Impact of alternative protein fining agents on the phenolic composition and color of Syrah red wines from warm climate

Belén Gordillo, Francisco Chamizo-González, M. Lourdes González-Miret*, and Francisco J. Heredia

Food Colour and Quality Laboratory, Facultad de Farmacia, Universidad de Sevilla, 41012 Sevilla, Spain

Belén Gordillo: bgordillo@us.es

Francisco Chamizo-González: fchamizo@us.es

Francisco J. Heredia: heredia@us.es

M. Lourdes González-Miret: miret@us.es

* Corresponding author:

M. Lourdes González-Miret

Food Colour & Quality Lab., Área de Nutrición y Bromatología. Facultad de Farmacia. Universidad de Sevilla. 41012-Sevilla, Spain

Tel.: +34 955420938

e-mail: miret@us.es

ABSTRACT

Currently, the wine industry has an increasing interest in developing alternative solutions to traditional animal proteins fining agents. In this study, the impact of different protein fining agents on the turbidity, phenolic composition and color of 2-month and 12-month Syrah red wines was assessed. Wines fined with egg albumin and plant-based proteins from potato, pea, and grape seed as recent alternative, were compared to unfined control wines. Changes on turbidity, phenolic composition and color (by Differential Colorimetry) showed that animal and plant proteins differed in their clarifying efficiency and ability to interact with colorless phenolics and anthocyanins, depending on the age of wine, with important consequences on color quality and stability. Plant proteins showed lower effectiveness to reduce wine turbidity than egg albumin but modified in different way the phenolic composition, inducing lower color differences with respect to control wine and similar stability, especially potato and grape seed proteins.

Keywords: Protein fining agents; grape seed protein; phenolic composition; color; red wine.

1. Introduction

Phenolic compounds are the main chemical substances responsible for the organoleptic characteristics of wines such as color, bitterness and astringency, especially in red wines. The color of red wine is one of the most important parameters to decide its quality since it is the first attribute perceived, so it influences consumer's preferences and market decisions. Color is directly related to the anthocyanin composition of wine and the interactions among them or with other wine components (mainly colorless phenolics) by copigmentation, which contributes to the color evolution and stabilization (ordillo Ce udo- astante odr ue - ulido on le -Miret & Heredia, 2013; Escribano-Bailón & Santos-Buelga, 2012).

On the other hand, the bitterness of wine is mainly due to flavan-3-ols, and also caused by some flavonols, hydroxycinnamates and benzoic acid derivatives (Ferrer-Gallego, Hernández-Hierro, Rivas-Gonzalo, & Escribano-Bailón, 2014; Ferrer-Gallego, Brás, García-Estévez, Mateus, Rivas-Gonzalo & De Freitas, 2016). Moreover, low molecular weight flavanols and their oligomeric/polymeric derivatives (procyanidins or tannins) are the main phenolics contributing to the astringency, which tend to decrease during red wine maturation and aging by polymerization and precipitation reactions (Quijada-Morín, Williams, Rivas-Gonzalo, Doco, & Escribano-Bailón, 2014; Ramos-Pineda, García-Estévez, Brás, Martín del Valle, Dueñas, & Escribano Bailón, 2017). Thus, to elaborate full-bodies red wines with stable deep colors and equilibrated taste sensation of bitterness and astringency it is crucial to control and modulate the phenolic composition during vinification.

After fermentative processes, wines are turbid and unstable media mainly due to the presence of microorganisms (yeast and bacteria), tartrate crystals, rests of grape skin and pulp, and aggregates of macromolecules (mainly pectin and protein materials) formed during the fermentative maceration (Vernhet, 2018). In advanced stages of vinification, the presence of colloidal unstable species is related to the formation of less soluble phenolic species that tend to co-aggregate progressively during wine aging, which determine its natural slow precipitation and sedimentation (González-Neves, Favre & Gil, 2014).

This diversity of particles, responsible for hazes and deposits, can indistinctly aggregate the coloring matter and colorless phenolics affecting the sensory quality of wines, so they need to be removed or stabilized to prevent alterations of taste, flavor, or color previous to bottling and consumption.

Clarification with fining agents is very common operation in oenology, which consists of adding an exogenous substance in a turbid wine that drags down other suspended particles by flocculation or adsorption (Gambuti, Rinaldi, Romano, Manzo & Moio, 2016; Vernhet, 2018). The main benefits rely on increasing the wine limpidity, color stability and modulating mouthfeel perception by eliminating or reducing some phenolic compounds of colloidal nature implicated on oxidation processes or aggressive taste sensations (Marangon, Vincenzi & Curioni, 2019). However, this is a major challenge for red wines having insufficient levels of phenolics since clarifying in excess can negatively affect the stabilization processes related to small solutes and to macromolecules affecting especially the color, as typically occurs in warm climate regions.

Among clarifying substances, the protein fining agents are of great interest for wine fining because they have good ability to interact with wine phenolics and have demonstrated different affinity to diverse phenolic classes (Maury, Sarni-Manchado, Poinsaut, Cheynier, & Moutounet, 2016; Maury, Sarni-Manchado, & Cheynier, 2019; Río Segade, Paissoni, Vilanova de la Torre, Gerbi, Rolle & Giacosa, 2020). However, despite its effectiveness, traditional animal-derived fining agents like milk and egg proteins has been subjected in the last decade to increased regulation by the European Union, Australia, or New Zealand because of their potential allergenic risk or food intolerance (Tschiersch, Nikfardjam, Schmidt & Schwack, 2010; Marangon et al., 2019). For this reason, the use of plant-derived macromolecules such as proteins, cell wall material, or fiber from different vegetal sources have been recently proposed as alternative solutions for the clarification of white, rose, and red wines (Guerrero, Smith & Bindon, 2013; Cosme, Capão, Filipe-Ribeiro, Bennett & Mendes-Faia, 2012; Bautista-Ortín, Cano-Lechuga, Ruiz-García & Gomez-Plaza, 2014; Gambuti et al., 2016; Marangon et al., 2019; Jiménez-Martínez, Bautista-Ortín, Gil-Muñoz & Gómez-Plaza, 2019). At this respect, special attention

has been paid toward the use of protein fining agents endogenous to grapes and byproducts as proteins from seeds (Vincenzi et al., 2013; Gazzola, Vincenzi, Marangon, Pasini & Curioni, 2017). Notwithstanding, the effectiveness of different protein fining sources strongly differs depending on the type and age of wines in which are applied (Gonzalez-Neves et al., 2014; Martínez-Lapuente, Guadalupe & Ayestarán, 2017; Marangon et al., 2018; Río-Segade et al., 2020). Moreover, several studies have also demonstrated controversial effects on wine sensory characteristics, stability, and composition depending on the origin of the protein source and their hydrolysis grade, the dose applied, or the contact time (Tschiersch et al., 2010; Oberholster, Carstens & Du Toit, 2013; Ghanem et al., 2017; Kang, Niimi & Bastian, 2018; Jimenez-Martinez et al., 2019). In this sense, further studies about the effectiveness of alternative protein agents are still needed, which could be of great interest especially in warm climate vinifications.

Thus, the main objective of this work was to assess the impact of different vegetal protein fining agents (potato, pea, and grape seed proteins) in the turbidity, phenolic composition, and color of Syrah wines from warm climate (2 and 12-month from the end of fermentation), and compare them as potential alternatives to traditional animal-derived proteins such as egg albumin. Special attention was focused on color quality and stability by Differential Colorimetry, which provides relevant color information related to visual perception of qualitative and quantitative color variations.

2. Material and methods

2.1. Fining agents and preparation of grape seed protein concentrate

Fining agents used in the clarification trials were commercial powdered protein isolates from egg (OVOVIN, egg albumin), potato (PROVEGET FINE, *Solanum tuberosum*) and pea (PROVEGET 100, *Pisum sativum*), all of them provided from Agrovin S.A. (Ciudad Real, Spain) and approved by the International Oenological Codex and EC 606/2009 Regulation.

With the aim of comparing the fining efficiency of the selected protein agents, a grape seed protein concentrate (GSP) was experimentally prepared and included in this study as alternative protein-fining

source. GSP was obtained from defatted grape seed flour (industrial wine by-product, ALVINESA Natural Ingredients, Ciudad Real, Spain) by alkaline solubilisation with isoelectric precipitation, according to Gazzola et al., (2017). The alkaline solubilisation of grape seed proteins was carried out at pH 10.5 in a Bioreactor Bio Bundle Microbial System (Applikon Biotechnology®, Holland) using the pH-stat method. For this purpose, 1 kg of defatted grape seed flour was re-dissolved in 5 L of distilled water (20% p/v) during 12 h in agitation (300 rpm). After that, the alkaline aqueous solution was centrifuged (9000 rpm, 15 min, 10 ºC), decanted 24 h in refrigeration (5 ºC), and then acidified to pH 3.0 with 6 M HCl. The precipitated material was recovered by centrifugation (9000 rpm, 30 min, 10 °C) and lyophilized to obtain a fine powder (GSP concentrate), which was stored at -20 ºC until its use. The chemical composition of the GSP extract was determined according to the AOAC standard protocols (AOAC, 1990) and shown in Table S1.

Protein content (%) of fining agents were: egg albumin (82.3 % ± 0.3), potato isolate (76.9 % ± 0.3), pea isolate (68.7 % ± 0.5) and GSP (32.1 % ± 0.6). Protein content was determined using a LECO TruSpec® CHNS MICRO microsample elemental analyzer (Leco Instrumentos S.L., Madrid, Spain) based on the nitrogen determination by thermal conductivity detection system, after sample combustion. Protein content was computed using a nitrogen-to-protein conversion factor of 6.25 for animal protein and of 5.53 for plant based proteins (Zhou et al., 2011).

2.2. SDS-PAGE analysis

The range of molecular weight of protein fining agents was determined by sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE) usin the Invitro en™ system (Thermo Fisher Scientific Inc.). Fining agents were re-dissolved in Tris-HCl buffer pH 7.5 at 2 mg/mL. Aliquots of these concentrates (20 µL) were mixed with 5 µL of 0.02 M Tris-HCl loading buffer pH 6.8 (containing 40% w/v glycerol, 4.6% w/v SDS, 20% v/v 2-mercaptoethanol, and 0.01% Bromophenol Blue, all from Sigma-Aldrich, Milan, Italy), and then denatured at 100°C for 5 minutes. Denatured protein samples were loaded into 12% polyacrylamide gel 1.5 mm (acrylamide: bisacrylamide, 30:1) and electrophoresis

136 was run with constant voltage mode (160 V) at room temperature for 50-60 min until the tracking dye
137 Bromophenol Blue ran off the gel. Gels were stained with Quick Coomassie Stain (Quimigen S.L.,
138 Madrid) and then de-stained with water. The molecular weight standard proteins (ranging from 10 to
139 200 kDa) used were the a e uler™ Unstained rotein Ladder (Thermo Fisher Scientific Inc.).

140 **2.3. Winemaking protocols and fining treatments**

141 Two Syrah red wines elaborated in warm climate ("Condado de Huelva" Desi nation of Ori in
142 southwestern Spain) at different stages of vinification (2-months (W2) and 12-month (W12) from the
143 end of fermentation) were used for clarification assays. W2 corresponded to a very recent wine, and
144 W12 corresponded to a more stabilized wine in which some reactions (aggregations or sedimentation)
145 have occurred along the winemaking period. These are two stages of the vinification process in which
146 clarification could be performed: when wine is ready to initiate the stabilization (around 2 months)
147 process and when is ready to be bottled (around 12 months).

148 W2 and W12 red wines were made from grapes *Vitis vinifera* var. Syrah by traditional fermentative on-
149 skin maceration for 6 days. Healthy grapes were harvested at optimum technological maturity (average
150 13.5 ºBé and 13.9 ºBé in W2 and W12, respectively), destemmed and crushed, and distributed into
151 stainless steel tanks for maceration, where alcoholic fermentation (20-25 ºC) was induced by inoculating
152 selected yeast (*Saccharomyces cerevisiae* 25 g/hL, Viniferm BY, Agrovin, Ciudad Real, Spain).
153 Fermentative maceration occurred along 5 days (fermentation caps were punched down once a day);
154 after this, the mash was drawn off to remove the solid parts, and the free run wine was racked to
155 stainless steel tanks to finish the fermentation. Malolactic fermentation was induced by inoculating
156 selected lactic acid bacteria (*Oenococcus oeni* VINIFERM Oe 104, 14 mL/hL, Agrovin, Ciudad Real,
157 Spain). When fermentative processes finished, sulfur dioxide and total acidity were adjusted at the same
158 levels for all wines. W2 and W12 wines were maintained in 50 L stainless-steel tanks until fining trials.
159 The mean conventional oenological parameters of wines, assessed according to the Official Methods
160 established by European Union (UE, 2003) were: W2 (alcohol content = 13.5 v/v; reducing sugars =

1.88 g/L; titratable acidity = 6.07 g/L as tartaric acid; pH = 3.38; volatile acidity = 0.40 g/L as acetic acid; free sulfur dioxide = 13.3 mg/L; total sulfur dioxide = 119.3 mg/L) and W12 (alcohol content = 13.8 v/v; reducing sugars= 1.75 g/L; titratable acidity = 5.63 g/L as tartaric acid; pH = 3.35; volatile acidity = 0.60 g/L as acetic acid; free sulfur dioxide = 25.8 mg/L; total sulfur dioxide = 112.5 mg/L).

The clarification assays were performed in triplicate, in 200 mL glass containers, for each wine (W2 and W12) and each fining agents, which were prepared following the recommendation of manufacturers and the doses applied of each product were the maximum recommended, being 10 g/hL for egg albumin and potato protein isolates, and 30 g/hL for pea protein isolate. For comparative purposes, GSP was applied at two doses: 10 and 30 g/hL (GSP_{10} and GSP_{30}, respectively). The fining agents were added and homogenized with the wines, the containers completely filled were then closed and maintained at room temperature (10 ± 2 ºC, in the dark) for 6 days. These are the mean conditions for the normal treatment in wineries. Moreover, triplicates of 200 mL of untreated W2 and W12 wines were used as control. After 6 days of clarification, wine samples were separated from lees and stored closed in glass containers at room Tª (10 ± 2 ºC in dark) during 1 month.

Wine samples were taken for chemical and colorimetric analysis at day 1 (before clarification), day 6 (end of clarification), and at the end of storage (30 days after clarification).

2.4. Analysis of turbidity

Turbidity of wines was measured using a 2100P Portable Turbidimeter (HACH® *Be Right*™, Loveland, CO, USA) before treatment and after 6 days of fining. Wine samples were analyzed inside a glass vial and measured four times rotating the vial after each measurement. Results were expressed in NTU (Nephelometric Turbidity Unit).

2.5. HPLC-DAD analysis of phenolic compounds

High performance liquid chromatography (HPLC) was applied for the determination of the monomeric anthocyanins, phenolic acids, monomeric flavan-3-ols and flavonols by direct injection of the samples, previously filtered through a 0.45 μm Nylon filter. An Agilent 1200 (Palo Alto, CA), equipped with

186 quaternary pump, UV-Vis diode-array detector, automatic injector, and the ChemStation software was
187 used for the analyses. All analyses were made in triplicate. The separation, identification and
188 quantification of compounds was performed following a modification of the method described in
189 Gordillo et al. (2013). Phenolic compounds were separated on a Zorbax C18 column (250 x 4.6 mm, 5
190 μm particle size) maintained at 38 ºC. Acetonitrile-formic acid-water (3:10:87) as solvent A, and
191 acetonitrile-formic acid-water (50:10:40) as solvent B were used. Acetonitrile and formic acid were
192 HPLC grade (Merck, Darmstadt, Germany) and purified water was obtained from a NANOpure
193 Diamond system (Barnsted Inc., Dubuque, IA, USA). The elution profile was as follows: 0-10 min with
194 6% B; 10-15 min with 30% B; 15-25 min with 40% B; 25-30 min with 45% B; 30-33 min with 50% B;
195 33-34 min with 60% B; 34-35 min with 6% B. The flow-rate was 0.8 mL/min and the injection volume
196 was 50 μl. UV-Vis spectra were recorded from 200 to 800 nm with a bandwidth of 2.0 nm. The
197 wavelengths of detection were 525 (monomeric anthocyanins), 280 nm (benzoic acids and monomeric
198 flavanols), 320 nm (hydroxycinnamic acids and their tartaric esters) and 360 nm (flavonols).
199 Identification of phenolics was performed according to the spectra features and retention times with
200 those of the available pure standards and our data library of the standards. The quantification was made
201 by external calibration comparing the areas with the following commercial standards: malvidin 3-*O*-
202 glucoside (≥ 97%, Extrasynthese, Genay, France), catechin, *p*-coumaric acid, and quercetin (≥ 96%,
203 Sigma-Aldrich, Madrid, Spain). The concentration of compounds was expressed as mg/L.
204 The determination of procyanidins (dimeric and oligomeric flavan-3-ols) were performed, in triplicate,
205 according to Jara-Palacios, Gordillo, Gonzalez-Miret, Hernanz, Escudero-Gilete & Heredia (2014) by
206 rapid resolution liquid chromatography (RRLC). After filtration through a 0.45 μm Nylon filter, samples
207 were injected (0.5 μL injection volume) in an Agilent 1290 chromatographic system, equipped with
208 quaternary pump, UV-VIS diode-array detector, automatic injector, and ChemStation software (Agilent
209 Technologies, Palo Alto, USA). A C18 Poroshell 120 column (2.7 μm, 5 cm x 4.6 mm) was used. The
210 solvents were formic acid and water (1:999 mL:mL) as solvent A, and acetonitrile as solvent B at the

following gradients: 0-5 min of 5% B linear; 5-20 min of 50% B linear; and 20-25 min of washing, which was followed by re-equilibration of the column. The flow-rate was 1.5 mL/min, and the column temperature was set to 25 ˚C. Identification of compounds was performed according to the retention times of the standards (when available), UV-vis spectra and mass spectra, as described by Jara-Palacios et al. (2014). The quantification was made at 280 nm by external calibration comparing the areas with the commercial standard of procyanidin B1 and B2.

The total monomeric anthocyanins, phenolic acids contents (benzoic and hydroxycinnamic derivatives), monomeric flavan-3-ols, procyanidins (dimeric and oligomeric forms), and flavonols were calculated as the sum of individual phenolic compounds identified by HPLC and RRLC.

2.6. **Colorimetric measurement**

The absorption spectra (380-770 nm) of wines were recorded at constant intervals ($\Delta\lambda$=2 nm) with an Agilent 8453 UV-Vis spectrophotometer (Agilent Technologies, Palo Alto, USA), using 2 mm path length glass cells and distilled water as reference. The CIELAB parameters were calculated from the absorption spectra by using the original software CromaLab® (Heredia lvare on le -Miret & Ramírez, 2004), following the recommendations of the Commission International de L'Eclaira e: the CIE 1964 10° Standard Observer and the Standard Illuminant D65, corresponding to the natural daylight (CIE, 2004). CIELAB parameters were L* (the correlate of lightness, ranging from 0, black, to 100, white), and two color coordinates, a* (the green-red axis) and b* (the blue-yellow axis). From these coordinates, other color parameters are defined: the hue angle (h_{ab}, the correlate of chromatic tonality), and the chroma (C^*_{ab}, the correlate of color intensity). Saturation, s_{uv}, is defined only in the CIELUV space, and it is calculated from the chroma and lightness of CIE 1976 L*, u*, v* color space, according to the following formula: $s_{uv} = (C^*_{uv} / L^*)$. It was included in the colorimetric analysis because it is considered the best correlate for the visually perceived saturation and CIELAB space cannot define a similar correlate (Gómez-Míguez, González-Miret & Heredia, 2007; Gordillo, Lopez-Infante, Ramírez-Perez, González-Miret & Heredia., 2010).

236 Applying Differential Colorimetry (Gordillo et al., 2015) the color differences (ΔE^*_{ab}) (CIE, 2004)
237 among wines during vinification were calculated by the Euclidean distance between two points in the
238 three-dimensional space defined by L* a* and b*: $\Delta E^*_{ab} = [(\Delta L^*)^2 + (\Delta a^*)^2 + (\Delta b^*)^2]^{1/2}$. In addition,
239 the relative contribution of li htness (%ΔL) chroma (%ΔC) and hue (%ΔH) that makes a iven color
240 difference (ΔE^*_{ab}) expressed as percentages, were calculated as follows:

241 - elative contribution of li htness: $\%\Delta L = [(\Delta L^*)^2/(\Delta E^*_{ab})^2] \times 100$

242 - elative contribution of chroma: $\%\Delta C = [(\Delta C^*_{ab})^2/(\Delta E^*_{ab})^2] \times 100$

243 - elative contribution of hue: $\%\Delta H = [(\Delta H)^2 /(\Delta E^*_{ab})^2] \times 100$

244 bein ΔH mathematically deduced from: $\Delta H = [(\Delta E^*_{ab})^2 - (\Delta L)^2 - (\Delta C)^2]^{1/2}$

245 **2.7. Statistical analysis**

246 All statistical analyses were performed using Statistica® 8.0 software (Stat Soft). Univariate analysis of
247 variance (Tukey test, $p<0.05$) was applied to establish statistical differences for the chemical and
248 colorimetric characteristic among wine treatments.

249 **3. Results and discussion**

250 **3.1. Impact on wine turbidity**

251 Table 1 shows the turbidity values (Nephelometric Turbidity Units, NTU) of 2-month and 12-month old
252 Syrah wines (W2 and W12, respectively) after 6 days of clarification. The effectiveness of the selected
253 fining agents significantly varied ($p<0.05$) according to the protein source and the degree of clarification
254 was notably different depending on the age of the wines.

255 The turbidity of W2 control wines after fining treatments was 38.8±0.79 NTU. In comparison, all the
256 protein fining agents produced a significant higher degree of clarification, except for pea proteins. The
257 best clarifying agent was egg albumin followed by potato protein, which reduced W2 turbidity by 77.5%
258 and 62% respect to control (8.67 and 14.6 NTU, respectively) when applied at similar amounts (10 g/hL;
259 82-77% protein richness). Grape seed proteins (GSP), however, showed lower fining efficiency at 10
260 and 30 g/hL than the aforementioned protein sources. Probably the lower protein content of the GSP

extracts (32% protein richness) and differences on the protein composition between the fining sources characterized by electrophoretic analysis under non-reducing conditions (Figure S1) could affect the coagulation/flocculation processes leaving them in some cases incomplete (Gambutti et al., 2016). Notwithstanding, GSP reduced W2 wine turbidity by around 15% respect to control at the two doses applied demonstrating being better plant-based fining agent than pea proteins (by 4.6%) in spite of its lower protein concentration (32% versus 68.7% protein richness in GSP extract and pea protein isolate, respectively). Similar results were obtained by Gazzola et al., (2017), which recently demonstrated the potential use of grape-derived proteins as promising alternative fining agents for white, rosé and red wines clarifications.

Considering the oldest Syrah wines (12-month old, W12), the impact of the fining treatments on wine turbidity was comparatively lower than in youngest ones (W2), at the same amounts applied. The W12 control wines had a mean value turbidity of 4.20±0.40 NTU and the W12 wines fined with protein agents ranged from 2.8 to 4.2 NTU. There were no significant differences among the effects of pea protein and GSP treatments respect to control wines. As expected, the high turbidity levels typical of the earlier stages of vinification tend to progressively decrease due to the sedimentation/precipitation processes that naturally occur in stainless steel tanks during the first year of the stabilization. Our results agree with the study of Gonzalez-Neves (2014), which reported a lower clarifying effect in 14-month aged wines due to a greater degree of stability achieved naturally over the time. Even so, taking into account than target values to obtain bright red wines are in the order or below 2 NTU (Verhnet, 2018), egg albumin and potato proteins demonstrated being more effective protein agents to significantly reduce the residual turbidity of wines in advanced stages of vinification.

3.2. Impact on phenolic composition

In this study, 32 phenolic compounds were identified and quantified in W2 and W12 Syrah wines, which had the same phenolic profile (Figure S2 and S3). Figure 1 shows the concentration (mg/L, mean±SD, n=3) of the main phenolic families (Total Monomeric Anthocyanins, Total Phenolic Acids, Total

286 Monomeric Flavanols, Total Procyanidins, and Total Flavonols) of W2 and W12 wines after the
287 clarification treatments, indicating the statistical differences (p<0.05) with respect to control wines (CW,
288 unfined).
289 Regarding W2 wines (Figure 1A), the mean contents of the aforementioned compounds in control
290 treatment were: Total Monomeric Anthocyanins (112.59±0.25 mg/L), Total Phenolic Acids (57.09±1.65
291 mg/L), Total Monomeric Flavan-3-ols (99.80±0.25 mg/L), Total Procyanidins (28.97 ±0.07 mg/L) and
292 Total Flavonols (38.94±2.29 mg/L). All the fining treatments applied affected the phenolic composition
293 of the youngest wine. The impact was significant for the Total Monomeric Flavan-3-ols, Total
294 Procyanidins, and Total Monomeric Anthocyanins. A higher effect in the removal of Total Monomeric
295 Flavan-3-ols was observed between fining treatments with losses ranging from 7% to 23% with respect
296 to the content of control wines (CW). The lowest reduction was found in wines fined with pea proteins
297 (PE by 6%), followed by egg albumin and GSP at the lowest dose (EA and GSP10 by 10%). In contrast,
298 potato protein and GSP at the higher dose led to significant highest reductions of Monomeric Flavan-3-
299 ols (PT and GSP30 by 23% and 17%, respectively) by decreasing the contents of both (+)-catechin
300 (33.8 and 37.9 mg/L, respectively) and (-)-epicatechin (39.2 and 45.4 mg/L, respectively) (Table S2).
301 At the same time, the levels of the Total Procyanidins were significantly (p<0.05) diminished by all of
302 the fining agents (Figure 1A). Among them, a higher reduction of the dimeric and oligomeric flavanols
303 was produced by egg albumin followed by GSP at the highest dose (GSP30) and potato protein (Total
304 procyanidins=22.9 ±0.6, 23.4± 0.4 mg/L, and 23.6 ± 0.3 mg/L, respectively). Differences for the effect
305 on the individual dimeric (procyanidin B1, B2, B2-gallate, B7) and oligomeric compounds (tetramer 1
306 and 2) is shown in Table S2. Different efficiency in the removal of flavanols has been reported related to
307 the different molecular size, hydrophobicity, and conformation of animal/vegetal proteins. The results
308 obtained by Granato et al. (2018), who described native pea proteins as less effective agents to remove
309 monomeric and dimeric flavanols, agreed with our study. Likewise, our results are in agreement with
310 those of Gambuti, Rinaldi and Moio (2012), Tschiersch et al., (2010), and Gazzola et al., (2017), which

311 showed that potato and grape seed proteins are good alternative plant sources to animal proteins to
312 reduce the content of compounds mainly responsible for the astringency in wines (monomeric or
313 oligomeric flavanols).
314 On the other hand, although there was no significant impact on the Total Flavonols and Total Phenolic
315 Acids contents, some of these individual compounds was affected by the clarification treatments. In
316 particular, wines fined with pea proteins showed a higher reduction of *t*-caftaric and *t*-coutaric acids than
317 wines treated with other fining sources (Table S2). These differences were significant ($p<0.05$) for the *t*-
318 caftaric content. This result disagrees with Ghanem et al. (2017), who did not find any impact in the
319 phenolic acids content by different kind of fining agents, oenological tannins, and mannoproteins
320 suggesting that there was no interaction between small phenolic compounds and such macromolecules.
321 Considering the compounds directly responsible for the color, the Total Monomeric Anthocyanins
322 content in fined W2 wines was reduced by 6% to 12.5% with respect to the content in control ones. All
323 the wines fined with plant-based proteins had significant ($p<0.05$) lower contents of Total non-
324 acylglucosides (monoglucosides), acetylglucosides and *p*-coumaroilated derivatives than control wines
325 while wines fined with egg albumin mainly affected the monoglucosides derivatives (Table 2). The
326 impact of interactions was also dissimilar for the individual compounds depending on the origin of the
327 fining source. Pt-3glc, Pn-3glc, Mv-3glc and Pt-3-acetylglc were the most affected anthocyanins by egg
328 albumin. On the other hand, plant-based proteins had a significantly higher impact on Df-3gl, Pt-3gl,
329 Mv-3gl, and the acetylated and *p*-coumaroylated derivatives of Mv-3gl.
330 By comparing the Total Anthocyanin content between fining treatments (Figure 1A), the lowest global
331 reductions corresponded to wines fined with pea proteins, egg albumin and potato proteins (by 6%, 7%
332 and 8%, respectively) suggesting a lower affinity to monomeric pigments than grape seed proteins
333 (GSP10 and GSP30 reduction by 11-12.5%). However, the Total Monomeric Anthocyanins content
334 among them was comparatively no significant (ranging from 98.5 and 105.1 mg/L), as well the content
335 of Total monoglucosides (non-acylated) and Total acetylglucosides. In the case of *p*-coumaroilated

derivatives, there were significant differences between wines fined with GSP30 and egg albumin due to the higher losses of Pt-3gl and Mv3gl derivatives by the former.

Concerning the 12-month Syrah wines (W12), the mean contents of the phenolic families in control treatment after clarification were: Total Monomeric Anthocyanins (64.24±1.08 mg/L), Total Phenolic Acids (44.18±0.24 mg/L), Total Monomeric Flavanols (90.82±1.84 mg/L), Total Procyanidins (27.23±0.57 mg/L) and Total Flavonols (21.35±0.41 mg/L). As observed in Figure 1B, clarification treatments had a significant impact on the Total Monomeric Flavan-3-ols and in a lesser extent in Total Procyanidins. With respect to the content in control wines, the highest reduction of the monomeric forms was found with pea proteins followed by egg albumins (PE and EA by 20% and 17%, respectively). Thus, these protein sources had higher impact in removing low molecular weight flavanols (both in (+)-catechin and (-)-epicatechin contents, Table S2) when applied in advanced stages of vinification than in younger wines. Conversely, potato protein showed a lower interaction with Monomeric Flavanols reducing the global content by 12% (instead by 23% in W2 wines) due a significant decrease only in (+)-catechin. Regarding procyanidins, a higher efficiency in the removal of the total content was observed only for grape seed protein and potato proteins (Total procyanidins=24.73±0.5, 25.98 ±0.3, and 26.05± 0.4 mg/L, in GSP30, GSP10 and PT, respectively) although most of the individual dimeric and oligomeric flavanols were not affected by the fining treatments (Table S2).

In agreement with previous studies (Gonzalez-Neves et al., 2014), the behavior of each fining agent can vary with different wines due to differences in the wine composition (concentration and structural characteristics of both proteins and phenolics), which is highly influenced by the stage of vinification. However, in the case of grape seed proteins, the impact on removing the Total Monomeric Flavanols was quite similar in W12 and W2 wines at the two amounts tested (GSP10 by 10% and GSP30 by 13-17%). Probably the lower protein purity of GSP and the presence of other plant components in the obtained extracts (polysaccharides, carbohydrates, among others) could interfere in the interactions of

grape seed proteins with wine phenolics (Table S1). Indeed, further studies focused on increasing the purity of grape protein extracts are still needed in order to optimize their efficacy as fining agents in relation with the dose applied and contact time (Jiménez-Martínez et al., 2019; Marangon et al., 2019).

As in W2 wines, the content of some individual phenolic acids was affected by fining treatments in W12 wines, although this effect was not reflected in the total content. In particular, wines fined with pea proteins and GSP30 showed significant (p<0.05) lowest contents of gallic acid than control wines (90.2, 92.7, and 94.1 mg/L, respectively; Table S2), which proved its higher ability to bind such as small colorless phenolics than the other fining sources.

As shown in Table 2, most of the individual anthocyanin monomers were not affected in W12 wines by any of the fining treatments, as well the total monoglucosides and acetylated derivatives. Consequently, although the global levels of Total Monomeric Anthocyanins (Figure 1B) were slightly decreased after clarification, the differences in relation to the content of control W12 wines were not significant. These results agreed with Granato et al. (2018), who showed that different fining agents had higher impact in the anthocyanin composition of young wines than in one-year older ones. Nevertheless, a more comprehensive assessment of the impact of fining treatments in the phenolic composition of aged wines should include the evaluation of the anthocyanin-derived pigments, which are progressively formed during vinification contributing in a higher extent to the total pigment content in advanced stages of vinification (González-Neves et al., 2014; De Freitas & Mateus, 2011).

3.3. Impact on wine color by Tristimulus Differential Colorimetry

Table 3 shows the effect of the clarification treatments on the colorimetric characteristics (mean±SD, n=3) of 2-month and 12-month Syrah wines. In W2, all the fining treatments significantly affected most of the CIELAB (L*, a*, b*, C^*_{ab}, h_{ab}) and CIELUV (s_{uv}) color parameters compared to those of control wines (CW, unfined). In general, the lightness (L*) and hue (h_{ab}) values slightly increased in fined wines while the chroma (C^*_{ab}) and saturation (s_{uv}) values decreased. These trends indicate that clarification induced both quantitative and qualitative color changes in young Syrah wines. From a quantitative point

386 of view (L*, C^*_{ab}, and s_{uv}), fined wines showed clearer, less intense and less saturated colors than
387 control wines, which was also confirmed by the decreases of C^*_{ab} values. Likewise, the variations of the
388 qualitative attribute of color (h_{ab}) denote a slight reduction of the bluish component of the red tonality in
389 fined wines regarding control ones, as observed by the increases of the negative b* values.
390 Notwithstanding, the rate of the quantitative and qualitative color changes varied between the fining
391 sources. Egg albumin and pea proteins produced the highest impact on the CIELAB quantitative
392 attributes of color by decreasing the wine color intensity by 5-4% (C^*_{ab}=48.6 and 49.1 versus 51.2 in
393 CW, p<0.05) and increasing the lightness by 5% (L*=55.4 and 54.8 versus 52.5 in CW, p<0.05). Even
394 so, these fining sources had not significant effect on the hue values with respect to CW (h_{ab}= -1.30° and
395 -1.40° versus -1.45° in CW), which indicate that maintained better the bluish-red tonality of young
396 Syrah wines. On the other hand, fining with GSP had the lowest impact in color intensity and lightness
397 (C^*_{ab} values decreases and L* increases by 2% with respect to CW), but the influence on the tonality of
398 wines was higher in comparison to the other fining sources (h_{ab} increases of +0.9 with respect to CW).
399 In this case, grape seed proteins at the higher dose applied (GSP30) showed no significant differences
400 for any of the color parameter with respect to CW, except for the lightness L*. Similarly, although the
401 CIELUV saturation (s_{uv}) values decreased in all fined wines, those treated with GSP showed the lowest
402 reductions in relation to CW making the wine to keep the purity and intensity of their original color. The
403 higher effect of egg albumin in reducing the color intensity in young wines with respect to the most
404 plant-based fining agents tested (mainly potato and GSP proteins) agree with the results reported by
405 Gazzola et al. (2017) and Gambutti et al. (2012).The differences found in the quantitative and qualitative
406 color effects between the fining sources could be explained by their selectivity to remove specific
407 anthocyanins compounds and families, which influenced not only the global pigment contents of wines
408 but also the proportions of individual anthocyanins, as reported by Granato et al. (2018). Moreover, it
409 was observed different rates on the reduction of copigments families such as flavanols, flavonols and
410 phenolic acids (González-Neves et al., 2014).

411 On the other hand, most of the CIELAB and CIELUV parameters were not significantly different
412 between fined wines and control ones (CW) when applied in advanced stages of vinification (W12
413 wines). Quantitatively, only wines fined with pea proteins had significant (p<0.05) higher values of L*
414 and lower of C^*_{ab} than CW (L*=60.6 versus 59.2; C^*_{ab}= 41.2 versus 42.6), which meant lighter and less
415 intense colors (L* increased by 2% and chroma decreased by 3%). Similarly, these wines showed less
416 saturated colors than CW (lower values of $s_{uv;}$ p<0.05). Regarding the hue, W12 wines showed higher
417 values than W2 wines (h_{ab}=8-9° versus -1.45°/-0.59°) corresponding to the redness region of the
418 CIELAB space (between 0°-10° and positive values of b*), which indicates an important reduction of
419 the bluish tonalities typical of the earlier stages of vinification. Qualitatively, although wines fined with
420 egg albumin, potato and pea proteins had significant differences for the hue values compared to those of
421 control wines, the changes in the tonality between wines were quite small and thus, could be considered
422 negligible.
423 The impact of clarification observed in the color of W12 wines agree with the changes found in the
424 phenolic composition. On the one hand, all the fining treatments did not affect the content of most of the
425 anthocyanin compounds and the total levels of monomeric pigments. In fact, pea proteins were the
426 fining source that most reduced the content of copigments such as monomeric flavanols and flavonols
427 which could lead to higher loses on the color intensity of wines.
428 To quantify the color changes due to the fining treatment, we have used the Color Difference (ΔE^*_{ab})
429 defined by CIELAB, which provide relevant color information related to visual perception (Gordillo et
430 al., 2015). For each ΔE^*_{ab}, it is possible to calculate the relative contributions of color attributes
431 changes: %ΔL (relative difference of lightness), %ΔC (relative difference of chroma) and %ΔH (relative
432 difference of hue). These contributions allow comparing objectively the quantitative and qualitative
433 effects of the different treatments on color.
434 The mean color differences (ΔE^*_{ab}) between each fining treatments and its corresponding control wine
435 (not-treated) were calculated for W2 and for W12 wines, to compare the magnitude of the clarification

436 effects on the color of wines when this treatment is applied at different stages of vinification. As
437 observed in Figure 2, the ΔE^*_{ab} values between control and fined treatments were comparatively higher
438 for W2 than for W12 wines (ΔE^*_{ab} = 1.6-3.9 and 0.38-2.1, respectively) confirming that clarification had
439 more impact on color when applied in earlier stages of vinification (2 months versus 12 months). In the
440 case of W2, the highest color differences with respect to control wines (treated-W2 vs not-treated-W2)
441 was found in wines fined with egg albumin (ΔE^*_{ab}= 3.9) compared to the ΔE^*_{ab} values obtained for the
442 rest of the fining agents (ranging from 1.6 to 3.2). These results indicate that the selected plant-based
443 proteins had less impact on the color of young Syrah wines than traditional fining agents based on
444 animal proteins, which confirm those found by Gonzalez-Neves et al. (2014). However, taking into
445 account that ΔE^*_{ab} around or higher than 3 CIELAB units indicates that color differences can be
446 perceived by the human eyes (art ne el osa re , Hita & Negueruela, 2001), the effects of egg
447 albumin and pea proteins on wine color could be considered visually discernible. In both cases, the
448 differences were mainly due to a higher contribution of changes on the quantitative attributes of color
449 (%ΔL=56% and 51%; Δ%C= 44% and 47%, respectively). These results suggest that excessive fining
450 can be detrimental to the sensory quality of red wines. In contrast, the lowest color differences in
451 relation to control wines were found in wines fined with GSP and potato proteins (ΔE^*_{ab} <2.5, not
452 clearly perceptible), in agreement with Gazzola et al. (2017). When fining treatments were applied in
453 advances stages of vinification (W12), wines fined with pea proteins led to the highest color differences
454 with respect to unfined wines mainly due to lightness and chroma changes (higher weight of Δ%L and
455 Δ%C), which agree with the effect observed in the individual color attributes. Nevertheless, in all cases
456 the ΔE^*_{ab} values were lower than 2 units, and thus, considered not visually appreciable.
457 The assessment of the global color differences occurring during 30 days of storage after clarification
458 allowed evaluating the color variation of each wine over time ($\Delta E^*_{ab}= [(L_{30}-L_0^2 + (a^*_{30}-a^*_0)^2 + (b^*_{30}-$
459 $b^*_0)^2]^{1/2}$, and compare the impact of fining treatments on color stability in W2 and W12 wines (Table 4).
460 In W2, all the fined wines showed significant lower values of color differences with respect to control

461 wines (ΔE^*_{ab} ranging from 7.2 to 9.1 versus 9.8 in CW), which indicate lower color variation and thus,
462 higher color stability. Among fining treatments, the higher stabilizing effect was found in wines fined
463 with e albumin (lower ΔE^*_{ab}) but the differences in relation to the plant-based protein agents were not
464 significant, except for S at the hi her dose (ΔE^*_{ab} =7.2 versus 9.1, respectively). In fact, wines fined
465 with GSP at the two doses (GSP10 and GSP30) showed higher color variation (lower stability)
466 compared to wines fined with potato and pea proteins. Probably the presence of other plant components
467 co-extracted with grape seed proteins in GSP extract could exert a negative impact on the color stability
468 of wines when used as fining agents (Table S1). The trend of the color attributes during storage (positive
469 values of ΔL^* and Δh_{ab}, negative of ΔC^*_{ab}) indicates that all wines increased the lightness and hue
470 values but decreased the chroma, that is, showed clearer and less intense red-orange colors with the
471 time. The magnitude of the changes reflects that the most affected parameters were chroma and hue.
472 Among fining treatments, wines fined with egg albumin maintained better the color intensity while those
473 fined with potato proteins showed more stable hues (significant lower decreases of ΔC^*_{ab} and increases
474 of Δh_{ab}, respectively).
475 In the case of the older Syrah wines (W12), the global color variations were comparatively lower than in
476 youngest ones (W2), mainly due to increases of hue during storage while the changes on chroma and
477 lightness were in most cases negligible (slight increases or decreases of C^*_{ab} and L^*). At this regard, the
478 higher color stability (lower ΔE^*_{ab} values) was found in wines fined with pea proteins and GSP at the
479 lowest dose (GSP10), but the differences with respect to control wines were only significant for the
480 formers. In both cases, the higher color stability achieved was due to their lower increases of hue, that is,
481 these wines showed more stables hues.

482 **4. Conclusions**

483 Results showed than plant-based proteins (such as potato and pea, and grape seed proteins as recent
484 proposal) are suitable sources to be used as clarifying agents both in earlier and advanced stages of
485 vinification of red wines from warm climate as alternative to traditional fining agents from animal origin

such as egg albumin. The effectiveness at reducing wine turbidity, and the impact on the phenolic composition (colorless phenolics and anthocyanin pigments) and color quality and stability, have demonstrated in most cases sensory benefits in fined wines in relation to unfined ones, compared to egg albumin. Therefore, the exploitation for their potential use in the wine industry could be of great interest. Although the use of seed proteins from grape by-products has the advantages of being endogenous to grape, not allergenic, and adds value to this byproduct, further studies are still needed to optimize their efficacy as fining agent in relation with the protein purity of the extracts, the dose applied and contact time, and hence, the wine industry application.

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Conflict of interest statement

Authors declare no conflict of interest

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FIGURE CAPTIONS

Figure 1. Concentration (mg/L, mean±SD, n=3) of the main phenolic families (Total Anthocyanins, Total Phenolic Acids, Total Monomeric Flavanols and Total Flavonols) of Syrah wines after clarification treatments (6 days): a) 2-month wine, W2; b) 12-month wine, W12. Abbreviations: CW: Control wine; and wines treated with protein fining agents (EA: Egg albumin, PT: Potato protein, PE: Pea protein, GSP_{10} and GSP_{30}: Grape seed protein at 10 and 30 g/hL). Different letters on the bars indicate significant differences ($p<0.05$) with respect to control wine.

Figure 2. Mean color differences (ΔE^*_{ab}), with the relative contribution of lightness, chroma, and hue (%ΔL %ΔC %ΔH) calculated between control wines and wines treated with protein fining agents after clarification (6 days). Abbreviations: EA: Egg albumin, PT: Potato protein, PE: Pea protein, GSP_{10} and GSP_{30}: Grape seed protein at 10 and 30 g/hL; W2 and W12: 2-month and 12-month Syrah wines.

HIGHLIGHTS

1. Commercial proteins and grape seed protein were assessed as fining agents
2. The study considered two different stage of vinification of warm climate red wine
3. Fining agents showed different impact on phenolics and color of red wine
4. Differences were also depending on the age of the wine
5. Potato and grape seed proteins induced lower color changes regarding control wines

***Declaration of Interest Statement**

Conflict of Interest and Authorship Conformation Form

Please check the following as appropriate:

- ✓ All authors have participated in (a) conception and design, or analysis and interpretation of the data; (b) drafting the article or revising it critically for important intellectual content; and (c) approval of the final version.
- ✓ This manuscript has not been submitted to, nor is under review at, another journal or other publishing venue.
- ✓ The authors have no affiliation with any organization with a direct or indirect financial interest in the subject matter discussed in the manuscript
- ✓ The following authors have affiliations with organizations with direct or indirect financial interest in the subject matter discussed in the manuscript:

Author's name	Affiliation
Belén Gordillo	Universidad de Sevilla, Spain
Francisco Chamizo-González	Universidad de Sevilla, Spain
M. Lourdes González-Miret	Universidad de Sevilla, Spain
Francisco J. Heredia	Universidad de Sevilla, Spain

***Credit Author Statement**

Belén Gordillo: Investigation, Writing - Original Draft

Francisco Chamizo: Investigation

M. Lourdes González-Miret: Investigation, Review & Editing

Francisco J. Heredia: Conceptualization, Supervision

Table 1

Table 1. Turbidity (NTU, mean value ± SD, n=4) of 2-month and 12-month Syrah wines (W2 and W12, respectively) after clarification treatments (6 days).

Fining agents	**W2 wine**	**W12 wine**
Control wine (unfined)	38.8 ± 0.79 a	4.20 ± 0.50 a
Egg albumin (10 g/hL)	8.67 ± 0.45 b	2.86 ± 0.30 b
Potato protein isolate (10 g/hL)	14.6 ± 0.36 c	2.78 ± 0.34 b
Pea protein isolate (30 g/hL)	37.02 ± 0.81 a	3.98 ± 0.40 a
GSP_{10} (grape seed protein concentrate 10 g/hL)	33.20 ± 1.56 d	4.20 ± 0.39 a
GSP_{30} (grape seed protein concentrate 30 g/hL)	32.72 ± 0.50 d	3.59 ± 0.22 a

Different letters in the same column indicate significant differences ($p < 0.05$).

Table 2

Table 2. Anthocyanin composition (mg/L; mean±SD, n=3) of 2-month and 12-month Syrah wines (W2 and W12, respectively) after clarification (6 days) with protein fining agents.

	Control	Egg albumin	Potato protein	Pea protein	GSP_{10}	GSP_{30}
W2 wine						
Dp-3-glc	9.88±0.08 $_a$	9.62±0.38 $_a$	9.04±0.38 $_b$	9.06±0.20 $_b$	8.76±0.15 $_b$	8.31±0.53 $_b$
Cy-3-glc	1.32 ±0.02 $_a$	1.37±0.07 $_a$	0.98±0.60 $_a$	1.23±0.01 $_b$	1.30±0.03 $_a$	1.19±0.05 $_b$
Pt -3-glc	11.16±0.18 $_a$	10.09±0.70 $_b$	10.02±0.14 $_b$	10.18±0.24 $_b$	10.07±0.12 $_b$	9.36±0.53 $_b$
Pn- 3-glc	6.48±0.17 $_a$	5.51±0.54 $_b$	6.17±0.09 $_a$	6.26±0.07 $_a$	5.39±0.09 $_b$	5.76±0.32 $_b$
Mv-3-glc	51.95±0.20 $_a$	48.64±2.12 $_b$	49.38±0.77 $_b$	49.18±0.54 $_b$	45.45±0.34 $_b$	45.95±2.34 $_b$
Pt-3-acetylglc	2.86±0.09 $_a$	2.47±0.14 $_b$	2.78±0.09 $_a$	2.89±0.04 $_b$	2.83±0.05 $_a$	2.68±0.13 $_a$
Pn-3-acetylglc	2.76±0.09 $_a$	2.74±0.32 $_a$	2.69±0.14 $_a$	2.49±0.03 $_a$	2.52±0.13 $_a$	2.61±0.19 $_a$
Mv-3-acetylglc	14.22±0.31 $_a$	13.18±1.00 $_a$	13.40±0.04 $_b$	13.07±0.14 $_b$	12.65±0.15 $_b$	12.53±0.75 $_b$
Pt -3-*p*-coumglc	1.65±0.11 $_a$	1.77±0.05 $_a$	1.32±0.05 $_b$	1.38±0.04 $_b$	1.37±0.16 $_a$	1.23±0.09 $_b$
Pn- 3-*p*-coumglc	2.25±0.04 $_a$	1.88±0.39 $_a$	1.97±0.02 $_b$	2.06±0.05 $_b$	2.27±0.57 $_a$	1.89±0.17 $_b$
Mv -3-*p*-coumglc	8.05±0.08 $_a$	7.77±0.07 $_a$	7.11±0.03 $_b$	7.35±0.18 $_b$	6.75±0.25 $_b$	6.67±0.60 $_b$
Total non-acylglc	80.80±0.10 $_a$	75.25±3.82 $_b$	75.59±1.74 $_b$	75.92±1.03 $_b$	70.96±0.34 $_b$	70.57±3.77 $_b$
Total Acetylglc	19.83 ±0.13 $_a$	18.40±1.47 $_a$	18.87±0.24 $_b$	18.41±0.21 $_b$	18.00±0.28 $_b$	17.82±1.04 $_b$
Total *p*-Coumglc	11.95±0.23 $_a$	11.41±0.51 $_a$	10.42±0.06 $_b$	10.80±0.28 $_b$	10.40±0.91 $_b$	9.79±0.86 $_b$
W12 wine						
Dp-3-glc	2.22±0.06 $_a$	2.17±0.07 $_a$	2.23±0.02 $_a$	2.22±0.17 $_a$	2.21±0.01 $_a$	2.24±0.14 $_a$
Cy-3-glc	0.69±0.02 $_a$	0.62±0.05 $_a$	0.67±0.05 $_a$	0.65±0.06 $_a$	0.61±0.05 $_a$	0.63±0.04 $_a$
Pt -3-glc	3.82±0.14 $_a$	3.73±0.06 $_a$	3.81±0.08 $_a$	3.79±0.18 $_a$	3.72±0.04 $_a$	3.73±0.13 $_a$
Pn- 3-glc	3.95±0.13 $_a$	3.72±0.07 $_a$	3.88±0.11 $_a$	3.81±0.05 $_a$	3.80±0.04 $_a$	3.79±0.11 $_a$
Mv-3-glc	30.95±0.43 $_a$	30.51±0.04 $_a$	30.93±0.14 $_a$	30.80±0.95 $_a$	30.33±0.14 $_a$	30.45±0.32 $_a$
Pt-3-acetylglc	1.30±0.07 $_a$	1.35±0.01 $_a$	1.36±0.08 $_a$	1.36±0.05 $_a$	1.36±0.08 $_a$	1.29±0.11 $_a$
Pn-3-acetylglc	2.08±0.02 $_a$	1.98±0.03 $_b$	2.12±0.10 $_a$	1.99±0.06 $_a$	2.03±0.04 $_a$	2.08±0.04 $_a$
Mv-3-acetylglc	11.37±0.21 $_a$	11.42±0.18 $_a$	11.33±0.24 $_a$	11.39±0.36 $_a$	11.10±0.03 $_a$	11.20±0.08 $_a$
Pt -3-*p*-coumglc	0.88±0.11 $_a$	0.88±0.08 $_a$	0.87±0.10 $_a$	0.86±0.03 $_a$	0.90±0.02 $_a$	0.90±0.06 $_a$
Pn- 3-*p*-coumglc	1.61±0.02 $_a$	1.58±0.05 $_a$	1.46±0.05 $_b$	1.48±0.15 $_a$	1.41±0.10 $_b$	1.44±0.01 $_b$
Mv -3-*p*-coumglc	5.36±0.13 $_a$	5.36±0.09 $_a$	5.19±0.16 $_a$	5.23±0.30 $_a$	5.04±0.06 $_b$	5.08±0.08 $_b$
Total non-acylglc	41.63±0.68 $_a$	40.72±0.05 $_a$	41.50±0.20 $_a$	41.28±1.39 $_a$	40.68±0.10 $_a$	40.84±0.63 $_a$
Total Acetylglc	14.75±0.26 $_a$	14.75±0.22 $_a$	14.81±0.27 $_a$	14.74±0.43 $_a$	14.48±0.11 $_a$	14.57±0.21 $_a$
Total *p*-Coumglc	7.86±0.18 $_a$	7.82±0.11 $_a$	7.52±0.22 $_a$	7.57±0.34 $_a$	7.36±0.06 $_b$	7.41±0.11 $_b$

Abbreviations: Dp: delphinidin; Cy: cyanidin; Pt: petunidin; Pn: peonidin; Mv: malvidin; glc: glucose; non-acylglc (non-acyl glucosides; acetylglc: acetylglucosides; *p*-coumglc:*p*-coumaroylglucosides.
Different letters in the same row indicate significant differences ($p<0.05$) for each fining treatment respect to control wine.

Table 3

Table 3. Colorimetric parameters (mean±SD, n=3) of 2-month and 12-month Syrah wines (W2 and W12, respectively) after clarification (6 days) with protein fining agents.

	Control	**Egg albumin**	**Potato protein**	**Pea protein**	**GSP_{10}**	**GSP_{30}**
W2 wine						
L*	52.53 ± 0.06 $_a$	55.42 ± 0.40 $_b$	54.67 ± 0.07 $_b$	54.81 ± 0.16 $_b$	53.63 ± 0.83 $_b$	53.66 ± 0.68 $_b$
a*	51.15 ± 0.21 $_a$	48.56 ± 0.19 $_b$	49.46 ± 0.44 $_b$	49.05 ± 0.40 $_b$	50.02 ± 1.02 $_b$	50.30 ± 0.83 $_a$
b*	-1.29 ± 0.08 $_a$	-1.10 ± 0.13 $_a$	-1.21 ± 0.06 $_a$	-0.95 ± 0.13 $_b$	-0.52 ± 0.55 $_b$	-0.58 ± 0.45 $_a$
C^*_{ab}	51.16 ± 0.21 $_a$	48.57 ± 0.19 $_b$	49.47 ± 0.45 $_b$	49.06 ± 0.39 $_b$	50.02 ± 1.01 $_b$	50.29 ± 0.83 $_a$
h_{ab}	-1.45° ± 0.08 $_a$	-1.29° ± 0.16 $_a$	-1.40° ± 0.04 $_a$	-1.11° ± 0.15 $_b$	-0.59° ± 0.65 $_b$	-0.67° ± 0.53 $_a$
s_{uv}	1.45 ± 0.01 $_a$	1.31 ± 0.01 $_b$	1.35 ± 0.01 $_b$	1.35 ± 0.02 $_b$	1.40 ± 0.06 $_b$	1.41 ± 0.02 $_a$
W12 wine						
L*	59.21 ± 0.02 $_a$	59.68 ± 0.53 $_a$	59.61 ± 0.45 $_a$	60.62 ± 0.02 $_b$	58.82 ± 0.30 $_a$	59.02 ± 0.04 $_b$
a*	42.12 ± 0.71 $_a$	41.32 ± 0.75 $_a$	41.80 ± 0.47 $_a$	40.69 ± 0.75 $_b$	42.17 ± 0.20 $_a$	41.81 ± 0.12 $_a$
b*	6.50 ± 0.15 $_a$	5.97 ± 0.02 $_b$	5.90 ± 0.10 $_b$	6.43 ± 0.19 $_b$	6.55 ± 0.21 $_a$	6.54 ± 0.08 $_a$
C^*_{ab}	42.62 ± 0.69 $_a$	41.75 ± 0.75 $_a$	42.21 ± 0.48 $_a$	41.20 ± 0.72 $_b$	42.68 ± 0.23 $_a$	42.32 ± 0.13 $_a$
h_{ab}	8.78° ± 0.33 $_a$	8.23° ± 0.14 $_b$	8.04° ± 0.07 $_b$	8.98° ± 0.41 $_b$	8.82° ± 0.24 $_a$	8.89° ± 0.09 $_a$
s_{uv}	1.15 ± 0.03 $_a$	1.11 ± 0.03 $_a$	1.12 ± 0.02 $_a$	1.08 ± 0.03 $_b$	1.16 ± 0.01 $_a$	1.14 ± 0.01 $_a$

Different letters in the same row indicate significant differences (p<0.05) for each fining treatment respect to control wine.

Table 4

Table 4. Color variations (ΔE^*_{ab}, ΔL^*, ΔC^*_{ab}, Δh^*_{ab}, mean±SD, n=3) of control wines and wines treated with protein fining agents after 30 days of clarification. Abbreviations: W2 and W12 wine: 2-month and 12-month Syrah wines.

	Control	**Egg albumin**	**Potato protein**	**Pea protein**	**GSP_{10}**	**GSP_{30}**
W2 wine						
ΔE^*_{ab}	9.81 ± 0.06 $_a$	7.21 ± 0.48 $_b$	7.70 ± 0.44 $_b$	7.88 ± 0.34 $_b$	8.64 ± 0.12 $_b$	9.13 ± 0.07 $_a$
ΔL^*	+1.65 ± 0.02 $_a$	+1.06 ± 0.51 $_a$	+0.73 ± 0.54 $_a$	+0.89 ± 0.12 $_a$	+0.53 ± 0.11 $_a$	+0.72 ± 0.27 $_a$
ΔC^*_{ab}	-5.10 ± 0.14 $_a$	-1.67 ± 0.79 $_b$	-3.77 ± 0.88 $_a$	-2.96 ± 0.24 $_b$	-4.88 ± 0.68 $_a$	-4.77 ± 1.19 $_a$
Δh_{ab}	+9.68 ± 0.02 $_a$	+8.25 ± 0.90 $_a$	+7.99 ± 0.01 $_b$	+8.76 ± 0.37 $_b$	+8.51 ± 0.06 $_b$	+9.21 ± 0.79 $_a$
W12 wine						
ΔE^*_{ab}	5.09 ± 0.15 $_a$	5.27 ± 0.28 $_a$	5.67 ± 0.20 $_b$	4.62 ± 0.09 $_b$	4.76 ± 0.25 $_a$	5.05 ± 0.27 $_a$
ΔL^*	-1.23 ± 0.43 $_a$	+0.64 ± 0.33 $_b$	+0.62 ± 0.36 $_b$	-1.39 ± 0.72 $_a$	-1.44 ± 0.10 $_a$	-0.02 ± 0.01 $_b$
ΔC^*_{ab}	+0.63 ± 0.03 $_a$	-0.19 ± 0.26 $_a$	-0.64 ± 0.05 $_a$	+1.55 ± 0.04 $_a$	+1.00 ± 0.57 $_a$	+0.20 ± 0.63 $_a$
Δh_{ab}	+6.47 ± 0.25 $_a$	+7.18 ± 0.20 $_b$	+7.65 ± 0.14 $_b$	+5.66 ± 0.59 $_a$	+5.84 ± 0.16 $_b$	+6.75 ± 0.35 $_a$

$\Delta E^*_{ab} = [(L_{30}-L_0)^2 + (a^*_{30}-a^*_0)^2 + (b^*_{30}-b^*_0)^2]^{1/2}$, $\Delta L^* = L_{30}-L_0$, $\Delta C^*_{ab} = C^*_{ab30}-C^*_{ab0}$, $\Delta h_{ab} = hab_{30}-h_{ab0}$.
Different letters in the same row indicate significant differences (p<0.05) for each fining treatment respect to control wine.

Figure 1
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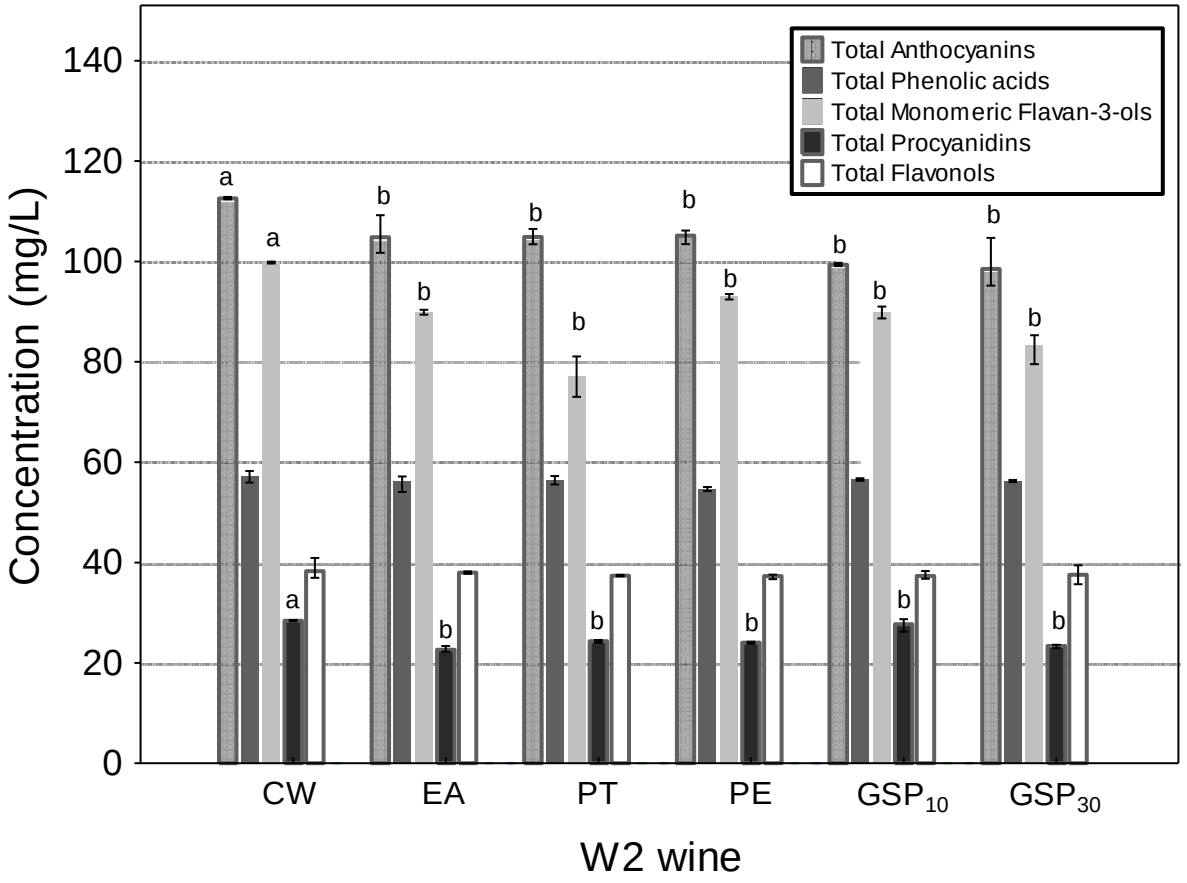
Figure 1.

A.



B.

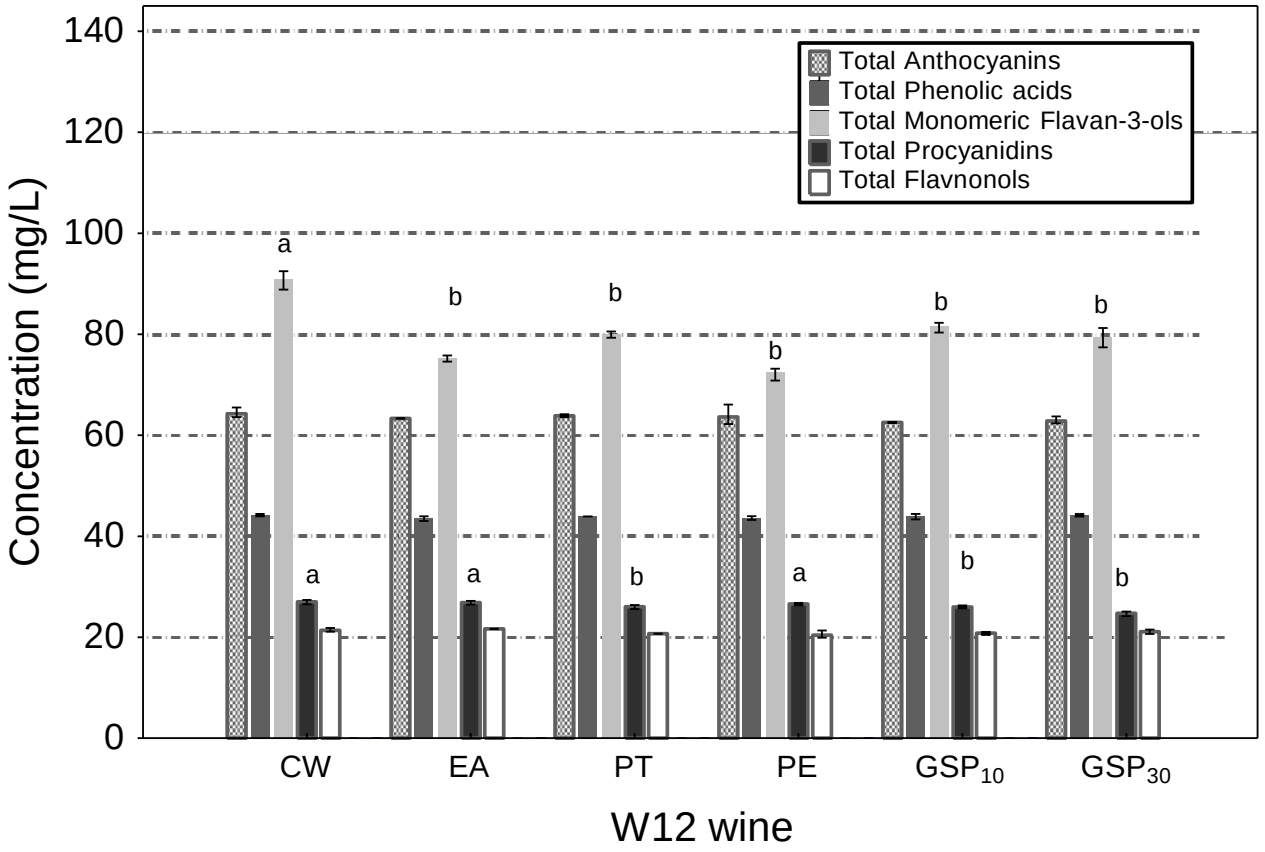

Total Anthocyanins
Total Phenolic acids
Total Monomeric Flavan-3-ols
Total Procyanidins
Total Flavnonols
Concentration (mg/L)
0
20
40
60
80
100
120
140
a
b
b
b
b
b
a
a
b
a
b
b
CW
EA
PT
PE
GSP10
GSP30
W12 wine

Figure 2
Click here to download Figure(s): Gordillo_AlterProtFining_Figure 2.doc

Figure 2.

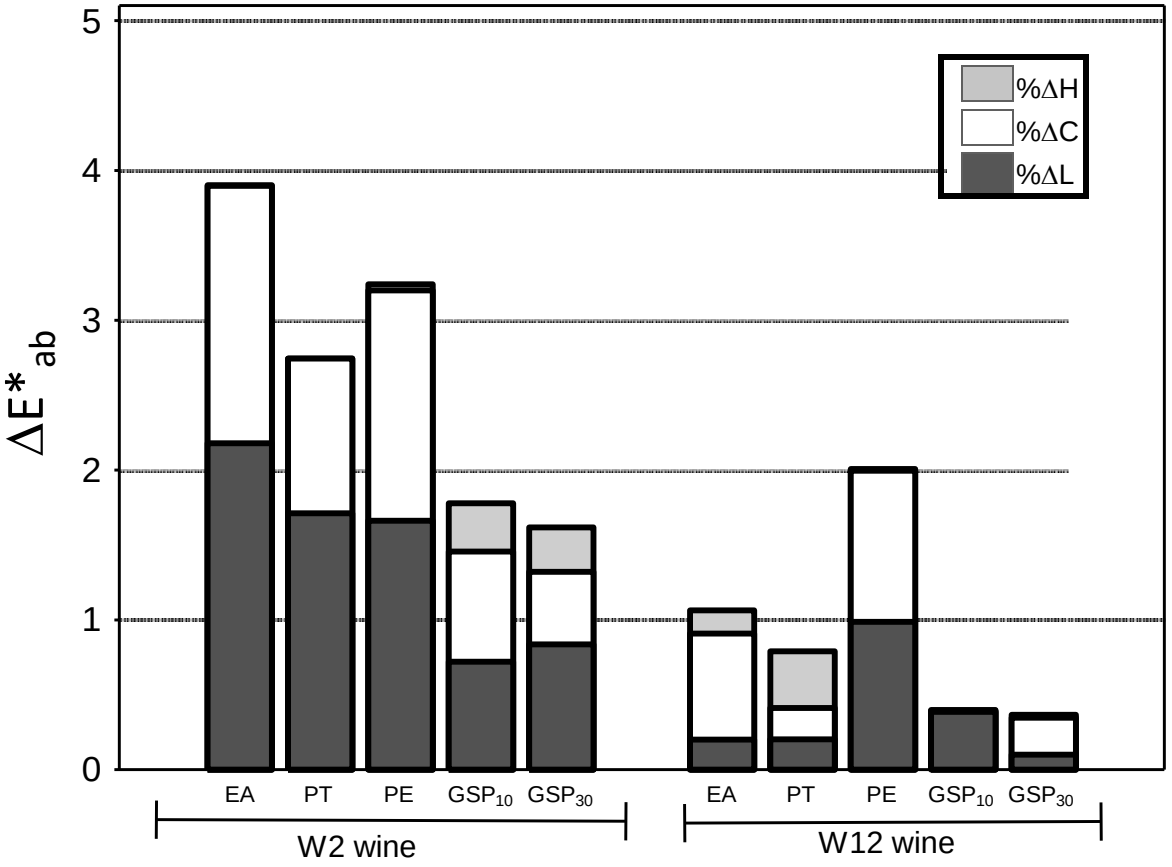


Figure S1
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Figure S1. SDS-PAGE analyses of the fining agents (MMP, molecular weight protein marker; EA: Egg albumin; PT: potato protein; PE: Pea protein; GSP: grape seed protein).

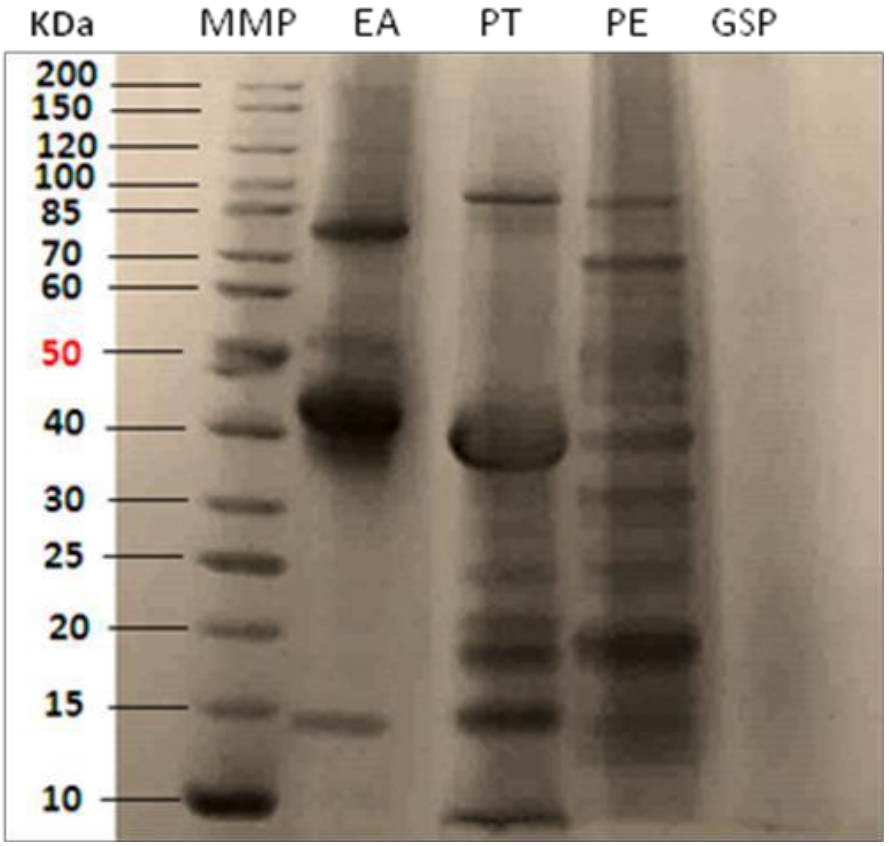

Figure S2
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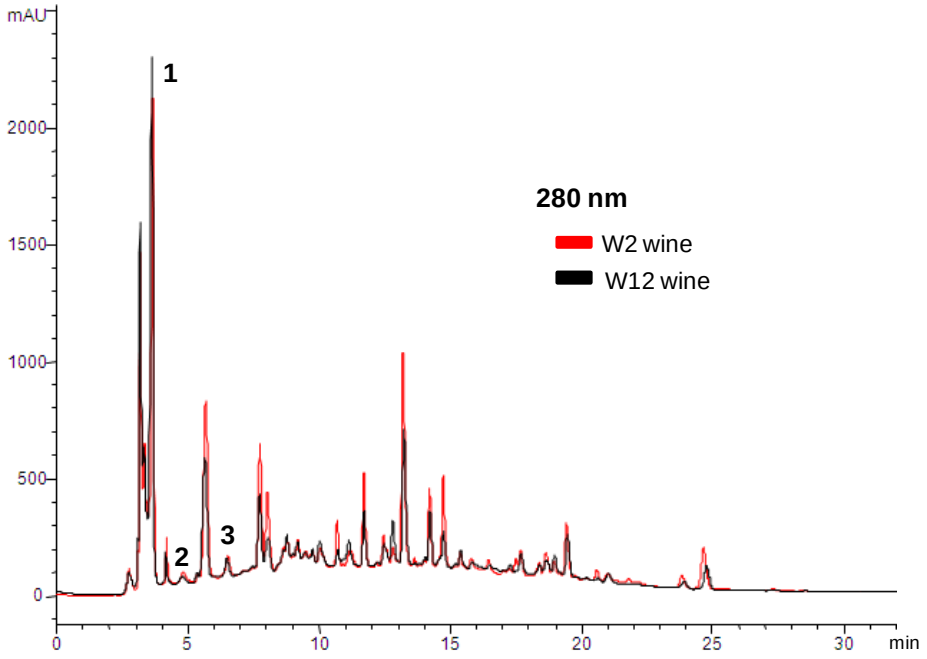
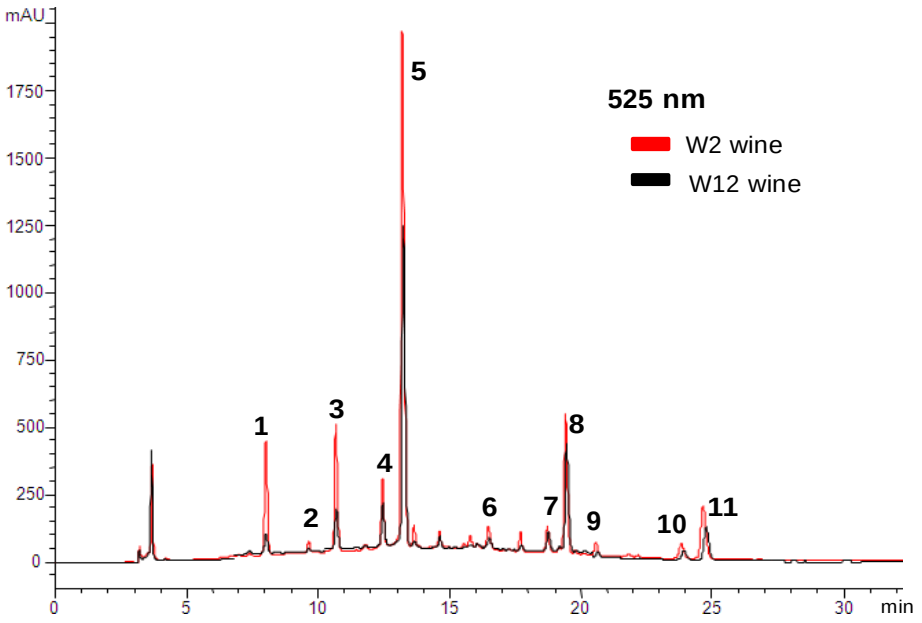
Figure S2. HPLC chromatograms recorded at 280, 525, 320 and 360 nm of W2 and W12 wines. Peaks: **A)** 1, gallic acid; 2, (+)-catechin; 3, (-)-epicatechin; **B)** 1, delphinidin 3-glucoside; 2, cyanidin 3-glucoside; 3, petunidin 3-glucoside; 4, peonidin 3-glucoside; 5, malvidin 3-glucoside; 6, petunidin 3-acetylglucoside; 7, peonidin 3-acetylglucoside; 8, malvidin 3-acetylglucoside; 9, petunidin 3-*p*-coumaroylglucoside; 10, peonidin 3-*p*-coumaroylglucoside; 11) malvidin 3-*p*-coumaroylglucoside; **C)** 1) *t*-caftaric acid; 2) *t*-coutaric acid; 3) *p*-coumaric acid; **D)** 1) myricetin-3-glucuronide; 2) myricetin-3-glucoside; 3) quercetin-3-glucuronide; 4) quercetin-3-glucoside; 5) laricitrin-3-glucoside; 6) kaempferol-3-glucoside; 7) isorhamnetin-3-glucoside; 8) syringetin-3-glucoside.

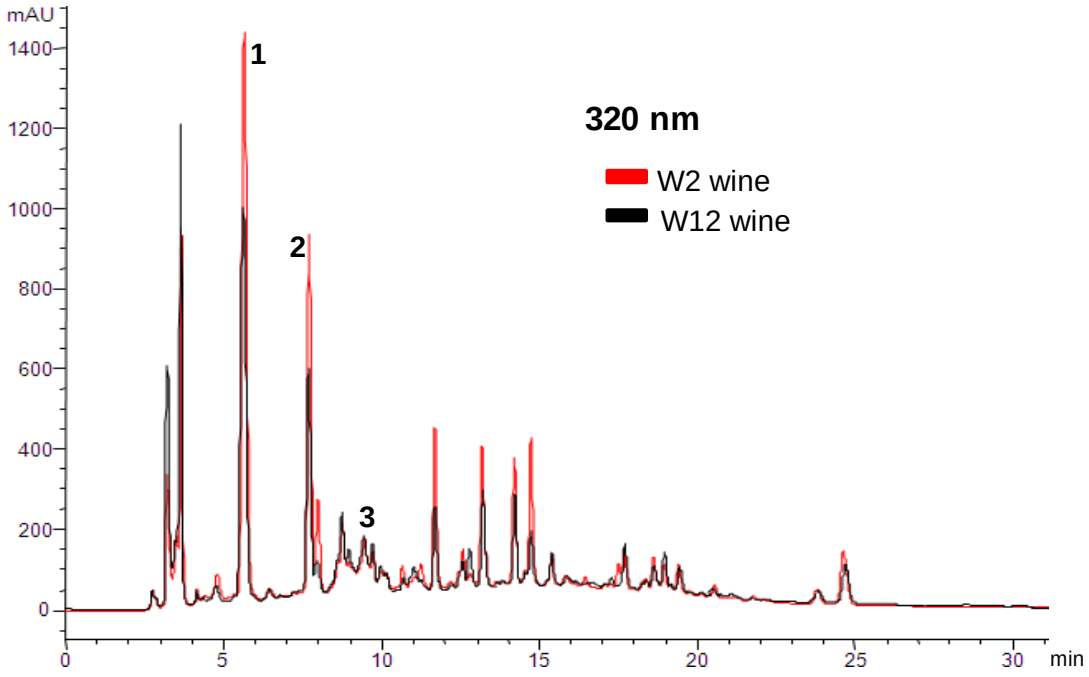
A.



B.



C.


mAU
1400
1200
1000
800
600
400
200
0
1
2
3
320 nm
W2 wine
W12 wine
0
5
10
15
20
25
30
min

D.

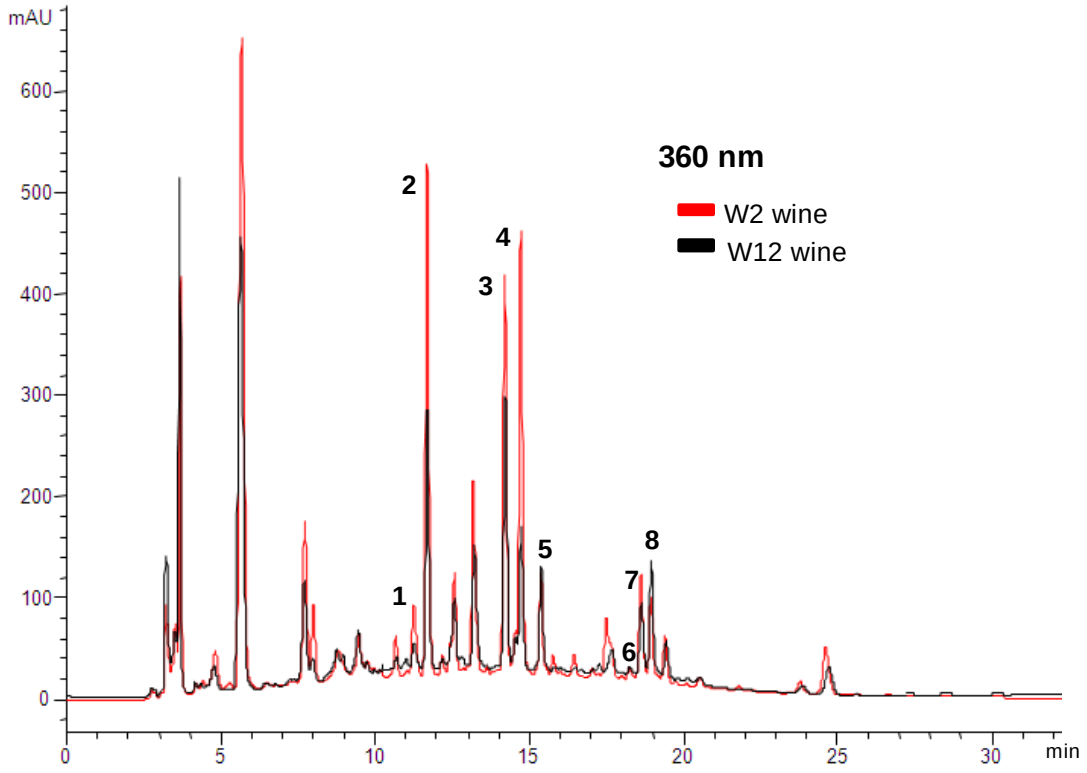

mAU
600
500
400
300
200
100
0
1
2
3
4
5
6
7
8
360 nm
W2 wine
W12 wine
0
5
10
15
20
25
30
min

Figure S3
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Figure S3. RRLC chromatogram recorded at 280 nm of W2 and W12 wines. Peaks: 1, Procyanidin B1; 2, Tetramer 1; 3, Procyanidin B2; 4, Procyanidin B2 3-*O*-gallate; 5, Procyanidin B7; 6, EC Gallate; 7, Tetramer 2.

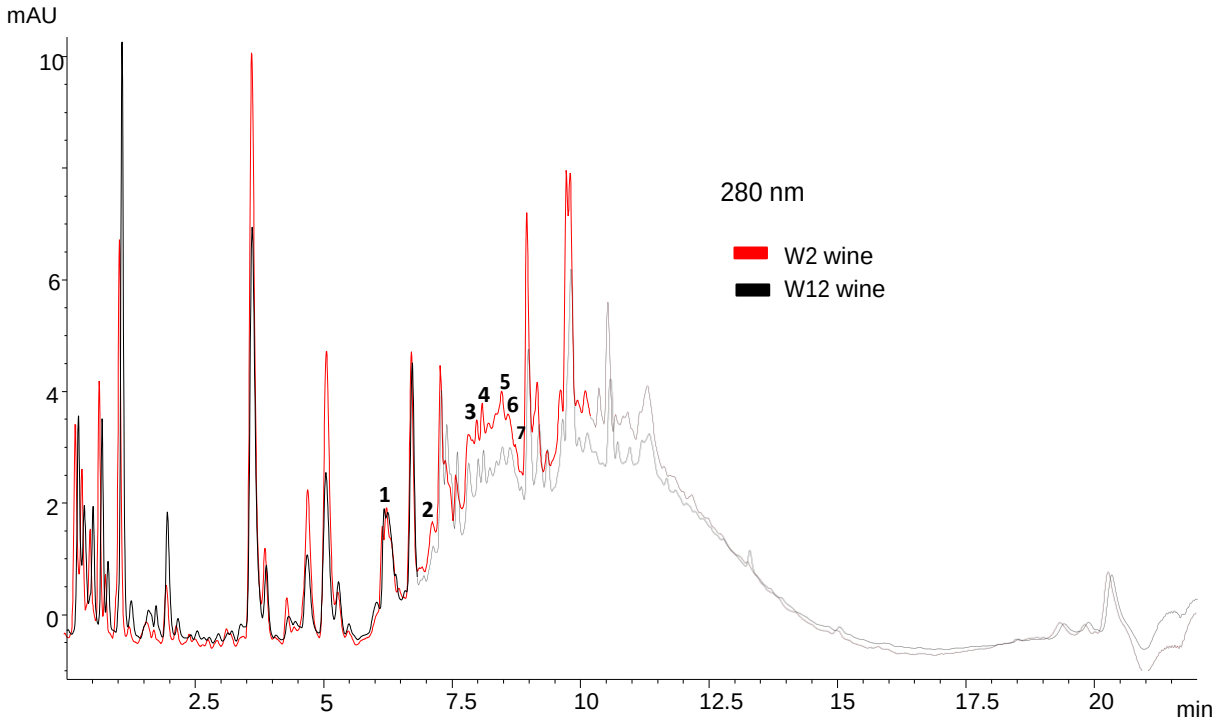

Table S1. Chemical composition the GSP extract (mean values ±SD; n=3).

% w/w	**GSP**
Protein	32.16 ± 0.60
Fat	0
Carbohydrates	53.45± 1.33
Ash	5.76 ± 0.18
Total Fiber	8.03 ± 0.5
Total Phenolics (Folin Ciocalteau)	0.70 ± 0.10

Table S2
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Table S2. Phenolic acid, monomeric flavan-3-ol, procyanidin, and flavonol composition (mg/L; mean±SD, n=3) of 2-month and 12-month Syrah wines (W2 and W12, respectively) after clarification (6 days) with protein fining agents.

	Control	Egg albumin	Potato protein	Pea protein	GSP_{10}	GSP_{30}
W2 wine						
Phenolic acids						
gallic acid	81.89±3.46 $_a$	81.84±0.41 $_a$	85.45±3.13 $_a$	79.29±0.37 $_a$	80.04±0.77 $_a$	78.78±1.35 $_a$
t-caftaric acid	34.02 ±0.68 $_a$	34.11±0.25 $_a$	33.99±0.17 $_a$	32.68±0.43 $_b$	33.72±0.24 $_a$	33.86±0.10 $_a$
t-coutaric acid	17.77±0.41 $_a$	17.75±0.25 $_a$	17.90±0.16 $_a$	17.17±0.11 $_a$	17.71±0.08 $_a$	17.78±0.11 $_a$
p-coumaric acid	4.69±0.51 $_a$	4.91±0.23 $_a$	4.53±0.78 $_a$	4.71±0.49 $_a$	5.10±0.05 $_a$	4.52±0.03 $_a$
Monomeric Flavan-3-ols						
(+)- catechin	43.74±1.69 $_a$	42.59±0.95 $_a$	33.82±2.98 $_b$	43.31±2.15 $_a$	38.95±0.80 $_b$	37.89±1.82 $_b$
(-)-epicatechin	54.81±3.45 $_a$	47.28±0.19 $_a$	39.21±2.70 $_a$	49.72±2.19 $_b$	50.87±1.11 $_a$	45.41±3.23 $_b$
Procyanidins						
procyanidin B1	11.35±0.16 $_a$	7.12±0.49 $_b$	7.93±0.19 $_b$	8.25±0.38 $_b$	8.76±1.26 $_b$	8.50±0.39 $_b$
procyanidin B2	3.89±0.02 $_a$	2.80±0.01 $_b$	3.08±0.09 $_b$	2.81±0.04 $_b$	3.86±0.03 $_a$	2.87±0.19 $_b$
procyanidin B2 3-*O*-gallate	3.72±0.06 $_a$	3.12±0.18 $_b$	2.97±0.09 $_b$	3.17±0.14 $_b$	3.54±0.30 $_a$	2.86±0.04 $_b$
procyanidin B7	2.07±0.04 $_a$	2.12±0.02 $_a$	2.11±0.05 $_a$	2.09±0.02 $_a$	2.13±0.02 $_a$	2.02±0.02 $_a$
EC Gallate	2.47±0.08 $_a$	2.63±0.10 $_a$	2.55±0.13 $_a$	2.75±0.32 $_a$	2.55±0.17 $_a$	2.35±0.04 $_a$
tetramer 1	3.02±0.05 $_a$	2.46±0.03 $_b$	2.28±0.02 $_b$	2.49±0.11 $_b$	2.76±0.34 $_a$	2.44±0.02 $_b$
tetramer 2	2.46±0.03 $_a$	2.69±0.08 $_a$	2.65±0.23 $_a$	2.47±0.05 $_a$	2.68±0.38 $_a$	2.32±0.13 $_a$
Flavonols						
myricetin-3-glucuronide	1.23±0.15 $_a$	1.20±0.06 $_a$	1.08±0.10 $_a$	1.12±0.07 $_a$	1.19±0.07 $_a$	1.13±0.07 $_a$
myricetin-3-glucoside	12.10±0.44 $_a$	11.99±0.06 $_a$	11.85±0.14 $_a$	11.84±0.18 $_a$	1.96±0.17 $_a$	11.74±0.51 $_a$
quercetin-3-glucuronide	9.24±0.49 $_a$	9.18±0.09 $_a$	8.90±0.05 $_a$	8.77±0.09 $_a$	8.89±0.22 $_a$	8.80±0.55 $_a$
quercetin-3-glucoside	10.49±0.64 $_a$	10.57±0.19 $_a$	10.40±0.12 $_a$	10.38±0.32 $_a$	10.38±0.15 $_a$	10.35±0.75 $_a$
laricitrin-3-glucoside	1.46±0.13 $_a$	1.43±0.20 $_a$	1.37±0.04 $_a$	1.42±0.20 $_a$	1.33±0.04 $_a$	1.34±0.15 $_a$
kaempferol-3-glucoside	0.56±0.28 $_a$	0.64±0.14 $_a$	0.63±0.28 $_a$	0.54±0.16 $_a$	0.50±0.15 $_a$	0.44±0.13 $_a$
isorhamnetin-3-glucoside	2.07 ±0.12 $_a$	2.05±0.05 $_a$	1.90±0.19 $_a$	1.97±0.06 $_a$	1.94±0.05 $_a$	1.90±0.16 $_a$
syringetin-3-glucoside	1.13±0.08 $_a$	1.25±0.16 $_a$	1.25±0.26 $_b$	1.24±0.09 $_a$	1.12±0.09 $_a$	1.13±0.05 $_a$
W12 wine						
Phenolic acids						
gallic acid	94.07±0.35 $_a$	93.23±1.00 $_a$	93.54±1.68 $_a$	90.2±2.31 $_b$	94.43±0.60 $_a$	92.97±0.39 $_b$
t-caftaric acid	26.75±0.24 $_a$	26.61±0.76 $_a$	26.52±0.25 $_a$	26.32±0.20 $_a$	26.77±0.02 $_a$	27.18±0.22 $_a$
t-coutaric acid	12.14±0.16 $_a$	12.26±0.12 $_a$	11.71±0.14 $_b$	11.92±0.32 $_a$	11.98±0.28 $_a$	11.99±0.17 $_a$
p-coumaric acid	5.27±0.15 $_a$	5.23±0.41 $_a$	5.66±0.40 $_a$	5.29±0.14 $_a$	5.24±0.37 $_a$	4.98±0.33 $_a$
Monomeric Flavan-3-ols						
(+)- catechin	45.09±3.65 $_a$	36.34±0.23 $_b$	30.90±1.33 $_b$	33.24±0.23 $_b$	34.70±4.21 $_b$	35.26±1.45 $_b$
(-)-epicatechin	45.73±1.93 $_a$	38.35±1.79 $_b$	49.02±0.88 $_a$	39.12±1.56 $_b$	46.86±3.16 $_a$	44.17±0.58 $_b$
Procyanidins						
procyanidin B1	10.12±0.60 $_a$	9.76±0.48 $_a$	9.15±0.30 $_a$	9.55±0.28 $_a$	9.09±0.27 $_a$	8.50±0.18 $_b$

procyanidin B2	3.89±0.02 $_{a}$	3.86±0.05 $_{a}$	3.56±0.29 $_{a}$	3.81±0.02 $_{a}$	3.45±0.17 $_{b}$	3.70±0.12 $_{a}$
procyanidin B2 3-*O*-gallate	3.94±0.11 $_{a}$	3.91±0.05 $_{a}$	3.74±0.31 $_{a}$	3.72±0.06 $_{a}$	3.97±0.17 $_{a}$	3.65±0.32 $_{a}$
procyanidin B7	2.07±0.04 $_{a}$	2.06±0.02 $_{a}$	2.09±0.04 $_{a}$	2.06±0.01 $_{a}$	2.07±0.02 $_{a}$	2.08±0.03 $_{a}$
EC Gallate	2.47±0.08 $_{a}$	2.52±0.05 $_{a}$	2.73±0.10 $_{a}$	2.54±0.07 $_{a}$	2.72±0.19 $_{a}$	2.11±0.32 $_{b}$
tetramer 1	2.28±0.02 $_{a}$	2.28±0.03 $_{a}$	2.23±0.02 $_{a}$	2.24±0.02 $_{a}$	2.23±0.02 $_{a}$	2.21±0.05 $_{a}$
tetramer 2	2.46±0.03 $_{a}$	2.46±0.05 $_{a}$	2.54±0.11 $_{a}$	2.47±0.07 $_{a}$	2.47±0.03 $_{a}$	2.48±0.02 $_{a}$
Flavonols						
myricetin-3-glucuronide	tr	tr	tr	tr	tr	tr
myricetin-3-glucoside	6.06±0.06 $_{a}$	6.14±0.09 $_{a}$	5.97±0.01 $_{a}$	5.88±0.23 $_{a}$	6.02±0.06 $_{a}$	6.05±0.05 $_{a}$
quercetin-3-glucuronide	6.75±0.10 $_{a}$	6.82±0.08 $_{a}$	6.27±0.06 $_{b}$	6.29±0.11 $_{b}$	6.28±0.19 $_{b}$	6.52±0.01 $_{b}$
quercetin-3-glucoside	2.96±0.05 $_{a}$	3.04±0.05 $_{a}$	2.63±0.01 $_{b}$	2.61±0.12 $_{b}$	2.62±0.34 $_{a}$	2.60±0.31 $_{a}$
laricitrin-3-glucoside	1.95±0.03 $_{a}$	1.99±0.04 $_{a}$	1.93±0.01 $_{a}$	1.88±0.11 $_{a}$	1.95±0.03 $_{a}$	1.96±0.03 $_{a}$
kaempferol-3-glucoside	0.05±0.09 $_{a}$	0.24±0.06 $_{b}$	0.10±0.03 $_{a}$	0.04±0.07 $_{a}$	0.10±0.05 $_{a}$	0.15±0.08 $_{a}$
isorhamnetin-3-glucoside	1.22±0.04 $_{a}$	1.26±0.03 $_{a}$	1.26±0.01 $_{a}$	1.23±0.08 $_{a}$	1.27±0.02 $_{a}$	1.34±0.10 $_{a}$
syringetin-3-glucoside	2.35±0.04 $_{a}$	2.38±0.03 $_{a}$	2.53±0.01 $_{b}$	2.48±0.11 $_{a}$	2.56±0.02 $_{b}$	2.47±0.14 $_{a}$

Different letters in the same row indicate significant differences (p<0.05) for each fining treatment respect to control wine.
tr (traces)