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ORIGINAL RESEARCH ARTICLE

Identification and quantification of resolubilised polyphenols from fining precipitation

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ABSTRACT

The aim of this study was to better understand the colloidal phenomenon involved in the fining process and to determine how many polyphenols are impacted in this process. Different types and compositions of fining agent were used to fine the wine. Some of them were pure and based on animal proteins and plant proteins, while others comprised a mixture of different matter, like PVPP and plant proteins, or PVPP, plant proteins and bentonite. Before and after fining, five different analyses were performed on the wine to characterise the polyphenolic composition and content. In order to determine polyphenol loss more precisely during fining, a new method was developed to quantify and characterise polyphenol precipitate using fining agents. This new method allowed us to find some drastic differences between the fining agents in term of total polyphenol precipitation, as well as in the composition of the precipitated compounds. Indeed, a group of anthocyanins present in low levels in wine (i.e., *p*-coumaroylated anthocyanins) became the most represented in the fining precipitate. Similarly, differences were also observed between the fining agents in the composition of precipitated condensed tannins. Fining agents without PVPP did not precipitate monomeric or dimeric flavan-3-ol or crown tannins. Some differences were also observed between the fining agent composed of plant-derived protein and that comprising gelatin.

KEYWORDS: Polyphenols, fining, precipitate, condensed tannins, anthocyanins

INTRODUCTION

Polyphenolic compounds are secondary metabolites that are widely found in the plant kingdom, as well as in plant-derived foods and beverages (Cheynier, 2012). The study of these compounds is essential for improving wine quality in terms of flavour, colour and taste (e.g., astringency and bitterness) (Kennedy, 2008; Li & Sun, 2019). In grape and wine, polyphenols mainly fall into two different groups; non-flavonoid and flavonoid compounds. Non-flavonoid compounds comprise phenolic acids and stilbene, while flavonoid compounds mostly comprise flavanols, condensed tannins (also called proanthocyanidin) and anthocyanins. These polyphenols are known as defence metabolites in plants and they also have good antioxidant properties (Castaldo *et al*., 2019). During the winemaking process, polyphenols are extracted from the skins and seeds of the grape berries to the wine (Hennig & Burkhardt, 1960). Proanthocyanidins and anthocyanins are flavonoid polyphenols related to quality markers, often targeted for quantification in wine (Singleton, 1988). The main anthocyanins in *V. vinifera* cultivars and in the obtained red wine are 3-*O*-monoglucosides of delphinidin, cyanidin, petunidin, peonidin and malvidin. Moreover, these monoglucoside forms can be acylated at the C6″ position of the glucose moiety by aromatic or aliphatic acids, with the most common acylated anthocyanins in *V. vinifera* grape being 3-*O*-(6″-*p*-coumaroyl)-glucosides, 3-*O*-(6″-acetyl)-glucosides and 3-*O*-(6″-caffeoyl)-glucosides (Pinasseau *et al*., 2017). In grape and wine, condensed tannins are composed of five different flavan-3-ol monomers: (+)-catechin, (–)-epicatechin, (–)-epigallocatechin, (–)-epicatechin-3-*O*-gallate and (–)-epigallocatechin-3-*O*-gallate. These monomers can be linked by two types of inter-flavanoid linkages: the B-type linkage, which is a carbon-carbon linkage between the C4-C6 or C4-C8 inter-flavanoid bonds, and the A-type linkage which is a B-type linkage with an additional ether linkage C2-O-C7 or C2-O-C5 (De Freitas *et al*., 2000).

Condensed tannins are known to protect plant cell walls against microorganism and fungi (Cooper & Owen-Smith, 1985). They are also involved in protecting against browsing ruminant and insect herbivores due to their ability to inhibit the fermentation of cell walls and bind proteins that disturb digestive tracts (Cooper & Owen-Smith, 1985). In wine, condensed tannins are known for their astringency due to their interaction with saliva proteins in the mouth (Cheynier *et al*., 2006). Anthocyanins in plants, on the other hand, are known for contributing to the colour of flowers or fruits (Stintzing & Carle, 2004). In wine, anthocyanins are involved in red wine colour stabilisation and evolution due to interaction with condensed tannins to form polymeric pigments (He *et al*., 2012).

The content and composition of polyphenolic compounds in wine is mainly influenced by grape ripeness and variety, as well as the winemaking process used (González-Neves *et al.*, 2014; Maza *et al*., 2019). Fining is a key step in winemaking during which the clarification and colloidal stability of the wine are finalised before bottling (González-Neves *et al.*, 2014). The fining agents used to perform this step mainly interact with the polyphenolic fraction of the wine and they induce the modification of the wine's organoleptic properties. Fining agents can comprise different matter, for example, animal proteins, plants, synthetic polymers like PVPP or mineral matter like bentonite. Depending on the fining agent that is used, its impact on wine colloidal matrix and the polyphenolic content and composition differs (González-Neves *et al.*, 2014). The usual strategy for estimating the impact of a fining agent on wine composition is to analyse wine composition before and after fining (Castillo-Sánchez *et al*., 2008). However, in order to better understand the precipitated polyphenol by the fining agent, resolubilisation via SDS treatment or fractionation on size exclusion gel can be carried out (Sarni-Manchado *et al*., 1999; Maury *et al*., 2001; Maury *et al*., 2003). Unfortunately, due to the experimental conditions of the SDS treatment, not all the common quantifications can be carried out.

The aim of this new method is to directly quantify any polyphenols which are precipitated by different fining agents.

MATERIALS AND METHODS

1. Experimental materials

1.1. Chemicals

Deionised water was from a Milli-Q system (Millipore, Bedford, MA, USA). Ethanol, methanol and acetonitrile were high-performance liquid chromatography quality and were purchased from VWR Chemicals. N,N-Dimethylformamide (DMF) was purchased from Sigma-Aldrich. Formic acid, acetic acid and hydrochloric acid (HCl) were analytical reagent grade and were purchased from Fisher Chemical.

1.2. Harvesting and winemaking

Cabernet-Sauvignon grapes were harvested from a vineyard in the Bordeaux wine region, with a maturity level of 110 days after flowering. The values of the standard oenological parameters in the must were about 213.22 g/L sugar, about 2.75 g/L total acidity and a pH of about 3.51. Microvinifications were conducted in duplicate at the vinification platform (Bordeaux Vinif) of the Institute of Vine and Wine Sciences of Bordeaux (ISVV). The grapes were destemmed, crushed by hand, sulphited with 3 g/hL sulphur dioxide (10 %) and put into 30 L stainless-steel tanks. Alcoholic fermentation began with inoculation using 20 g/hL *Saccharomyces cerevisiae* yeast (Actiflore F33) from Laffort Industry France and the addition of thiazote to reach 200 mg/L of total assimilable nitrogen (half being added at the beginning and the other half at mid-fermentation). Fermentation was carried out at 22°C and monitored by density measurement using electronic densimeter DMA 35 basic (Anton Paar France, Ulis, France). At the end of the alcoholic fermentation, the wine was racked into 5-L glass tanks. After this transfer, malolactic fermentation was performed by inoculation using *Oenoccocus oeni* bacteria from Laffort Industry France (SB3 Direct).

At the end of malolactic fermentation, the temperature of the 5-L glass tank was set to 4°C. Three weeks later, the wine was racked into a bag-in-box sulphited with 5 g/hL suphfur dioxide (10 %) and stocked at 15°C. The values of the standard oenological parameters were approximately: 12.56 % vol. average alcohol by volume (ABV), 2.14 g/L total acidity (TA), 0.15 g/L volatile acidity (VA) and a pH of 3.66.

2. Fining and resolubilisation of the fining precipitate method

2.1. Fining conditions

Fining was performed with fining agents purchased from Laffort Industry France. The studied fining agents and their composition were as follows: Vegecoll (Vege) composed of patatine, Gecoll (Gec) composed of gelatin, Polymust V (PolyV) composed of PVPP and pee protein, and Polymust Press (PPress) composed of bentonite, PVPP and patatine. The doses of fining agent were chosen following the technical recommendations for each product given by the producer: 3 g/hL for Vege, 10 cL/hL for Gec, 80 g/hL for PolyV and 50 g/hL for PPress. Each fining agent corresponded to one treatment and for each treatment the fining was carried out in triplicate. 50 mL of wine was fined in 50-mL plastic flasks. One mL of milli-Q water was added to each fining agent to rehydrate it before addition to the wine. In the case of gelatine, which was liquid, 950 µL of milli-Q water was added to the 50 µL of liquid gelatin needed for 50 mL of wine. After fining, the 50-mL flasks were closed, sealed and stocked at 15°C for 4 days.

2.2. Fining precipitate resolubilisation method

After fining, the 50-mL flasks contained wine as the supernatant with the fining precipitate underneath. The supernatant was carefully removed from each flask and transferred to and stored in new 50-mL flasks at 15°C until analysis. The fining precipitate was transferred into glass tubes and then centrifuged for 5 min at 4500 rpm. After centrifugation, the supernatant was removed and 5 mL of model wine solution (aqueous solution with 12% of ethanol (v/v) and pH adjusted to 3.4 using formic acid) was added. The glass tube was vortexed and centrifuged again for 5 min at 4500 rpm, after which the supernatant was removed. Model solution addition, vortexing, centrifugation and supernatant removal were carried out until the supernatant was clear. After this, 5 mL of acidified DMF containing 10 % formic acid (v/v) was added to the fining precipitate and the whole was vortexed in order to solubilise the precipitate; in the case of the fining agents composed of PVPP or bentonite, centrifugation was carried out for 5 min at 4500 rpm. The supernatants were then filtered using 0.45 µm Agilent PTFE filters prior to analysis.

3. Chemical impact of fining

3.1. Total anthocyanins quantification

Total anthocyanins were determined according to Ribéreau-Gayon and Stonestreet (1965), using a discolouring method. 200 µL of wine or resolubilised fining precipitate, 200 µL of ethanol acidified with 0.1 % of HCl (v/v) and 4 mL of milli-Q water acidified with 2 % of HCl (v/v) were added to a glass tube. Then 1 mL of this mixture was transferred to 2 different tubes: 400 µL of sodium bisulphite at 20 % (v/v) was added to one (tube A) and 400 µL of milli-Q water was added to the other (tube B). After 20 min, the absorbances were measured at 520 nm and the total anthocyanin concentrations were calculated using the following formula: concentration (mg/L) = 875 x (Abs tubeB – Abs tubeA).

3.2. Total condensed tannins quantification

Total condensed tannins were determined according to Ribéreau-Gayon & Stonestreet (1966) based on an adaptation of the Bate-Smith method. This is a specific reaction used to quantify only condensed tannins linked by the B-type interflavanoid linkage; it involves depolymerisation in acidic and high temperature conditions and the formation of anthocyanidin. 80 µL of wine or resolubilised fining precipitate, 5.92 mL of milli-Q water and 6 mL of hydrochloric acid (37%, v/v) were added to two separate hydrolysis tubes. One tube (tube A) was placed in a water bath at 100°C and the other (tube B) tube was placed in an ice bath. After 30 min, the tubes were cooled down and 250 µL of ethanol was added prior to the measurement of their absorbances at 550 nm in a 1-cm-path-length cuvette. The total condensed tannin concentrations were calculated using the following formula: concentration (g/L) = 19.33 x (Abs tubeA – Abs tubeB).

3.3. Quantification and composition of anthocyanins by HPLC-DAD

Anthocyanin content and composition were determined in red wines before and after fining, as well as in the resolubilised fining precipitate according to Ćurko *et al*. (2014). Each sample was filtered through a 0.45 µm Agilent PTFE filter injected on a Thermo-Finnigan Accela HPLC system composed of an autosampler (Accela autosampler), a pump (Accela 600 Pump) and a diode array detector (Accela PDA Detector) coupled to a Finnigan Xcalibur data system. Separation was performed on a reversed phase Agilent Nucleosil C18 column (250 mm x 4 mm, 5 µm). Solvent A comprised 95% milli-Q water (v/v) and 5% formic acid (v/v) and solvent B comprised 95% acetonitrile and 5% formic acid (v/v). The gradients were: from 10 to 35% B in 25 min, then from 35 to 100% B until 35 min, isocratic at 100 % B until 40 min, 100% to 10 % B until 40 to 41 min and finally isocratic at 10 % B until 45 min with a flow rate set at 1 mL/min. Detection was conducted at 520 nm and the concentration of each anthocyanin was expressed as malvidin-3-*O*-glucoside equivalent using a calibration curve.

3.4. Quantification and composition of monomeric and dimeric flavan-3-ol

Monomeric and dimeric flavan-3-ol content and composition were determined in red wines before and after fining, as well as in the resolubilised fining precipitate according to Ćurko *et al*. (2014). Each sample was filtered on 0.45 µm Agilent PTFE filter prior to injection on a Thermo Vanquish HPLC system composed of an Autosampler,

a Thermo Vanquish Pump, and a Thermo Dionex Ultimate 3000 RS Fluorescence detector coupled to Chromeleon software. Separation was performed on a reverse phase LiChrospher 100 RP-18 (250 mm x 2 mm, 5 µm) column using the following condition. The solvent were composed of 99.5 % of milli-Q water (v/v) acidified with 0.5 % formic acid (v/v) (solvent A) and 99.5 % of acetonitrile (v/v) acidified with 0.5 % formic acid (v/v) (solvent B) with the following gradients: from 5 to 18 % B in 30 min, from 18 to 100 % B in 1 min, then isocratic at 100 % B for 7 min, from 100 to 5 % B in 1 min and isocratic at 5 % B for 3 min with a flow rate set at 1 mL/min. The fluorimetric detector was set at an excitation wavelength of 280 nm and emission wavelength of 320 nm of and the concentrations were expressed as mg/L catechin equivalents using a calibration curve.

3.5. Quantification of tetrameric crown tannins

The tetrameric crown tannins content was determined in red wines before and after fining, as well as in the resolubilised fining precipitate according to Jouin *et al*. (2022); Zeng *et al*. (2019). Each sample was filtered using a 0.45 µm Agilent PTFE filter prior to injection on a UHPLC-UV-ESI-QTOF system composed of an Sampler and DAD Agilent 1290 UHPLC and an LC/MS Q-TOF 6530 Agilent Technologies coupled to Mass Hunter software. Separation was performed on a C18 reverse phase Agilent Eclipse Plus (2.1 x 100 mm, 1.8 µm) column. The solvents were composed of 99.9 % mili-Q water (v/v) acidified with 0.1 % formic acid (v/v) (solvent A) and 99.9 % methanol (v/v) acidified with 0.1 % formic acid (v/v) (solvent B) with the following gradients: 6 % B in 0.5 min, from 6 to 95 % B in 13.5 min, then isocratic at 95 % B for 4 min with a flow rate of 0.3 mL/min. The Q-TOF detector allowed quantification to be carried out, expressed as mg/L of tetrameric crown tannins, using a calibration curve.

4. Statistical analysis

Each statistical analysis was performed using "R" software. Normality of the residuals and homoscedasticity were first investigated. If both were respected then a parametric test was performed using the Anova test. If homoscedasticity was not respected, then a visual inspection was carried out to decide whether a non-parameter would be needed or not. If neither normality nor homoscedasticity were respected then the non-parametric test Kruskal Wallis was carried out. Samples were considered as means of the triplicate, and significant differences were identified using the Anova and Kruskal Wallis tests.

RESULTS AND DISCUSSION

Before the development of this new resolubilisation method, the main way of estimating polyphenol loss during fining was to simply analyse the wine before and after fining. With the classical analysis method fining precipitates cannot be investigated. The present new resolubilisation method for determining fining precipitates composition used DMF acidified with 10% formic acid (v/v), which was the best mixture for resolubilising the entire fining precipitate while breaking the interaction between the polyphenol and the fining agent. Their release into solution thus enabled the characterisation and quantification of the precipitated polyphenol. Therefore, in this new strategy, after fining, the upper wine was removed leaving precipitate at the bottom composed of fining agent-polyphenols aggregates and some remaining wine. Thus, the first step of the procedure was to remove the wine from around the aggregates to make sure that after resolubilisation the released polyphenol would only be the polyphenol linked to the fining agent and not the polyphenols from the residual wine around the fining aggregates. That is why a wine-like model solution (aqueous solution with 12% of ethanol (v/v) with pH adjusted to 3.4 using formic acid) was used to remove this residual wine. Thus, the wine-like model solution was added to the precipitate, which was vortexed to re-suspend the aggregate and then centrifuged to remove the supernatant containing the residual wine. This residual wine-removing step was performed 3 times to ensure the complete removal of the residual wine. Then, DMF acidified with 10 % formic acid (v/v) was added to break the interaction between the polyphenol and the fining agent in the aggregates, thereby releasing the trapped polyphenols into the solution. After the two latter steps (i.e., washing of the precipitate and resolubilisation of the precipitate), it was then possible to quantify and characterise the polyphenols precipitated by the fining agent using regular polyphenolic quantification methods.

In order to determine whether this new resolubilisation method was efficient, four fining agents of different compositions were used: i) "Vege" derived from plant protein (patatine), ii)"Gec" from animal protein (gelatin), iii)"PolyV" from plant protein (pea proteins) and polyvinylpolypyrrolidone (PVPP), and iv) "PPress" from plant protein (patatine), polyvinylpolypyrrolidone (PVPP) and bentonite. The three levels of complexity were used to determine whether the method was suitable for use with different types of fining agent commonly used in winemaking. It should be noted that the control triplicate did not have any precipitates, but it still underwent the same steps as the other samples (i.e., washing of the precipitate and resolubilisation of the precipitate). The content and composition of polyphenols in the fined wines and in the corresponding fining precipitates were determined by carrying out five different analyses to find total anthocyanins and total tannins content, composition of anthocyanins, content and composition of monomeric and dimeric flavan-3-ol and crown tannins.

Regarding only the wine after fining, no significant differences were found between the fined wines and the control wine in terms of concentrations of total anthocyanins and total tannins (Figure 1A and Figure 2A). This means that fining with the used concentration of each fining agent did not have enough impact to show significant differences in the wine at the end. However, significant differences were observed in the fining precipitates after resolubilisation in terms of total anthocyanin and total condensed tannin concentration. Regarding total anthocyanin concentration

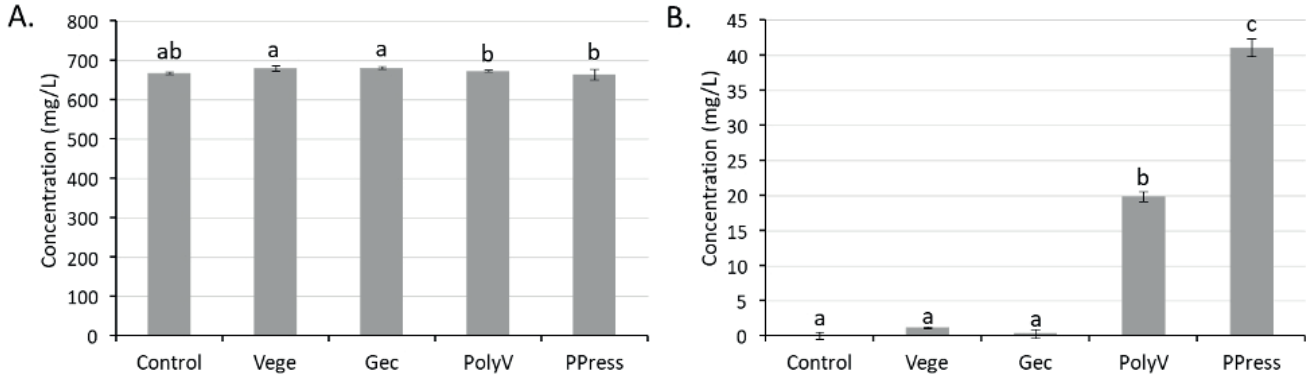

FIGURE 1. Total anthocyanins concentration (mg/L) in A) wines, and B) fining precipitates.

Letters indicate significant differences between treatments with the different fining agents at *p*-value ≤ 0.05. Vege = Vegecoll, Gec = Gecoll, PolyV = Polymust V, Ppress = Polymust Press.

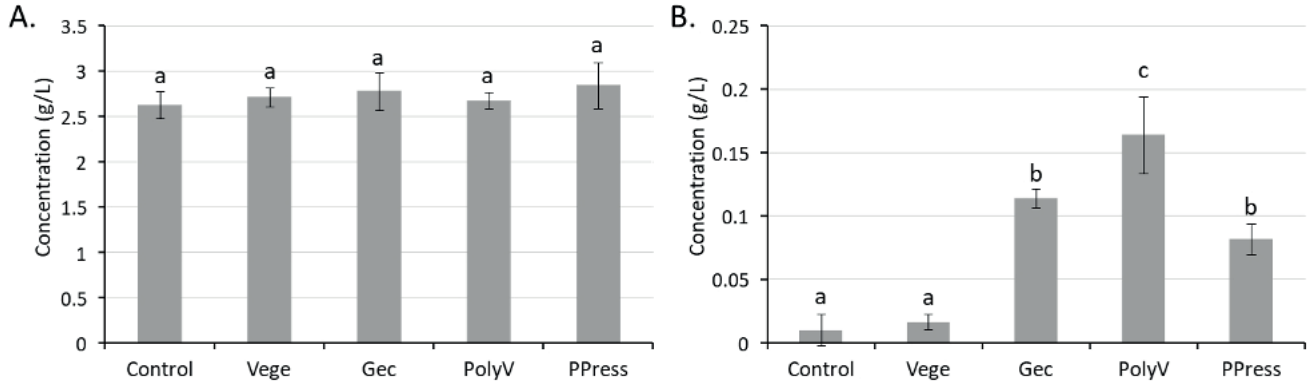

FIGURE 2. Total condensed tannins concentration (g/L) in A) fined wines, and B) fining precipitates.

Letters indicate significant differences between treatments with the different fining agents at *p*-value ≤ 0.05. Vege = Vegecoll, Gec = Gecoll, PolyV = Polymust V, Ppress = Polymust Press.

in the fining precipitates (Figure 1B), the protein-derived fining agents (i.e., Vege and Gec) show a very small release of anthocyanins, while PolyV and PPress show a significant concentration of released anthocyanins, 20 and 41 mg/L respectively. Fining agents containing only plant-derived protein or animal-derived protein appears not to be able to precipitate high concentrations of anthocyanins. On the other hand, PolyV and PPress, fining agents composed of PVPP and bentonite together with protein, were observed to precipitate high levels of anthocyanins. In light of these results, PVPP and bentonite seems to precipitate more anthocyanins than proteic-based fining agents.

In the case of total condensed tannins (Figure 2B), the analyses of the resolubilisation of the fining precipitates from Vege did not reveal any condensed tannins, while the other studied fining agents Gec, PolyV, and PPress showed significant concentrations of total condensed tannins of around 0.11 g/L, 0.16 g/L and 0.08 g/L respectively.

No significant differences in the anthocyanin concentrations in the fined wines, measured by HPLC-DAD, were found between the fining agents, except for a decrease with PPress (Figure 3A). As in the case of total anthocyanin concentrations determined by discoloration, the fining agents derived only from protein precipitated an extremely small amount of anthocyanins, while the quantities of anthocyanins in the PolyV and PPress precipitate were higher (Figure 3) The amount of anthocyanins determined by HPLC-DAD appears to be smaller than that obtained by SO_2 discoloration. This difference can be explained by the fact that SO_2 discoloration can also react with some small polymeric pigments resulting from the reaction between anthocyanins and tannins, leading to an over-estimation of anthocyanin. Moreover, these results also show that the precipitated anthocyanins have a different composition to that in the wine. In order to easily visualise the differences in anthocyanin composition between the wines and the fining precipitates, the amount of malvidin-3-*O*-glucoside (Mv-gluc), malvidin-3-*O*-acetylglucoside (Mv-acetyl) and malvidin-3-*O*-coumaroylglucoside (Mv-coum) found in the samples are shown as a percentage (Figure 3C and Figure 3D). These three different types of malvidin derivatives were considered as being representative, since they are the most abundant in each sub-family. It can thus be clearly seen that the proportion of malvidins derivative in the wine was almost the same no matter which fining agent was used: approximately 47.7 % Mv-gluc,

18.9 % Mv-acetyl and 4.2 % Mv-coum (Figure 3C). By contrast, the anthocyanin composition in the fining precipitates resulting from each fining agent was found to be drastically different to the regular wine anthocyanin composition (Figure 3D). Three different profiles emerged. The fining agents derived only from protein (i.e., Vege and Gec) exhibited an anthocyanin composition in which the three sub-families were almost in equal proportion. The proportions of precipitated malvidins derivatives that were obtained indicate that *p*-coumaroylated and acetylated anthocyanin has more affinity for and interaction with proteins than simple glucosilated anthocyanin. These anthocyanins have also been reported in the literature as being more astringent and having more affinity for salivary proteins (Paissoni *et al.*, 2020). By contrast, with PolyV, the fining agent composed of protein and PVPP, the precipitated anthocyanins were mainly Mv-coum (33.9 %), followed by Mv-acetyl (19.6 %) and then Mv-gluc (16.6 %). The drastic differences in terms of anthocyanin composition can be attributed to the presence of PVPP in this fining agent: due to the presence of the esterified coumaric acid on their glucose moieties, *p*-coumaroylated anthocyanins are more apolar and therefore induce stronger interaction with and affinity for PVPP (Gil *et al.*, 2017). Finally, with the PPress fining agent, which is composed of protein, PVPP and bentonite, the precipitated anthocyanin composition is similar to that of wine: the main precipitated anthocyanin is Mv-gluc, followed by acetylated (23.9 %) and then *p*-coumaroylated (12.5 %) anthocyanins. The fact that the precipitated anthocyanin composition is similar to that of wine in the case of PPress can be explained by the presence of bentonite. Bentonite is a negatively charged clay, and thus the interaction between bentonite and anthocyanin is ionic and is not influenced by the presence of a functional group on the glucose moieties of the anthocyanins.

The results of the flavan-3-ol analysis showed differences between the unfined wine and the fined wines (Figure 4A). The control wine contained the same concentration of flavan-3-ol as Vege and Gec (72.1 mg/L), while PolyV and PPress contained significantly lower concentrations (62.5 mg/L and 67.6 mg/L respectively). These results indicate that fining agents containing PVPP, like PolyV and PPress, are able to precipitate monomeric and dimeric flavan-3-ols. The results of the analysis of the fining precipitates show that Vege and Gec did not precipitate any flavan-3-ols and dimeric tannins, while the PolyV and PPress fining precipitates contained significant concentrations of monomeric and dimeric flavan-3-ols (Figure 4B). The differences between the fining precipitate and wine in terms of flavan-3-ol composition were also investigated and compared. As was observed for anthocyanin composition, the proportions of monomeric and dimeric flavan-3-ols were in the same order of magnitude in the fined wine regardless of the fining agent (Figure 4C). However, the proportions of monomeric and dimeric flavan-3-ols differed between the wines and the fining precipitates: the fining precipitate showed higher levels of (+)-catechin than (–)-epicatechin compared to the wine (Figure 4C and Figure 4D): the (+)-catechin and (-)-epicatechin levels in the fining precipates were

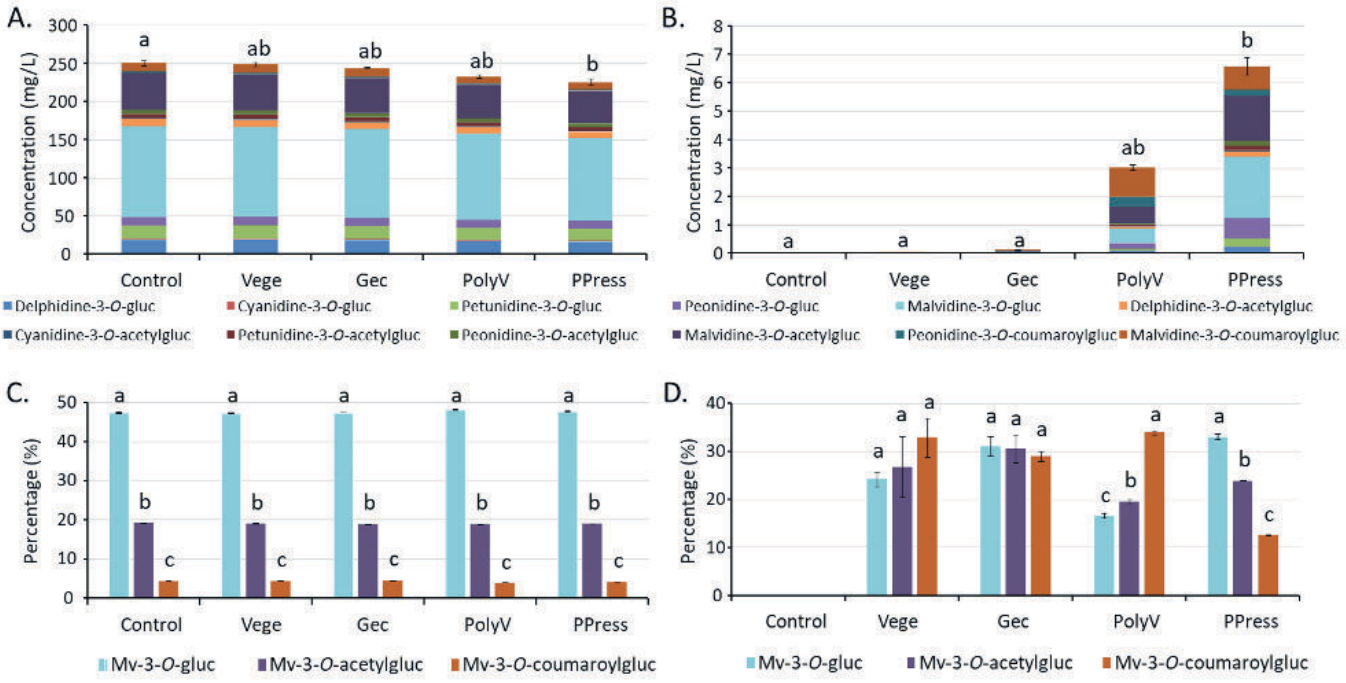

FIGURE 3. Anthocyanin concentrations determined by HPLC-UV-Vis (mg/L) in A) fined wines, and B) fining precipitates. Malvidins proportions according to substitution on the glucoside moieties, as % in C) in fined wines, and D) fining precipitates.

Letters indicate significant differences between treatments with the different fining agents at *p*-value ≤ 0.05. Vege = Vegecoll, Gec = Gecoll, PolyV = Polymust V, PPress = Polymust Press, Mv = malvidin, gluc = glucoside.

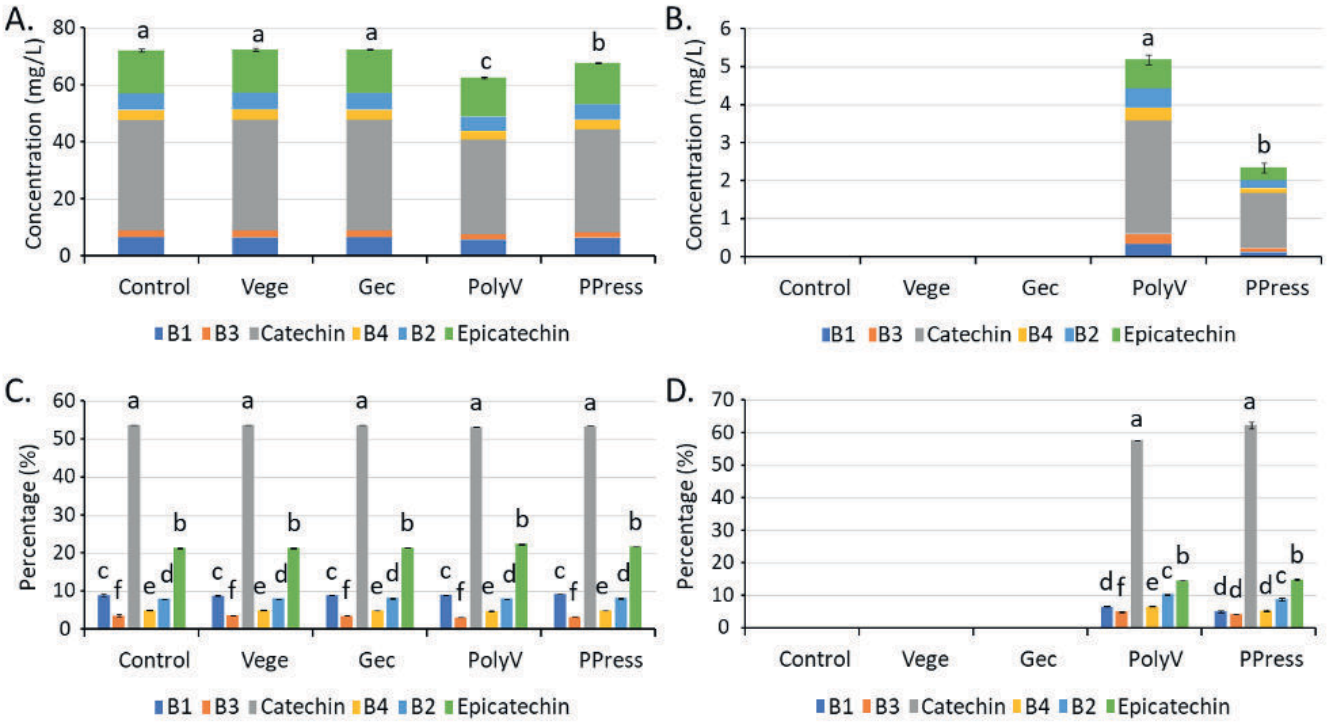

FIGURE 4. Monomeric and dimeric flavan-3-ol concentration (mg/L) in A) fined wines, and B) fining precipitates. Proportion of monomeric and dimeric flavan-3-ol as a percentage (%) in C) fined wines, and D) fining precipitates.

Letters indicate significant differences between treatments with the different finning agents at *p*-value ≤ 0.05. Vege = Vegecoll, Gec = Gecoll, PolyV = Polymust V, PPress = Polymust Press.

57.6 and 14.4 % respectively for PolyV and 62.3 and 14.7 % respectively for PPress, while they represented 53.4 and 21.6 % of the total monomeric and dimeric flavan-3-ol in the wine (Figure 4D). This observation leads to the conclusion that fining agents with PVPP are able to precipitate flavan-3-ols monomers and dimers that have a higher affinity for (+)-catechin than for (-)-epicatechin.

Significant differences were observed between the wines and the resolubilised fining precipitates in terms of crown tannins (Figure 5). The concentration of crown tannins in the fined wines Vege and Gec were significantly the same as in the control wine, but PPress contained a significantly lower concentration (Figure 5A). Regarding the fining precipitates, only the PolyV and PPress fining agents were able to precipitate crown tannins (Figure 5B): PolyV and PPress contained concentrations of 10.3 mg/L and 6.5 mg/L respectively, whereas the protein-based fining agents Vege and Gec did not precipitate any crown tannins, as was the case for favanol and dimeric condensed tannins. As in the case of favanol, the precipitation of crown tannins by the fining agents is mainly linked to the presence of PVPP.

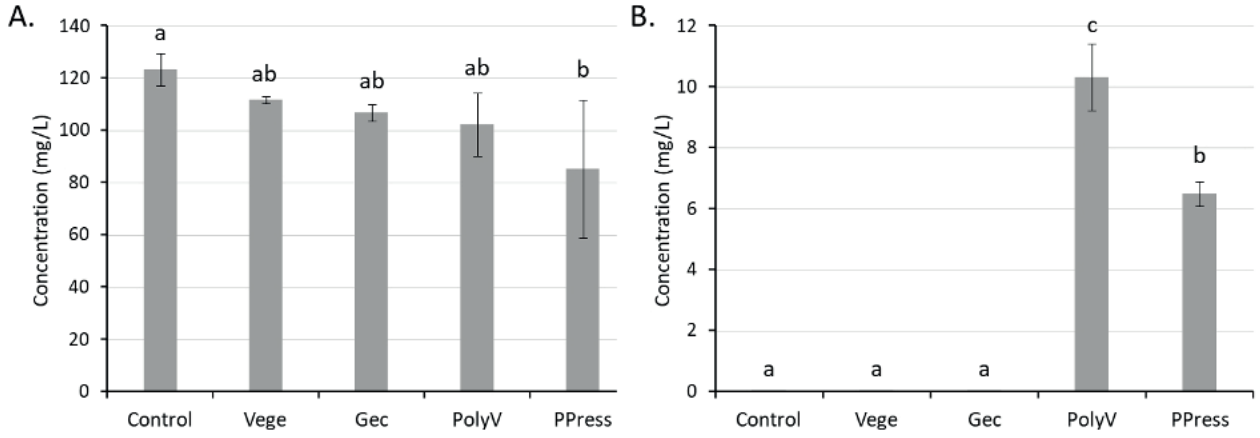

FIGURE 5. Concentration of tetrameric crown tannins (mg/L) in A) fined wines, and B) fining precipitates.

Letters corresponding to significant differences (ANOVA test) with *p*-value < 0.05. Vege = Vegecoll, Gec = Gecoll, PolyV = Polymust V, PPress = Polymust Press.

CONCLUSIONS

The data reported here confirm that polyphenols from fining precipitates are able to resolubilise, allowing them to be characterised and quantified. For the first time, a wide range of polyphenols from fining precipitates were characterised and quantified using this new resolubilisation method. The resolubilisation of the fining precipitates showed significant differences between the fining agents in terms of phenolic content and composition in the precipitates. In the fining precipitates, monomeric and dimeric flavan-3-ol and crown procyanidin were found to interact more with the fining agent composed of PVPP. The amount of precipitated anthocyanins also found in the fining precipitates was mainly influenced by the presence of PVVP and bentonite in the fining agent Moreover, the composition of the precipitated anthocyanins was drastically modified compared to those in the wine, with a minor group of anthocyanins in the studied wine (i.e., *p*-coumaroylated anthocyanins) becoming the most represented in the fining precipitates (except in the precipiate of the fining agent comprising bentonite).

Each component of the fining agents used in the study - as well as other fining agents like casein, egg albumin and fish proteins - would need to be investigated separately in their pure form to determine their respective specific impacts on the precipitation of polyphenols. Nonetheless, this new resolubilisation method is very innovative and could be an important tool for increasing the understanding of the impact of fining on wine composition and its organoleptic influence. This method was also found to be applicable to the investigation of colloidal precipitate, like the precipitation of coloured matter and filter cakes, which could increase knowledge of the colloidal phenomenon involved in fining precipitation and the stability and conservation of red wine. Further research would be required determine the structure and composition of the precipitated tannins (i.e., The monomeric composition, main degree of polymerization) and to determine which tannins are more susceptible to being precipitated in the presence of different fining agents. The structural parameters of condensed tannins could provide information about which tannins are more susceptible to being precipitated by the different fining agents. Similarly, the impact of fining on the polymeric pigments formed during ageing and on oak wood tannins could also be investigated using the reported strategy.

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