A recent review describes the different plant proteins currently used in winemaking as fining agents (Marangon et al. 2019). As with animal-based fining agents, they can remove different phenolic substances depending on molecular mass (Maury et al. 2016), degree of hydrolysis (Tschiersch et al. 2010), amino acids concentration (Maury et al. 2003) and grape cultivar (Gambuti et al. 2016) thereby contributing to a reduction in the perception of astringency (Gambuti et al. 2012, Kang et al. 2018) and bitterness (Tschiersch et al. 2010) in red wine.

Contrasting results have been obtained in fining trials comparing the effects of animal and plant proteins on red wine colour. Some authors observed a reduction in the anthocyanin concentration of red wines ranging between 8 and 38% using different gelatins (Ricardo-da-Silva et al. 1991, Karamanidou et al. 2011, Gambuti et al. 2012), with different effects on wine colour intensity and hue depending on protein type and dosage. The loss of colour is correlated with gelatin protein size (Cosme et al. 2009, Karamanidou et al. 2011), that is, the higher the molecular mass of proteins, the greater the loss of colour. The same effect was observed in wines that were fined with plant proteins (Tschiersch et al. 2010). A concentration-dependent decrease in monomeric anthocyanins was observed after fining with gelatin, but not with patatin, probably due to a saturation effect (Gambuti et al. 2012). Some changes in wavelength and colour coordinates may also happen. In some cases, treatment with gelatin diminished the red colour (Iturmendi et al. 2010) and enhanced the yellow tone of wine (Gambuti et al. 2012), while plant proteins (derived from gluten) equally reduced the absorbance measurement at 420 and 520 nm (Iturmendi et al. 2010). The use of potato protein resulted in the formation of new pigments, and a shift towards a higher wavelength (620 nm) occurred in wine (Gambuti et al. 2012). Different results on colour parameters were also found in four red wines that were

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fined with gelatin and plant proteins, but the dose rate varied according to recommendations made by the manufacturers (Río Segade et al. 2020).

In this work, the fining capability of a plant protein derived from potato tubers (*Solanum tuberosum* L.) was compared with that of an animal-derived protein in Sangiovese red wine. The effect of alternative clarification with patatin, which has never been studied in Sangiovese wines, was determined by comparing the colour and phenolic substances of Sangiovese wine, before and after fining with patatin compared to the standard gelatin. The astringency subqualities have never before been evaluated in red wine treated with fining agents.

## Materials and methods

### *Wine samples*

Sangiovese wines were produced in four wineries located in the Chianti DOCG area (Toscana, Italy) during the 2016 vintage. Wines were produced from Sangiovese grapes from four different vineyards following the same vinification protocol: grapes were destemmed and crushed; the resulting must treated with potassium metabisulfite (40 mg/kg) and inoculated with 20 g/hL of yeast (F83 Laffort, Bordeaux, France); the fermentation/maceration lasted 12 days at 25°C, during which yeast assimilable nitrogen (YAN), in the form of diammonium phosphate (containing ≈0.12% of thiamine hydrochloride), was added with the inoculum and then again at the third and sixth day of fermentation to a total concentration of 30 g/hL. Wines were then pressed to obtain softly [high anthocyanin (HA); low anthocyanin (LA)] (at 20 kPa) and strongly [high tannin (HT); low tannin (LT)] (at 150 kPa) pressed fractions, which were transferred to 53 L carboys. After the addition of pectolytic enzymes (3 g/hL), wines were inoculated with lactic bacteria (LF16 Direct, Laffort) at 1 g/hL. Potassium metabisulfite (6 g/hL) was then added to the wines, which were conserved under $N_2$ in stainless steel tanks (15 L) before commencing the fining trials in February 2018.

### *Fining trials*

The four Sangiovese wines, HT (high tannin), LT (low tannin), HA (high anthocyanin), and LA (low anthocyanin), were used in fining trials with gelatin (Gelatine Extra n°1, Laffort) and patatin (Vegecoll, Laffort) at a dose rate of 5 and 10 g/hL. The wines varied significantly in the concentration of phenolic substances, which was also maintained after fining (Table S1). Two replicates (1 L each) were prepared for each treatment as follows: gelatin and patatin were rehydrated in distilled water (1:10 w/v) for 20 min under gentle stirring. The temperature of the water for rehydration was 35°C for gelatin and 25°C for patatin. The protein solutions were added to wine at the concentration of 5 and 10 g/hL at a temperature of 25°C; an equivalent volume of distilled water was added to the Control wines. Wines were then stored for 12 days at 14 ± 2°C; they were then filtered under vacuum with Whatman glass microfiber filters (64 g/m$^2$) (Merk, Milan, Italy) and analysed.

### *Chemical analyses of wines*

All spectrophotometric determinations were made with a Shimadzu (Kyoto, Japan) UV-1800 spectrophotometer. Wine colour was measured by optical density (OD420 nm + OD520 nm + OD620 nm) and expressed as colour intensity (CI), with hue analysed according to Glories (1984). The CIE L*a*b* parameters, that is, L* (lightness), a* (from green to red), b* (from blue to yellow), C* (Chroma or saturation) and h° (hue angle) were determined with the Panorama software (Shimadzu, Milan, Italy). Vanillin reactive flavans (VRF) were determined according to Di Stefano and Guidoni (1989); anthocyanins, long polymeric pigments (LPP), short polymeric pigments (SPP), and bovine serum albumin (BSA) reactive tannins (BSA-tannins) according to Harbertson et al. (2003) and the saliva precipitation index (SPI) as described in Rinaldi et al. (2014). Analyses were in duplicate for each experimental replicate.

### *Sensory analysis of the wines*

A panel of 13 judges (comprising five women between 35–50 years of age, and eight men between 25–44 years of age) from the Division of Sciences of Vine and Wine, Department of Agriculture, University of Naples Federico II, in Avellino, Italy, was assembled. Panellists had experience in odour and aroma evaluation and were trained in evaluation of astringency and mouthfeel sensations (Rinaldi and Moio 2018). Training sessions consisted of six phases: (i) a selection phase, during which solutions of sucrose (10.0 g/L for sweetness), tartaric acid (1.0 g/L for acidity), caffeine (1.0 g/L for bitterness) and tannic acid (2.0 g/L for astringency) were presented in water and white wine; (ii) a taste recognition phase, during which solutions of sucrose (5.0 g/L for sweetness), tartaric acid (0.8 g/L for acidity), caffeine (0.5 g/L for bitterness) and tannic acid (1.0 g/L for astringency) were presented in water and in white and red wine; (iii) a binary phase, in which mixed solutions were presented in white wine at lower concentration; (iv) a rating phase, in which scaling solutions of caffeine (0.1 to 0.8 g/L) and five oenological tannins (0.2 to 1.5 g/L) were presented in water and white wine; (v) a subqualities familiarisation phase, during which panellists familiarised themselves with terms from the mouthfeel wheel (Gawel et al. 2001) and selected the most appropriate descriptors to use in the check-all-that-apply (CATA) questionnaire and (vi) a training phase for evaluation of subqualities using CATA and touch standards, during which six commercial red wines spiked with five oenological tannins (from 0.2 to 0.5 g/L) were tested in association with touch standards as described in Rinaldi and Moio (2018).

Wines were formally assessed during four sensory sessions, each comprising two brackets of five wine samples, with a 30 min break between brackets. Wines were presented in balanced random order at room temperature (18 ± 2°C) in black tulip-shaped glasses coded with three-digit random numbers. The sensations of astringency and bitterness were rated as the maximum perceived intensity, and the astringency subqualities, silk, velvet, dry, adhesive, hard, soft, rich, green, were evaluated by the CATA questionnaire. The odour (fruity-OD, floral-OD, spicy-OD, balsamic-OD, herbaceous-OD) and aroma (fruity-AR, floral-AR, spicy-AR, balsamic-AR, herbaceous-AR) profiles of the wines were rated according to a 0–5 point scale. Control and treated Sangiovese wines were evaluated in duplicate.

### *Data analysis*

All statistical analyses [ANOVA, principal component analysis, two-way ANOVA, chi-square test, Pearson's correlation] of data were carried out with the XLSTAT software package (Addinsoft, Paris, France). Tukey's test was performed on analytical data, Duncan's test on sensory data (astringency, bitterness, odour, aroma intensity) and the chi-squared test

on the citation frequencies (Cf%) of astringency subqualities. The confidence level $P < 0.05$ was considered to be statistically significant.

## Results

Four Sangiovese wines comprising different profiles of phenolic substances, that is low tannin (LT), high tannin (HT), low anthocyanin (LA) and high anthocyanin (HA) (Table S1), were treated with gelatin (a traditional, commonly used animal-based protein) and patatin (a plant-based protein) at a dose rate of 5 and 10 g/hL (G5 and G10, P5 and P10, respectively). Chemical and sensory analyses were subsequently performed on Control and treated wines to determine the suitability of patatin as an alternative, plant-derived fining agent.

### *Effect of fining on the colour of Sangiovese wines*

Wine colour was evaluated through spectrophotometric analysis, and the parameters such as CI, hue, anthocyanins, SPP, LPP and CIElab coordinates in Control and treated Sangiovese wines are shown in Table 1.

The CI of HT wine decreased following addition of gelatin at each concentration (−6.1 and − 11.6% for G5 and G10, respectively), while that of HA, depended more on the dose of the fining agent, with G10 the most effective (decreasing CI by 10.4%). In LA and LT, the P5 treatment did not significantly differ from that of the Control, and P10 gave a smaller decrease in CI (−3% in both wines) than G5 and G10. The colour data were subjected to a two-way ANOVA to assess the influence of fining agent and dose rate (Table 2). The lower concentration (5 g/hL) had less impact on CI than the higher 10 g/hL dose rate in HT and HA wines, but no impact in LT and LA wines. In these wines, treatment with patatin did not influence CI, whereas gelatin did. In HA wines, there was no colour difference between the proteins, while in HT wines, gelatin had the greatest effect on CI. For hue, a small but significant difference was found among HT wines only, with a decrease of 2.6 and 1.3% observed for the G10 and P10 treatments, respectively. For this wine, hue was affected more at the higher dose rates of both proteins (Table 2). The concentration of anthocyanin of HT also decreased following addition of the fining agents at the higher dose rate (by 12.3% for G10 and 9.2% for P10), but no difference was detected among HA wines. Anthocyanin decreased (by between 5.4 and 10.3%) in all treated LA wines and in gelatin-treated LT wine (by 4.6 and 7.6%, respectively). The 5 g/hL dose of both proteins did not affect anthocyanin in HT and HA wines, and gelatin showed much a greater impact than patatin in LT wines (Table 2). The SPP concentration of HA increased after the P10 fining treatment (by 6%) and decreased by gelatin in treated LT. There was no significant difference for the other wines. The LPP form over time between anthocyanins and tannins and can be precipitated by proteins such as BSA to stabilise wine colour in the same way as SPP. After fining, no difference in LPP was detected for HT and LT wines. In HA wine, a 10.4, 17.7 and 9.6% decrease in LPP was obtained, respectively, with P5, P10 and G10, while G5 was not significantly different from that of the Control wine. In contrast, treatment of the LA wine with 5 g/hL patatin effectively preserved the LPP.

In order to measure the colour perception in terms of three-dimensional space, the CIE L*a*b* coordinates were measured to evaluate the influence of fining on colour perception (Table 1). The lightness (L*) of wine increased after

**Table 1.** Effect of the dose rate of the protein fining agents gelatin and patatin on the colour and phenolic substances of Sangiovese wines of high and low anthocyanin and tannin concentration.

| | High tannin (HT) | | | | | High anthocyanin (HA) | | | | | Low anthocyanin (LA) | | | | | Low tannin (LT) | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **Parameter** | **C** | **G5** | **G10** | **P5** | **P10** | **C** | **G5** | **G10** | **P5** | **P10** | **C** | **G5** | **G10** | **P5** | **P10** | **C** | **G5** | **G10** | **P5** | **P10** |
| CI† | 15.6 ± 0.3 d | 14.6 ± 0.1 b | 13.7 ± 0.0 a | 15.0 ± 0.0 c | 14.5 ± 0.1 b | 13.5 ± 0.1 d | 12.7 ± 0.1 c | 12.1 ± 0.0 a | 12.7 ± 0.1 c | 12.4 ± 0.0 b | 9.4 ± 0.1 d | 8.8 ± 0.1 b | 8.6 ± 0.0 a | 9.3 ± 0.1 d | 9.1 ± 0.0 c | 11.4 ± 0.1 d | 10.9 ± 0.1 b | 10.5 ± 0.2 a | 11.5 ± 0.1 d | 11.1 ± 0.1 c |
| Hue‡ | 0.77 ± 0.0 d | 0.76 ± 0.0 bc | 0.75 ± 0.0 a | 0.76 ± 0.0 c | 0.76 ± 0.0 ab | 0.68 ± 0.0 | 0.68 ± 0.0 | 0.68 ± 0.0 | 0.68 ± 0.0 | 0.68 ± 0.0 | 0.83 ± 0.1 | 0.82 ± 0.0 | 0.83 ± 0.1 | 0.83 ± 0.0 | 0.82 ± 0.4 | 0.67 ± 0.0 | 0.66 ± 0.1 | 0.67 ± 0.0 | 0.67 ± 0.0 | 0.67 ± 0.0 |
| Anthocyanins§ | 243 ± 0.7 b | 237 ± 0.5 b | 213 ± 0.3 a | 236 ± 0.1 b | 221 ± 0.6 a | 300 ± 0.1 ab | 313 ± 0.7 b | 306 ± 0.3 b | 290 ± 0.6 a | 289 ± 0.7 a | 185 ± 0.43 b | 171 ± 0.4 a | 165 ± 0.3 a | 174 ± 0.5 a | 174 ± 0.6 a | 241 ± 0.12 cd | 230 ± 0.6 ab | 223 ± 0.7 a | 242 ± 0.2 d | 235 ± 0.6 bc |
| SPP¶ | 1.2 ± 0.2 | 1.1 ± 0.1 | 1.1 ± 0.1 | 1.1 ± 0.1 | 1.1 ± 0.1 | 2.0 ± 0.1 a | 2.0 ± 0.0 a | 2.0 ± 0.0 a | 2.1 ± 0.1 ab | 2.1 ± 0.0 b | 1.9 ± 0.1 | 1.9 ± 0.0 | 1.8 ± 0.1 | 1.9 ± 0.1 | 2.0 ± 0.2 | 2.8 ± 0.1 b | 2.6 ± 0.1 a | 2.5 ± 0.0 a | 2.8 ± 0.1 b | 2.7 ± 0.1 b |
| LPP¶ | 4.1 ± 0.3 | 3.9 ± 0.2 | 3.8 ± 0.1 | 4.0 ± 0.1 | 4.0 ± 0.1 | 2.5 ± 0.1 c | 2.4 ± 0.1 bc | 2.2 ± 0.0 ab | 2.2 ± 0.1 ab | 2.0 ± 0.1 a | 1.5 ± 0.1 b | 1.3 ± 0.1 a | 1.2 ± 0.0 a | 1.4 ± 0.0 ab | 1.3 ± 0.1 a | 1.6 ± 0.1 | 1.3 ± 0.2 | 1.3 ± 0.2 | 1.5 ± 0.1 | 1.4 ± 0.1 |
| **L***†† | 65.6 ± 0.5 a | 67.2 ± 0.2 c | 68.6 ± 0.3 d | 66.3 ± 0.3 b | 67.3 ± 0.2 c | 70.6 ± 0.1 a | 72.1 ± 0.1 b | 73.3 ± 0.1 d | 71.9 ± 0.1 b | 72.5 ± 0.1 c | 16.4 ± 0.2 a | 17.4 ± 0.1 c | 18.1 ± 0.2 d | 16.4 ± 0.1 a | 16.8 ± 0.0 b | 68.4 ± 0.7 ab | 69.2 ± 0.2 bc | 69.6 ± 0.6 c | 67.8 ± 0.3 a | 68.8 ± 0.5 abc |
| **a***†† | 38.2 ± 0.3 d | 37.2 ± 0.1 b | 36.3 ± 0.1 a | 37.8 ± 0.1 c | 37.2 ± 0.1 b | 39.4 ± 0.1 d | 38 ± 0.2 c | 36.9 ± 0.0 a | 38.2 ± 0.1 c | 37.4 ± 0.1 b | 36.0 ± 0.1 a | 37.6 ± 0.2 c | 38.4 ± 0.1 d | 36.3 ± 0.0 a | 36.8 ± 0.2 b | 29.4 ± 0.1 b | 28.8 ± 0.5 ab | 28.1 ± 0.5 a | 29.5 ± 0.1 b | 29.0 ± 0.2 ab |
| **b***†† | 18.8 ± 0.1 d | 18.1 ± 0.1 b | 17.3 ± 0.0 a | 18.4 ± 0.1 c | 17.9 ± 0.0 b | 19.3 ± 0.1 d | 18.6 ± 0.1 c | 18.1 ± 0.1 a | 18.7 ± 0.1 c | 18.3 ± 0.1 b | 26.0 ± 0.2 a | 28.6 ± 0.4 c | 30.0 ± 0.2 d | 26.4 ± 0.1 a | 27.3 ± 0.3 b | 14.0 ± 0.7 b | 13.8 ± 0.4 ab | 12.9 ± 0.2 a | 14.2 ± 0.2 b | 13.5 ± 0.2 ab |
| **C***†† | 42.6 ± 0.2 d | 41.3 ± 0.1 b | 40.3 ± 0.1 a | 42.0 ± 0.1 c | 41.3 ± 0.1 b | 43.9 ± 0.2 d | 42.3 ± 0.2 c | 41.1 ± 0.1 a | 42.6 ± 0.1 c | 41.7 ± 0.1 b | 44.4 ± 0.2 a | 47.2 ± 0.3 c | 48.7 ± 0.2 d | 44.9 ± 0.1 a | 45.8 ± 0.3 b | 34.1 ± 1.3 b | 33.4 ± 0.6 ab | 32.2 ± 0.5 a | 34.2 ± 0.2 b | 33.3 ± 0.1 ab |
| **h°**†† | 26.1 ± 0.1 c | 25.9 ± 0.2 b | 25.5 ± 0.1 a | 26.0 ± 0.2 bc | 25.7 ± 0.0 b | 26.1 ± 0.1 | 26.1 ± 0.0 | 26.1 ± 0.1 | 26.1 ± 0.1 | 26.1 ± 0.0 | 35.8 ± 0.1 a | 37.3 ± 0.2 c | 38.1 ± 0.1 d | 36.1 ± 0.1 a | 36.5 ± 0.2 b | 31.3 ± 0.3 c | 31.2 ± 0.4 bc | 30.4 ± 0.3 a | 31.2 ± 0.3 bc | 30.5 ± 0.5 ab |
| **ΔE**‡‡ | | 2.0 ± 0.2 b | 3.9 ± 0.1 c | 0.9 ± 0.2 a | 2.2 ± 0.2 b | | 2.2 ± 0.2 c | 3.9 ± 0.1 d | 1.8 ± 0.2 a | 2.9 ± 0.1 b | | 3.9 ± 0.5 c | 6.1 ± 0.3 d | 0.5 ± 0.1 a | 1.9 ± 0.4 b | | 1.1 ± 0.5 a | 2.3 ± 0.1 b | 0.6 ± 0.3 a | 1.0 ± 0.2 a |

Values are expressed as the means ± SD over four replications. Values with different letters in a row, within each Sangiovese wine treatment, are statistically different according to Tukey's test ($P < 0.05$). †CI, colour intensity, the sum of 420, 520, 620 nm; ‡hue is the 420 nm/520 nm ratio; §anthocyanin is expressed as mg/L of malvidin-3-glucoside equivalent; ¶SPP, LPP, short and long pigmented polymers expressed as 520 nm; ††CIE L*a*b* coordinates: L* (lightness), a* (from green to red), b* (from blue to yellow), C (chroma or saturation) and h° (hue angle); ‡‡$\Delta E = [(\Delta L^*)^2 + (\Delta a^*)^2 + (\Delta b^*)^2]^{1/2}$ in CIELAB units. *C*, Control; G5, gelatin at 5 g/hL; G10, gelatin at 10 g/hL; P5, patatin at 5 g/hL; P10, patatin at 10 g/hL.

**Table 2.** Effect of fining agent and dose of fining agent on colour parameters of Sangiovese wines of high and low anthocyanin and tannin concentration.

| | CI | Hue | Anthocyanin (mg/L) | SPP | LPP | L* | a* | b* | C | h° |
|---|---|---|---|---|---|---|---|---|---|---|
| **High tannin—fining agent** | | | | | | | | | | |
| *P*-value | < 0.0001 | 0.001 | n.s. | n.s. | 0.040 | < 0.0001 | < 0.0001 | 0.000 | < 0.0001 | 0.002 |
| Control | c | b | - | - | a | a | c | c | c | b |
| Gelatin | a | a | - | - | a | c | a | a | a | a |
| Patatin | b | a | - | - | a | b | b | b | b | a |
| **High tannin—dose (g/hL)** | | | | | | | | | | |
| *P*-value | < 0.0001 | < 0.0001 | < 0.0001 | n.s. | n.s. | < 0.0001 | < 0.0001 | < 0.0001 | < 0.0001 | < 0.0001 |
| 0 | c | c | b | - | - | a | c | c | c | c |
| 5 | b | b | b | - | - | b | b | b | b | b |
| 10 | a | a | a | - | - | c | a | a | a | a |
| FINING AGENT*DOSE | | | | | | | | | | |
| *P*-value | 0.019 | 0.019 | 0.019 | n.s. | n.s. | n.s. | n.s. | n.s. | n.s. | n.s. |
| **High anthocyanin—fining agent** | | | | | | | | | | |
| *P*-value | < 0.0001 | n.s. | < 0.0001 | 0.000 | 0.001 | < 0.0001 | < 0.0001 | < 0.0001 | < 0.0001 | n.s. |
| Control | b | - | b | a | b | a | b | b | b | - |
| Gelatin | a | - | c | a | b | b | a | a | a | - |
| Patatin | a | - | a | b | a | b | a | a | a | - |
| **High anthocyanin—dose (g/hL)** | | | | | | | | | | |
| *P*-value | < 0.0001 | n.s. | n.s. | n.s. | 0.001 | < 0.0001 | < 0.0001 | < 0.0001 | < 0.0001 | n.s. |
| 0 | c | - | - | - | b | a | c | c | c | - |
| 5 | b | - | - | - | ab | b | b | b | b | - |
| 10 | a | - | - | - | a | c | a | a | a | - |
| FINING AGENT*DOSE | | | | | | | | | | |
| *P*-value | n.s. | n.s. | n.s. | n.s. | n.s. | < 0.0001 | 0.041 | n.s. | n.s. | n.s. |
| **Low anthocyanin—fining agent** | | | | | | | | | | |
| *P*-value | < 0.0001 | n.s. | 0.000 | n.s. | 0.000 | < 0.0001 | < 0.0001 | < 0.0001 | < 0.0001 | < 0.0001 |
| Control | b | - | b | - | c | a | a | a | a | a |
| Gelatin | a | - | a | - | a | b | b | b | b | b |
| Patatin | b | - | a | - | b | a | a | a | a | a |
| **Low anthocyanin—dose (g/hL)** | | | | | | | | | | |
| *P*-value | 0.012 | n.s. | 0.001 | n.s. | 0.002 | 0.037 | 0.007 | 0.010 | 0.008 | 0.013 |
| 0 | b | - | b | - | b | a | a | a | a | a |
| 5 | ab | - | a | - | a | ab | ab | ab | ab | ab |
| 10 | a | - | a | - | a | b | b | b | b | b |
| FINING AGENT*DOSE | | | | | | | | | | |
| *P*-value | n.s. | n.s. | n.s. | n.s. | n.s. | 0.021 | n.s. | 0.044 | n.s. | 0.007 |
| **Low tannin—fining agent** | | | | | | | | | | |
| *P*-value | < 0.0001 | n.s. | 0.000 | < 0.0001 | 0.012 | 0.003 | 0.025 | n.s. | 0.027 | n.s. |
| Control | b | - | b | b | b | a | b | - | b | - |
| Gelatin | a | - | a | a | a | b | a | - | a | - |
| Patatin | b | - | b | b | ab | a | ab | - | ab | - |
| **Low tannin—dose (g/hL)** | | | | | | | | | | |
| *P*-value | 0.014 | n.s. | 0.033 | n.s. | 0.039 | n.s. | n.s. | 0.002 | 0.022 | 0.000 |
| 0 | b | - | b | - | b | - | - | b | b | b |
| 5 | ab | - | ab | - | a | - | - | b | ab | b |
| 10 | a | - | a | - | a | - | - | a | a | a |

The two-way ANOVA results according to the Tukey's HSD test, considering the effect of fining agent dose, and interaction (FINING AGENT*DOSE) on colour parameters shown in Table 1; n.s., not significant; different letters indicate a statistical difference according to Tukey's test ($P < 0.05$). CI, colourant intensity, the sum of 420, 520, 620 nm; hue is the 420 nm/520 nm ratio; anthocyanin is expressed as mg/L of malvidin-3-glucoside equivalent; SPP, LPP, short and long pigmented polymers expressed as 520 nm; CIE L*a*b* coordinates, L* (lightness), a* (from green to red), b* (from blue to yellow), C (chroma or saturation) and h° (hue angle).

fining relative to the Control wine, especially for the gelatin-treated HT and LA wines, G10- and P10-treated HA wines, and G10-treated LT wine. Fined Sangiovese wines resulted in an increased lightness (Table 2). The a* value (from green to red) decreased with gelatin treatment of HT, when the higher dose of protein was applied to HA (although G10 > P10), and of G10-treated LT. This parameter, however, increased in all treatments of LA, except for P5. Again, the dose rate had a significant effect on HT and HA wines, whereas only gelatin significantly affected LA and LT wines (Table 2). The other parameters b* and C, representing a shift from yellow to blue and chroma (vividness), followed the same trends as a*. Changes in the visual colour aspect depended again on the

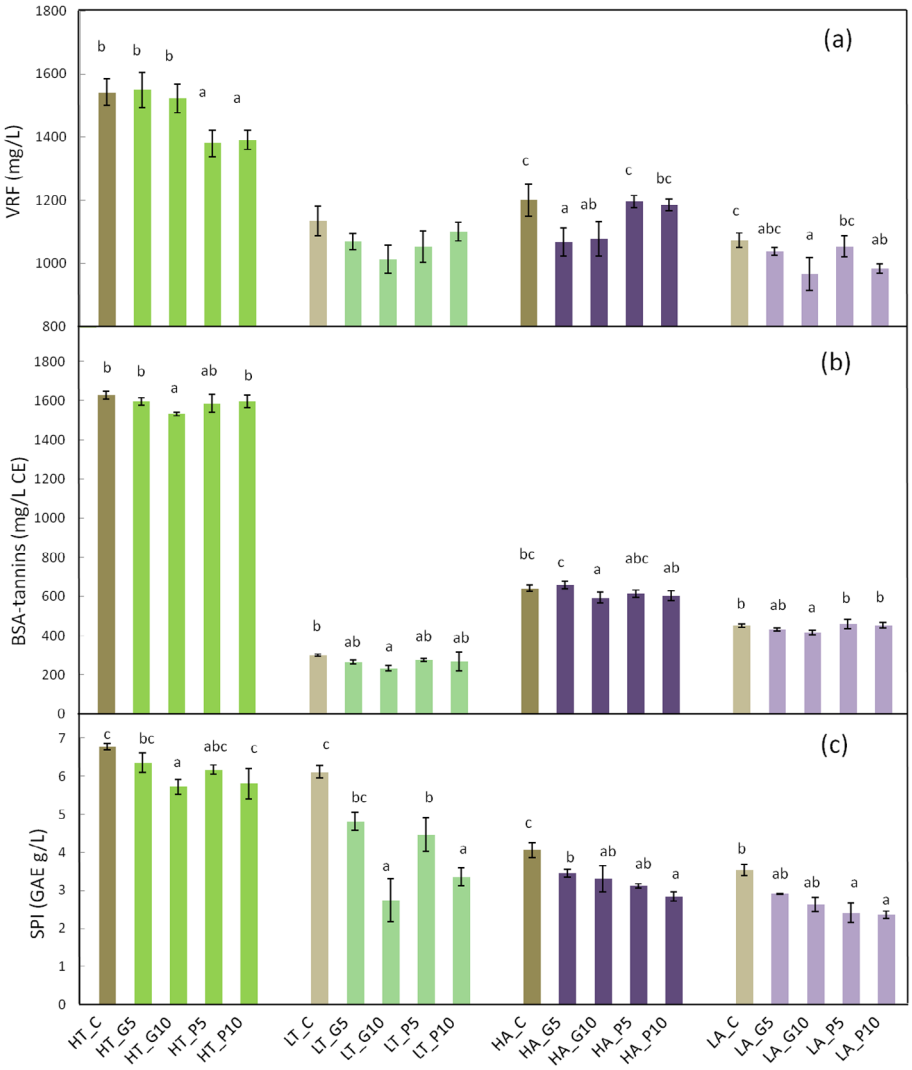


**Figure 1.** Effect of the protein fining agents gelatin and patatin on the reactive phenolic substances of Sangiovese wines: (a) vanillin reactive flavans (VRF) (mg/L); (b) bovine serum albumin (BSA)-tannin (mg/L catechin equivalent [CE]; (c) saliva precipitation index (SPI) (g/L gallic acid equivalent [GAE]). HT (high tannin), HA (high anthocyanin), LA (low anthocyanin), LT (low tannin) fined with gelatin (G) and patatin (P) at 5 and 10 g/hL (G5, G10, and P5, P10, respectively). C represents the Control. Error bars represent SD over four replications.

phenolic composition of wines. In HT wines, a decrease in redness, blueness and vividness followed the order: G10 > G5 = P10 > P5 and ranged between 2.7 and 5% for a*, 3.8 and 7.5% for b* and 2.9 and 5.4% for C. In HA wines, the order was: G10 > P10 > G5 = P5, that is the higher fining agent dose rate gave a larger colour difference relative to the Control, that is, on average, decreases between 3 and 6%. The HT wines treated with patatin were characterised by enhanced red and blue colour, together with the LA wines treated with the lower dose of the fining proteins.

In the LT wine, treatment with only 10 g/hL gelatin (G10) significantly decreased redness (−4.5%), blueness (−9.1%) and chroma (−5.4%). A different trend was observed in LA wines; instead, the colour parameters were increased in the following the order: G10 > G5 > P10. Gelatin was the most effective at increasing the redness (by 6.5%), blueness (by 1.5%) and vividness (by 9.6%), in wine with colour deficiencies (low CI, high hue). This increasing trend, however, was also observed for the hue angle (h°), indicating that the yellow tone was augmented with gelatin by between 1.7 and 6%, but no difference was detected between the Control and the patatin-treated wines (Table 2). A decrease in hue angle was observed with G10, in both HT and LT (by 2.4 and 2.9%, respectively), but depended more on the dose rate than on the protein used for fining. The ΔE represents the colour variation between Control and treated wines, and values greater than two CIELab units indicate that wines show a difference detectable to the human eye (Mokrzycki and Tatol 2011). In HT wine, G5, P10 and G10 treatments gave ΔE > 2, the highest value (3.8) corresponding to G10; these wines therefore had differences in colour perceivable by the simple observer. In HA wine, P5 did not differ from the Control. In other wines, increasing the dose rate of fining agents yielded colour differences, with gelatin having the most apparent effect. In LA, colour difference was perceived only after gelatin treatment at 5 or 10 g/hL (being 3.9 and 6.1, respectively). In LT, only G10 gave a significant colour difference that would be detectable by the expert human eye (ΔE = 2.3). Even at higher concentration, the plant-derived protein had less impact on colour than the animal protein, with the magnitude of the effect depending on the composition of the phenolic substances of the wine. These results are in accordance

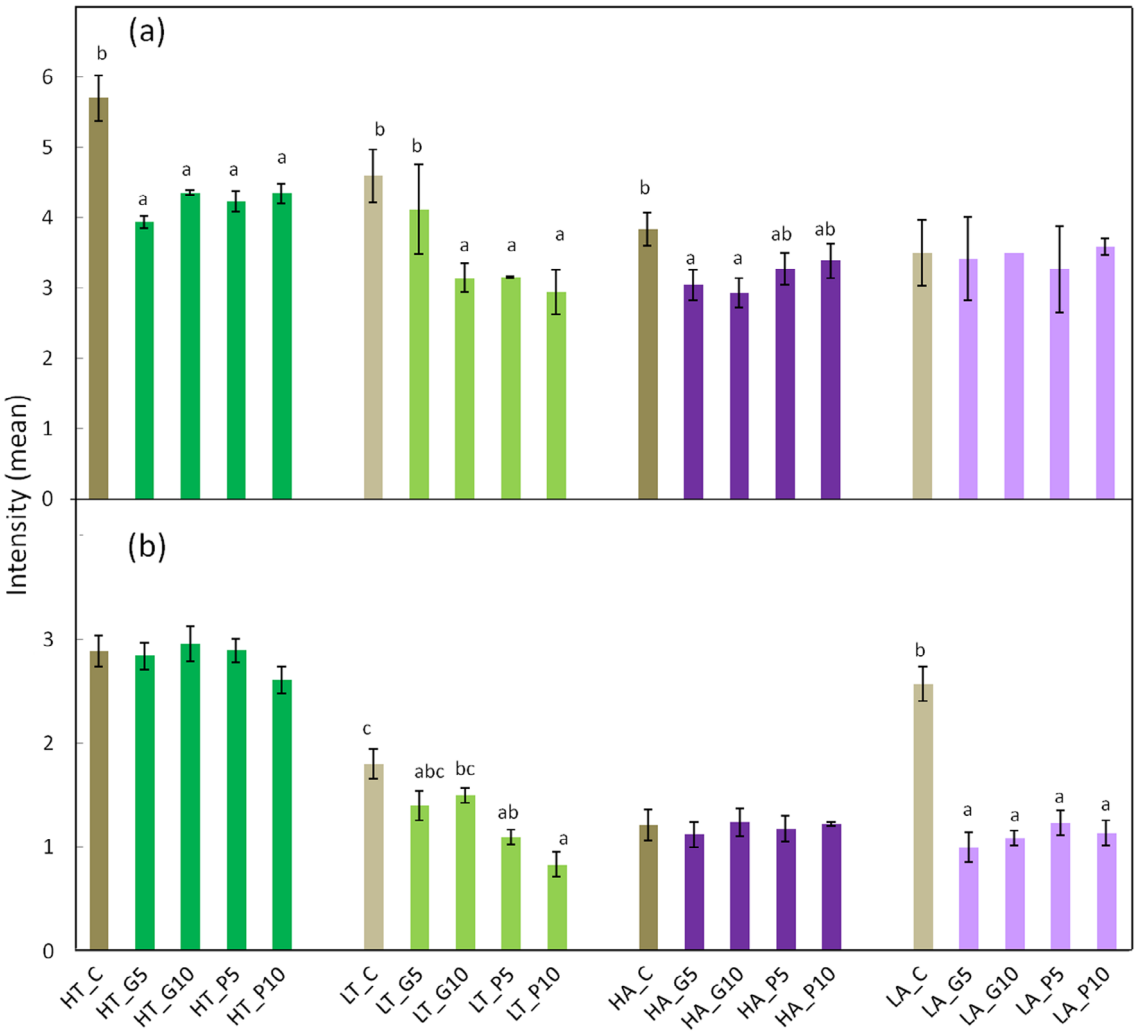


**Figure 2.** (a) The mean intensity of astringency and (b) of bitterness of high tannin (HT), low tannin (LT), high anthocyanin (HA), and low anthocyanin (LA) Sangiovese wines, before (C) and after fining with gelatin at 5 (G5) and 10 (G10) g/hL, and patatin at 5 (P5) and 10 (P10) g/hL. Error bars represent SD over two replications.

with similar studies involving fining of wine with animal and plant proteins (Kang et al. 2018, Rìo Sagade et al. 2020).

### *Effect of fining on reactive phenolic substances of Sangiovese wines*

Analytical parameters related to the compounds responsible for astringency and bitterness are essential to evaluate the effect of fining on Sangiovese wine, especially when there is no possibility of tasting the wines. Reactive phenolic substances include: (i) VRF comprising the low molecular mass proanthocyanidins, which are dimers, trimers and tetramers of flavan-3-ols, the compounds mainly responsible for the bitterness in wine (Peleg et al. 1999); (ii) BSA-tannins comprising the high molecular mass proanthocyanidins capable of precipitating BSA (Harbertson et al. 2003) and (iii) tannins that precipitate salivary proteins (SPI) and typically comprise the most astringent tannins (Rinaldi et al. 2012). The concentration of VRF, BSA-tannins and SPI of Sangiovese wines are shown in Figure 1, respectively.

After the clarification treatments, the VRF was significantly reduced in HT wines treated with patatin, and in HA wines treated with gelatin at each dose. In the LA wine, treatment with the fining agents at the higher dose decreased the concentration of VRF, while there was no difference in LT wines (Figure 1a). A significant decrease of the BSA-tannins was obtained with the gelatin treatment at 10 g/hL for all wines (Figure 1b). Gelatin, a high molecular mass protein (MM > 200 kDa), appears to show more affinity towards the polymerised tannins than does the low molecular mass protein of patatin (MM of about 40 kDa) (Gambuti et al. 2012). Not all condensed tannins, however, were considered in this work. Harbertson et al. (2014) showed that BSA precipitated up to 93% of octamers.

The SPI, simulating in vitro the interaction between saliva and wine, represents an indirect evaluation of astringency based on the precipitation of salivary proteins by tannins (Rinaldi et al. 2012). Following the clarification treatments, there was always a reduction in the SPI value, and indirectly in the astringency. This reduction was more significant for the treatments at 10 g/hL in HT, LT and LA wines, while patatin at each concentration gave the most significant reduction in SPI value in HA wines (Figure 1c).

### *Effect of fining on the sensory characteristics of Sangiovese wines*

The overall sensory evaluation of the Sangiovese wines after fining with gelatin and patatin included the intensity of

astringency and bitterness, the astringency subqualities and the aroma and odour profiles.

Gelatin and patatin impacted the mean intensity of astringency (Figure 2a) and bitterness (Figure 2b) of Control (C) and treated wines. The astringent sensation was reduced in HT wines after fining with gelatin and patatin at 5 and 10 g/hL ($P = 0.009$). Patatin at 5 and 10 g/hL (P5 and P10) and gelatin at 10 g/hL (G10) reduced the astringency in LT wines compared to that of the Control ($P = 0.023$). In HA wines, gelatin achieved a greater reduction in the astringency than patatin, although with a significance >0.05 ($P = 0.056$). While the astringency of LA wines was not affected by fining, the bitterness was. The LA-C wine was highly bitter, all treatments reduced this sensation significantly ranging between 52 and 61% ($P = 0.027$). A similar effect was observed in LT wines, with patatin at 5 and 10 g/hL the most effective in reducing the bitterness of Sangiovese wine [−39% (P5) and − 54% (P10) ($P = 0.046$)]. Fining affected the bitterness in LT wines but not in HT wines.

A correlation matrix was constructed between astringency and bitterness with the measured compounds (LPP, SPP, BSA-tannins, VRF, SPI) of Sangiovese wines grouped in high (HA, HT) and low (LA, LT) concentration of phenolic substances, in order to find relationships between the chemical and sensory data. Pearson's correlations ($P < 0.05$) revealed that in wines of high concentration of phenolic substances, astringency positively correlated with LPP (0.816), BSA-tannins (0.796), VRF (0.848), SPI (0.829) and negatively with SPP (−0.733); similar trends were observed for bitterness [LPP (0.977), BSA-tannins (0.990), VRF (0.932), SPI (0.959), and negatively with SPP (−0.983)] (Figure S1 and S2). For wines of low concentration of phenolic substances, only the SPI was positively correlated with astringency (0.723). The higher the concentration of phenolic substances, the higher the astringency and bitterness. In contrast, when the concentration of anthocyanin and tannin is low, other compounds may participate in eliciting astringency and bitterness, as previously observed (Rinaldi et al. 2020a). Polymeric compounds, however, can also provide qualitative sensory characteristics: LPP may confer positive tactile sensations of velvety and suppleness in the mouth; SPP may confer a flavour richness; BSA-tannins have been correlated with subqualities that classically define astringency, such as dry, pucker, adhesive, aggressive (Rinaldi and Moio 2018).

The qualitative traits of astringency after fining were also investigated. Eight definitions of astringency were the most significant in describing the differences between Sangiovese wines grouped for the high (HT and HA), and low (LT and LA) concentration of phenolic substances (Figure 3). An increasing proportion (citation frequency, Cf%) of the positive astringency subqualities, such as rich, soft and velvet, was achieved after the fining of wines of high concentration of phenolic substances (Figure 3a). In contrast, negative subqualities, such as dry, adhesive, green and hard, decreased. In particular, according to the chi-square test ($P < 0.05$), the treatment P5 reduced the astringency associated with bitterness (hard) significantly, G5 increased the soft astringency, the G10 reduced the dryness and increased the silk sensation. Treatment P10 increased the velvety and soft sensation the most while decreasing the dry and acidic astringency (green). The Control wines with a low concentration of phenolic substances were characterised by about 70% of negative subqualities (Figure 3b), which was less than that of the Sangiovese wine (85%) with a high concentration of phenolic substances. The

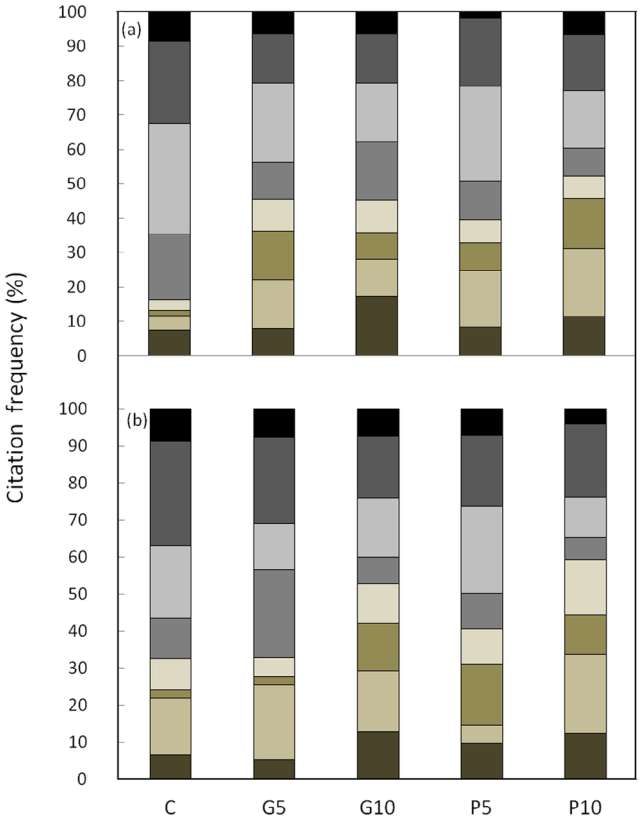


**Figure 3.** Citation frequency of the astringency subqualities, hard (■), adhesive (■), dry (□), green (■), rich (□), soft (■), velvet (□) and silk (■), *expressed as a proportion of the total citation frequency of the (a) high (b) low concentration of phenolic substances Sangiovese wines. C, Control wine; G5, G10, P5, and P10 represent the treatment at 5 and 10 g/hL of gelatin and patatin, respectively.*

treatment with gelatin at 5 g/hL appears unsuitable for this type of wine, because of an increase in the perception of acidity associated with astringency (green) and a reduction in the rich and silk terms. Greenness was finally reduced, however, by increasing the concentration of gelatin to 10 g/hL, and the soft sensation was improved. Patatin at 5 g/hL increased the positive subqualities (about +10% on the total), though, among these, the velvety sensation decreased while the soft sensation increased. The velvet and the rich (full aroma) sensations characterised the Sangiovese wine fined with10 g/hL patatin, with the dryness and greenness significantly reduced.

We also evaluated the effect of the fining on the odour and aroma profile of the Sangiovese wines (Figure 4). The principal component analysis obtained by the sensory data of the wines of high concentration of phenolic substances (Figure 4a) revealed that patatin and gelatin had the same efficacy in reducing the herbaceous notes characterising the Control wines and revealing the floral and fruity notes. The Control wines with low concentration of phenolic substances were still characterised by an herbaceous odour (Figure 4b). Only fining with patatin, however, enhanced the floral aroma and odour, typical of Sangiovese wine (Rinaldi et al. 2020b), in addition to the increase in the astringency subquality concerning aroma richness (rich).

## Discussion

Sangiovese wine is susceptible to colour instability and can display high bitterness and astringency (Gambuti et al. 2018, Rinaldi et al. 2020b); as a result, fining is necessary to

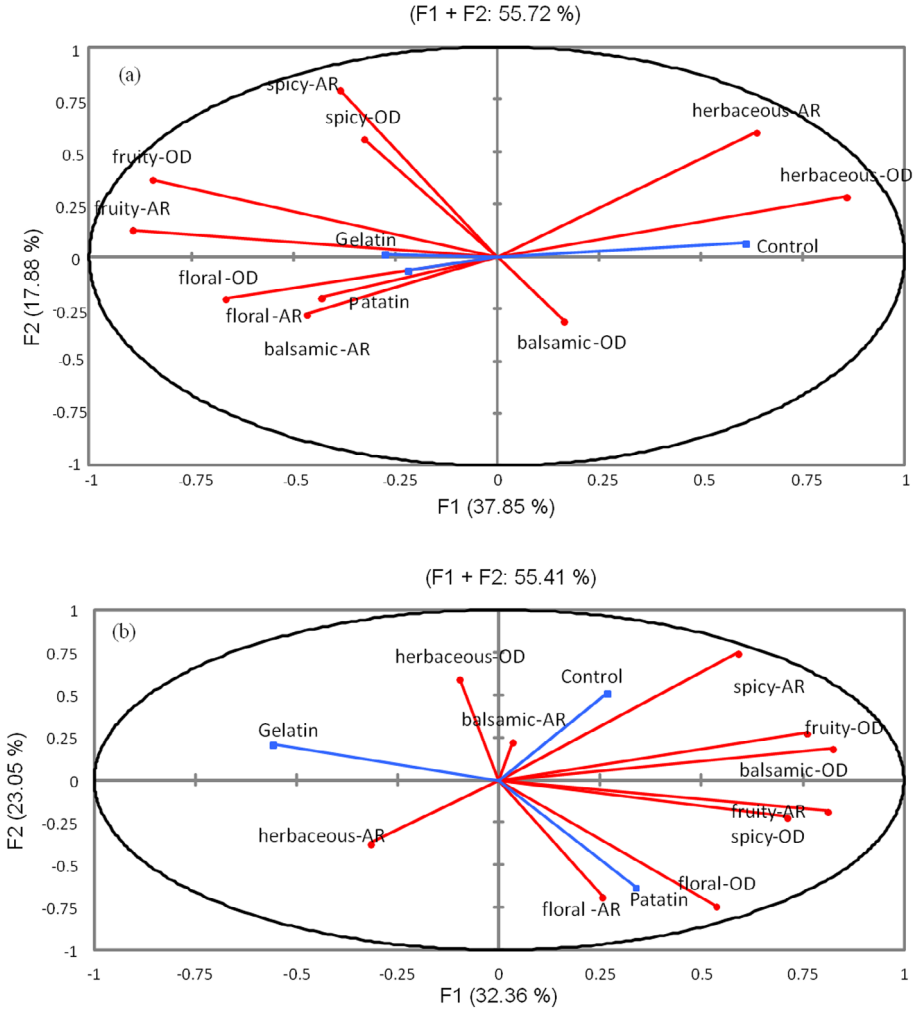


**Figure 4.** Principal component analysis of sensory descriptors related to aroma (AR) (fruity, floral, spicy, balsamic, herbaceous) and odour (OD) (fruity, floral, spicy, balsamic, herbaceous) of (a) high and (b) low concentration of phenolic substances Sangiovese wines grouped for fining treatment: Control, no fining; gelatin, fining with gelatin at 5 and 10 g/hL; Patatin, fining with patatin at 5 and 10 g/hL.

reduce the flavans and tannins and to assure colour stability during bottle ageing. Nowadays, in order to adapt to current strategies to reduce the allergenic risks in beverages such as wine, the use of plant-derived proteins is widely spread but has never been tested on Sangiovese wine.

The colour represents a critical parameter for quality assessment in red wine. The fining with plant and animal proteins led to a decrease in colour intensity in Sangiovese wines (Table 1), mainly when the protein agents were applied at high dose (10 g/hL). Patatin, however, had less impact than gelatin, which conforms to other studies that have shown that the vegetable proteins have less impact on colour compared to other fining agents (Granato et al. 2014, Ghanem et al. 2017). Anthocyanins are responsible for the colour of young red wine and are displaced progressively and irreversibly during ageing by more stable polymeric pigments (Ribéreau-Gayon 1982) combining with different co-factors (Brouillard et al. 2003). The SPP and LPP are polymeric pigments formed between anthocyanins and proanthocyanidins, which protect the chromophore of the anthocyanin from the action of $SO_2$ (Boulton 2001, Vidal et al. 2004) and contribute to the colour stability of the wine during ageing. Patatin preserved the pigmented polymers in LT wines (Table 2); Kang et al. (2018) also reported that patatin had a lower affinity towards polymeric pigments than gelatin. In HA wines (>300 mg/L) a concomitant decrease of LPP and an increase in SPP were observed with patatin at 10 g/hL (Table 1). In a Sangiovese wine with a lower concentration of anthocyanin (240 mg/L) but a higher tannin concentration (HT), the pigmented polymers were not affected by the fining treatments (Table 2). The different tannin concentration may explain the contrasting effect of fining on colour stability of HT and HA wines. Exogenous proteins bound the polymeric tannins preferentially to the polymeric pigments when the wine is high in tannin. In the fining trials, however, astringent tannins (SPI) were also significantly reduced by gelatin and patatin (Figure 1c), thus contributing to a strong decrease of astringency perception in HT Sangiovese wines (Figure 2a). Gelatin and patatin may have a higher affinity for proanthocyanidins with structural characteristics that were not measured in the current study, but they are known to contribute to astringency, as well as to the precipitation of salivary proteins, that is the large polymer size and high galloylation (Sarni-Manchado et al. 1999, De Freitas and Mateus 2001, Rinaldi et al. 2015).

Similar results on astringency have been previously obtained on Aglianico, a tannin-rich wine, fined with a higher concentration of gelatin and patatin (Gambuti et al. 2012). By comparing the two proteins at the same

concentration, the patatin showed greater efficiency in reducing astringency than gelatin, as also recently observed by Kang et al. (2018), however, they did not find a significant difference in bitterness. Few studies evaluate the bitterness after fining, and the variable results may depend on the concentration of phenolic substances in the wines and the different fining trials. In our study, bitterness decreased in LT wines after treatment (Figure 2b). In particular, the bitterness of LA wines was exceptionally high in respect of the concentration of VRF. Other compounds, such as syringic acid, kaempferol glucoside, may be responsible for the bitter sensation in Sangiovese wine (Rinaldi et al. 2020a). The correlation between chemical and sensory data (Figure S1 and S2) appears to suggest that in wines of high concentration of phenolic substances there is a synergism among compounds in eliciting bitterness and astringency, while in wines of low concentration of phenolic substance the concentration may represent the limiting factor.

The present study represents the first evidence of the effect of fining with the commonly used gelatin and the alternative patatin on the overall sensory evaluation of Sangiovese wine, comprising astringency subqualities, bitterness, aroma and odour of red wine. Other studies, confined to some sensory characteristics, found that fining with plant and animal proteins did not change flavour intensity (Cosme et al. 2012, Kang et al. 2018). Fined wines, however, were more appreciated and had slightly better scores than the Control wines (Karamanidou et al. 2011). Fining, which removes undesirable compounds, enhanced the mouthfeel of wine, but in some cases, results differed depending on the fining agent and the dose (Figure 3). The astringency subqualities (Figure 3a) and aroma (Figure 4a) of wines of high concentration of phenolic substance were improved by treatment with plant and animal proteins in a similar manner. Herbaceous notes and dry, green, adhesive tannins strongly characterised Control wines. Treatment with patatin at 10 g/hL, in particular, increased the velvety and soft sensations in wine. In wines of low concentration of phenolic substance (Figure 4b), patatin better expressed the characteristic floral aroma of Sangiovese (Rinaldi et al. 2020b), while the high dose of both proteins assured positive subqualities, such as silk, soft and rich (Figure 3b).

## Conclusions

The present study demonstrates the potential for plant proteins to be used as alternatives to traditional animal-based fining agents during production of Sangiovese wine. The clarification efficiency on phenolic substances and sensory characteristics of treated wine depends on the initial phenolic composition of Sangiovese wines. In wines with a higher concentration of tannin, the clarifying efficacy depended on the dose of fining agent (10 vs 5 g/hL) rather than the type of protein used. In wines with a low anthocyanin concentration, the protein type was more important; the most effective patatin treatment maintained colour stability in wine with a low concentration of anthocyanins and pigmented polymers, and with a greater tendency for loss of colour over time. By correlating analytical and sensory data, reactive phenolic substances appear suitable to represent the astringency and bitterness of Sangiovese only when the concentration of phenolic substances is high; in wines of low concentration of phenolic substances, only the SPI was correlated with astringency. Fining had a positive impact on the sensory perception of Sangiovese wines. In particular, the astringency subqualities and aroma characteristics of wines of high concentration of phenolic substances were improved by treatment with the higher dose of fining agents and by treatment with patatin in wines of low concentration of phenolic substance. In general, treated wines were less herbaceous and more floral than their corresponding Control wines. After fining, positive descriptors, such as soft, velvet and rich, were more frequently used to describe wines. Patatin is therefore considered a viable alternative to gelatin for the clarification of Sangiovese wines.

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## Supporting information

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**Table S1.** The ANOVA (Tukey's test) analysis of the phenolic substances of high and low tannin and anthocyanin Sangiovese wines fined with gelatin and patatin.

**Figure S1.** Pearson's correlation between the intensity of astringency and (a) long polymeric pigment (LPP) ($R$ = 0.816), (b) short polymeric pigment (SPP) ($R$ = −0.733), (c) BSA-tannins ($R$ = 0.796), (d) vanillin reactive flavans (VRF) ($R$ = 0.848) and (e) saliva precipitation index (SPI) of low (■) ($R$ = 0.723) and high (◆) ($R$ = 0.829) concentration of phenolic substances Sangiovese wines.

**Figure S2.** Pearson's correlation between the intensity of bitterness and (a) long polymeric pigment (LPP) ($R$ = 0.977), (b) short polymeric pigment (SPP) ($R$ = −0.983), (c) BSA-tannins ($R$ = 0.990), (d) vanillin reactive flavans (VRF) ($R$ = 0.932) and (e) saliva precipitation index (SPI) ($R$ = 0.959) of low (■) and high concentration of phenolic substances Sangiovese wines (◆).