

First Insight into Lignin Valorization as a Promising Biopolymer for the Modulation of the Physicochemical Properties of Port Wine

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ABSTRACT: Lignosulfonate (LS), kraft lignin (KL), and organosolv lignin (OL) were evaluated as potential modulating agents of the physicochemical properties of Port wine at two different concentrations for 7 and 30 days. KL and LS demonstrated the ability to remove proteins and potentiate the anthocyanin concentration. LS reduced the tannin content and the interaction of salivary acidic proline-rich proteins with wine phenolic compounds. None of the lignin promoted a perceptible color change; however, the yellowish color of KL and OL at 100 g/hL contributed to an increase in the yellow tones of wines. Lignin improved wine aroma by reducing the amount of unwanted volatiles by 30% and increasing the content of ethyl esters associated with fruity aromas by up to 60%. The results suggest that lignin, especially LS, can be employed as a modulating agent, positively impacting wine's physicochemical properties. This valorization of a byproduct opens up new opportunities for the wine industry.

KEYWORDS: lignosulfonates, kraft lignin, organosolv lignin, colloidal particles, protein haze, phenolic compounds, volatile compounds, astringency

1. INTRODUCTION

Winemaking is a dynamic and interactive process responsible for transforming grapes into wine. During fermentation and aging, various solids and colloidal particles, such as grape skin, tartrate crystals, and microorganisms (yeast and bacteria cells) can form.¹ These particles, solutes, and macromolecules can significantly affect wine's stability, clarity, texture, taste, and production efficiency. Even after wine bottling, the presence of suspended and colloidal particles increases the turbidity due to denaturation or agglomeration of unstable proteins, tannins, carbohydrates, and other macromolecules.^{1,2} Additionally, undesirable colors can appear as a consequence of reactions between wine components, or the oxidation of wine phenolic compounds.^{3,4} Besides color, wine aroma significantly influences consumer acceptability. It is a complex mixture of volatile molecules present at various levels (ng/L up to mg/mL) in the wine that exceeds the sensory threshold. A volatile compound may have a pleasant aroma at lower concentrations but can contribute to an unpleasant odor at higher concentrations. The occurrence of chemical reactions during wine aging can also form volatile compounds associated with off-odors.^{5,6} Imbalance among astringency, bitterness, and acidity is the main factor related to taste and tactile defects. Wine astringency is a consequence of the presence of proanthocyanidins (condensed tannins) responsible for sensations of drying and roughness.⁷ Among the several mechanisms proposed to explain astringency perception, the interaction between salivary proteins (SP) and tannins, with precipitation of the insoluble complexes, has been the most accepted one.⁸ All of the referred phenomena generally reduce the quality and/or safety of wine, resulting in economic losses

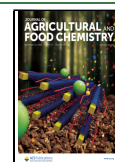
for the wine industry. To prevent their appearance, in addition to the natural sedimentation or filtration,^{3,4} the use of fining agents is a more common and straightforward procedure including bentonite, colloidal silica, phenolic substance complexes, and synthetic polyamides such as poly(vinylpyrrolidone) (PVP) and poly(vinylpolypyrrolidone) (PVPP).⁹ Extensive research suggests animal proteins (bovine proteins, porcine gelatin, egg albumin, and casein) as potential clarifying agents; however, they have various disadvantages, including ethical and religious considerations and they are well-known food allergens that can pose a risk to consumers.¹⁰ To overcome these issues, plant-based proteins (e.g., from grape seeds,¹¹ potatoes,¹² and vegetables¹³), as well as nonprotein plant-based materials (e.g., cell wall polysaccharides^{14,15} and pomace materials¹⁶) have been the focus of research in this area. This work presents a new approach for the wine industry, using lignin as a putative agent to modulate the physicochemical properties of wines. Lignin is a complex macromolecule found abundantly in nature (plant cells) and used as a raw material for several applications, including the chemical industry. Depending on the plant species (hardwood, softwood, or herbaceous plants) and the extraction method, lignin can have different proportions of linkages and functional

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groups, affecting their chemical and structural characteristics.¹⁷ This natural biopolymer has several advantages, including biodegradability, high stability, low cost, and low toxicity. The high molecular weight, the presence of several functional groups, and the structure with high porosity are crucial characteristics that can influence the efficiency of lignin in modulating the chemical composition and improving the wine quality. Various lignin types were applied, specifically lignosulfonate (LS), kraft (KL), and organosolv lignin (OL) determining their impact on the removal of colloids and unwanted particles, protein and tannin contents, anthocyanin concentration, color stability, and aroma profile. By assessing these physicochemical properties, the study explored the feasibility of using lignin as a sustainable and effective alternative for improving wine quality while maintaining its inherent flavor and color characteristics.

2. MATERIALS AND METHODS

2.1. Wine Sample. The wine used in this work was a ruby-style Port wine, produced from red grapes and supplied by Granvinhos, a company from the Douro region in Portugal. It was produced in the harvest of 2022 and was provided without any treatment six months after fermentation. The standard oenological parameters determined by the company's laboratory are summarized in Table 1. The pH of the wine samples was also measured after contact with lignin (Table S1).

Table 1. Physicochemical Analysis of the Port Wine Sample at 20 °C

parameters	values
alcohol content (ABV)	18.5% (v/v)
Baumé degree	3.15
density	1.02 g/L
total acidity (tartaric acid equivalents)	4.14 g/L
total sulfur dioxide	48 mg/L
volatile acidity	0.21 g/L
L-malic acid	1.24 g/L
pH	3.62

2.2. Lignin Samples. LS and KL, byproducts from the pulp and paper industry, were supplied by Portuguese companies. LS was obtained from a softwood sulfite liquor (SSL, total solids, 11.3% w/w liquor) after an ultrafiltration process using a Synder MT membrane (cutoff of 5 kDa) performed in a G.E. Osmonics SEPA CF II cross-flow filtration system. More details about the composition and structure of LS are described in the literature.¹⁸ KL was isolated from industrial *Eucalyptus globulus* kraft liquor collected in a Portuguese pulp mill. The extraction procedure and chemical characterization are reported in the literature.¹⁹ OL was isolated from corn cob supplied by a local farmer, involving two stages: autohydrolysis and ethanol organosolv treatment. Details about the experimental procedure and physicochemical properties are available in the literature.²⁰

2.3. Experimental Design. In this work, three types of lignin were studied as modulating agents, namely, the LS, KL, and OL at two concentrations (25 and 100 g/hL). These concentration values were chosen based on the amount of commercial bentonite used in the wine industry (40 g/hL), and two extreme values were selected to compare the effect. To achieve this, 100 mL of Port wine was combined with the specific type and concentration of lignin in a sealed glass bottle. Then, the mixture was shaken and maintained in the dark at room temperature. The experiments were carried out in triplicate for each type of lignin and concentration. Wine samples were analyzed after 7 and 30 days of contact with lignin. After this time, the control and wine samples treated with lignin were centrifuged at 10,000 rpm for 12 min. Bentonite (40 g/hL) was

also used as a reference since it is a widely used commercial fining agent. The experimental design is represented in Figure 1.

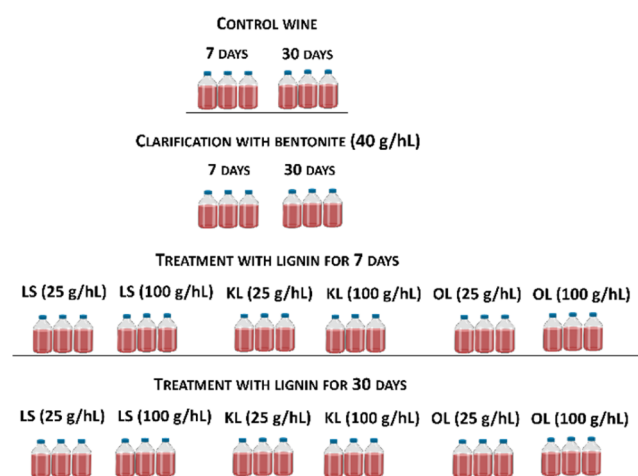


Figure 1. Experimental design used to evaluate the potential of different types of lignin as modulating agents for wine clarification (LS: Lignosulfonate; KL: Kraft Lignin and OL: Organosolv Lignin).

2.4. Physicochemical Analysis. **2.4.1. Determination of Wine Turbidity.** The assessment of wine turbidity allowed us to confirm the presence of suspended solids in the samples, proving wine clarity. The turbidity of the samples was measured before and after the centrifugation step (10,000 rpm for 12 min) using a turbidimeter HACH 2100 N equipped with a cell adapter of 100 × 12 mm.

2.4.2. Thermal Stability Test. The heat test is a simple method to predict the possibility of a wine becoming hazy. The samples were heated at 80 °C for 2 h to unfold the haze-forming proteins, followed by cooling at 20 °C, for 3 h. The turbidity of the samples was measured before and after heat treatment using a HACH 2100 N turbidimeter equipped with a cell adapter of 100 × 12 mm.

2.4.3. Protein Quantification. The concentration of proteins present in the wine samples was quantified using the bicinchoninic acid assay (BCA) and following the guidelines described by Thermo Scientific.²¹ It is based on the reduction of the copper cation in an alkaline medium by the proteins present in the samples, which results in a purple color formation by bicinchoninic acid. Before quantification, proteins were precipitated with 20% (m/v) trifluoroacetic acid (TCA) in acetone to eliminate interferences. Wine samples were mixed with the TCA solution (1:1), left for 1 h at −20 °C, and then centrifuged at 10,000 rpm for 10 min. Then, the pellet was removed, washed, and resuspended in water. In 96-well plates, 10 μL of each pellet solution or bovine serum albumin (BSA) standard solution were mixed with 200 μL of working reagent (50:1, Reagent A: B, Pierce BCA Protein Assay Kits). The plate was covered and incubated for 30 min at 37 °C. Then, the mixture was cooled to room temperature, and the absorbance was recorded at 562 nm on a plate reader (Biotek Powerwave XS). BSA was used as a standard for the calibration curve (Figure S1) to quantify the protein content in wine control, wine clarified with bentonite, and wine samples with the different types of lignin.

2.4.4. Phenolic Compound Quantification. The total phenolic index (TPI) is based on the ability of benzene rings to absorb UV light. The wines were diluted 20 times with a filtered wine model solution (12% ethanol, v/v, 5 g/L tartaric acid at pH 3.5) before being measured in a quartz cuvette using UV–vis spectroscopy (Cary 60—Agilent). TPI was calculated by multiplying the absorbance at 280 nm by the dilution factor. The total phenolic compounds were also estimated based on the Folin-Ciocalteu colorimetric method,²² where the intensity of the blue complex is proportional to the concentration of phenolic compounds. The wine samples were diluted 20 times and then mixed with the redox agents. The results were expressed as gallic

acid equivalent per milliliter of wine (mg of GAE/mL), considering the calibration curve in SI (Figure S2).

2.4.5. Quantification of Total Proanthocyanidins (Condensed Tannins). The concentration of PACs in wine samples was determined following the method described by Ribéreau-Gayon et al.²³ The total PACs were quantified based on their oxidative depolymerization in acidic medium at a high temperature (100 °C) to give colored anthocyanidin pigments.²³ 4 mL of wine sample (diluted 50 times with a wine model solution), 2 mL of deionized water, and 6 mL of 37% HCl (v/v) were added to a Pyrex tube. After that, the tubes were closed and heated at 100 °C for 30 min. The tubes were then cooled in cold water and mixed with 1 mL of ethanol. These samples were prepared in triplicates. A solution with the same composition but without heating was obtained for each wine sample as a reference. Then, the absorbance of all solutions was determined at 520 nm by using a glass cell with an optical path of 1 cm in a UV–visible spectrophotometer (Cary 60 Agilent). The concentration of condensed tannins was determined through a calibration curve ($Abs = 0.0456 [PACs] + 0.0178$) prepared by a mixture of oligomeric PACs and expressed in g/L.

2.4.5.1. Tannin-Specific Activity (TSA). This methodology is characterized by the interaction of phenolic compounds, namely, tannins, with proteins, resulting in the formation of aggregates that increase light diffraction. The protocol to determine the tannin-specific activity (TSA) is described in the literature²⁴ and it was performed with some modifications. For that, samples were diluted 50 times with a filtered wine model solution. Then, 4 mL of the diluted wine samples were placed in a glass tube, and the turbidity (blank) was recorded using a turbidimeter HACH 2100 N equipped with a cell adaptor of 100×12 mm. Then, 150 μ L of a solution of bovine serum albumin (BSA) at 0.8 g/L was added, mixed in the vortex, and left at room temperature in the dark for 30 min. After this time, the turbidity was determined. Data were expressed in turbidity units per milliliter of wine (NTU/mL), and determined using the equation:

$$\text{turbidity (NTU/mL)} = \frac{(\text{sample turbidity} - \text{blank sample})}{0.08} \quad (1)$$

where 0.08 corresponds to the dilution factor of wine.

2.4.6. Interactions of Saliva with Supplemented Wines. To obtain the first insights into the possibility of different types of lignin being able to modulate salivary protein (SP) precipitation, we studied the interaction between saliva and wine treated with lignin. Bentonite, a commonly used fining agent, was used as a commercial standard to compare the effectiveness of different types of lignin in reducing SP interaction and, consequently, astringency perception.

Saliva was collected from 20 healthy volunteers (aged 25 to 45 years old) who received specific instructions to avoid food/beverage intake before saliva collection, as already reported.^{25,26} Briefly, human saliva was collected at 2 p.m., pooled together (≈ 50 mL), and treated with TFA (final concentration of 0.1%) to inhibit intrinsic proteases and precipitate high-molecular-weight proteins. After centrifugation (8000 g, 5 min), the supernatant was recovered and the final pH was adjusted to 6–7 to represent the biological pH of human saliva. Before saliva collection, all the participants signed informed consent. This study was permitted by the Ethics Committee of the Faculty of Sciences of the University of Porto (EK84032011) and carried out according to the Declaration of Helsinki (The World Medical Association).

The interaction with wine before and after treatment (7 and 30 days) was carried out using a fixed volume of saliva (90 μ L). After the addition of 30 μ L of wine or wine model solution to saliva, the solutions were mixed and interacted for 10 min at 37 °C.^{27,28} Then, they were centrifuged and the supernatant (SP that did not interact) was analyzed by high-performance liquid chromatography (HPLC). Ninety microliters of each supernatant were injected into a JASCO HPLC-DAD system (LC-4000) with a Vydac C8 column (150×2.1 mm, 5 μ m particle diameter), and the detection was carried out at 214 nm. The eluents were 0.2% TFA in H₂O (A) and 0.2% TFA in ACN/H₂O 80:20 (v/v) (B). The gradient was linear from 11 to 70% eluent

B in 70 min at a flow rate of 0.3 mL/min. After each injection, a washing step was performed with 100% eluent B and then the initial conditions. A typical SP profile obtained by HPLC analysis with detection at 214 nm is represented in Figure S3.

2.4.7. Analysis of Anthocyanins. **2.4.7.1. Total Free Anthocyanins.** Free anthocyanins react with the bisulfite anion yielding the noncolored adduct which can be used to determine the concentration of free anthocyanins. The concentration of free anthocyanins was determined using the method described by Chris Somers et al.²⁹ with some modifications. For that, a solution with 40 mL of 2% HCl, 2 mL of ethanol, and 2 mL of wine sample was prepared. Then, 10 mL of this solution was added to four glass tubes. To three of them, 4 mL of 20% (v/v) sodium bisulfite 20% was added, and, to the fourth, 4 mL of deionized water (blank). The mixtures were homogenized in a vortex and left in the dark for 20 min. Next, the absorbance was determined at 520 nm by using a glass cuvette with an optical path of 1 cm in a UV–visible spectrophotometer (Cary 60 Agilent). The concentration of anthocyanins was determined by the difference between the blank absorbance and the samples treated with sodium bisulfite. The values expressed in mg/L were obtained by a calibration curve of standard solutions of malvidin-3-O-glucoside.

2.4.7.2. Quantification of Anthocyanins by High-Performance Liquid Chromatography with Diode-Array Detection (HPLC-DAD). Anthocyanins present in the wine samples were quantified using the HPLC-DAD (Thermo Scientific, Dionex Ultimate 3000) on a reversed-phase C18 column (Agilent) with 250×4.6 mm i.d., 2.7 μ m at 25 °C column chromatography. The solvents were A: H₂O/HCOOH (v/v) and B: HCOOH/CH₃CN (v/v), with a flow rate of 0.4 mL/min. The gradient consisted of 3–30% of solvent B for 31 min. Then, the column was washed with 100% of B for 10 min and stabilized with the initial conditions for 10 min. Twenty μ L of each sample were analyzed by HPLC-DAD in triplicates. The anthocyanins concentration was expressed in mg/mL using malvidin-3-O-glucoside as a standard.

2.5. Chromatic Properties. **2.5.1. Color Intensity (CI).** The color intensity (CI) of the wine samples was determined by the sum of the absorbances at 420, 520, and 620 nm. For this experiment, the wine samples were filtered with a filter of 0.45 μ m, and then, the absorbance was determined in a UV–vis spectrophotometer (Cary 60 UV–vis Agilent) using quartz cells with a 1 mm optical path.

2.5.2. CIELAB Color Coordinates. CIELAB color coordinates are a color system to quantify and measure the color of samples. The values were obtained by using Color software in a UV–vis spectrophotometer (Cary 60 Agilent). The CIELAB color coordinates of each sample were determined in triplicate by using glass cells with an optical path of 2 mm. The illuminant condition D65 and an observer angle of 10° were used.

2.6. Dialysis Index (DI). The dialysis index can be correlated with the structural complexity of the phenolic compounds present in the wine. It was performed according to the literature^{30,31} starting with the preparation of a reference solution using a volumetric flask (50 mL) to dilute 5 mL of wine control or wine-lignin sample with the model wine solution (D_0). At the same time, a dialysis membrane (cellulose, 6 mm i.d., cutoff 12–14 kDa) containing 5 mL of wine samples was placed inside the glass bottle (50 mL) with model wine solution (D). The bottles and the volumetric flasks were sealed, left at room temperature, and protected from the light for 24 h. After this time, the absorbance at 280 nm was determined in a quartz cuvette with an optical path of 1 mm by using the UV–visible spectrophotometer (Cary 60 UV–vis Agilent). Dialysis index (DI) was determined using the eq 2:

$$DI = \frac{(D_0 - D)}{D_0} \quad (2)$$

2.7. Analysis of Volatile Compounds by HS-SPME-GC/MS.

2.7.1. Chemicals. Ethanol (99.9%) was purchased from ERBA Reagents (Val de Reuil, France). 1-Hexanol (99.9%), 3-hexen-1-ol (98%), 5-methyl-2-furfural (99%), benzaldehyde ($\geq 99.5\%$), diethyl succinate ($\geq 99\%$), ethyl 2-methyl butanoate (99%), ethyl butanoate (99%), ethyl decanoate ($\geq 98\%$), ethyl hexanoate (99%), ethyl

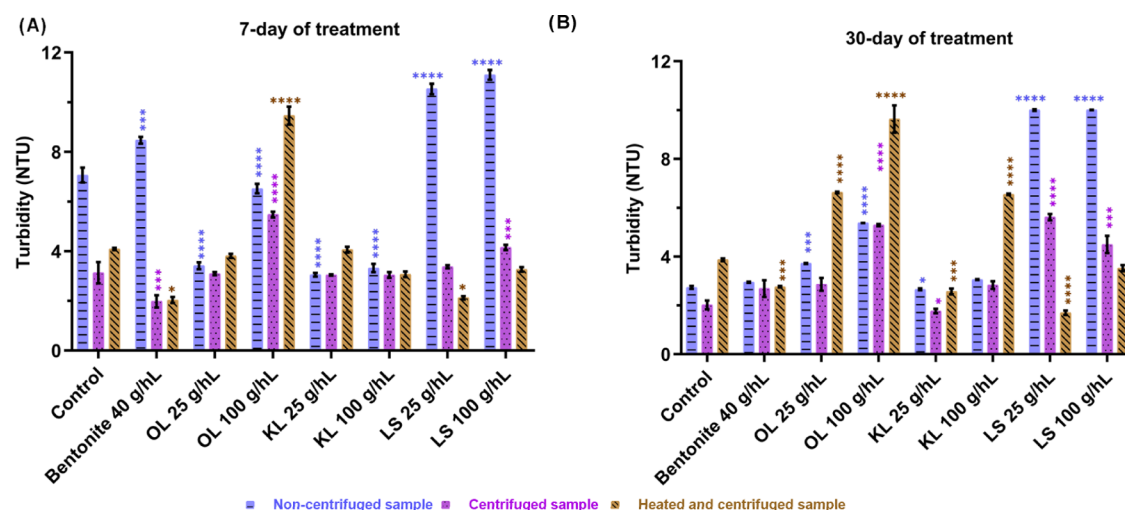


Figure 2. Turbidity of wine samples measured using nephelometry before (blue bars) and after (pink bars) centrifugation as well as after temperature treatment (brown bars). The results were determined after 7 (A) and 30 days (B) of lignin treatment (OL: organosolv lignin, KL: kraft lignin, and LS: liginosulfonate). The values are represented by the mean $\pm$ standard deviation ($n = 9$). Asterisks indicate significant differences from the wine control (* $p \leq 0.05$, ** $p \leq 0.01$, *** $p \leq 0.001$, **** $p \leq 0.0001$).

isobutanoate ($\geq 98\%$), ethyl isovalerate (98%), ethyl nonanoate ($\geq 98\%$), ethyl octanoate (99%), furfural (99%), hexyl acetate (98%), isoamyl acetate ($\geq 99\%$), isoamyl alcohol (98%), linalool oxide (97%), phenylacetaldehyde (90%), phenylethyl acetate (99%), phenylethyl alcohol (99%), tartaric acid ($\geq 99.5\%$), β -damascenone ($\geq 98\%$), α -ionone (85%), and β -linalool (80%) were supplied by Sigma-Aldrich (Madrid, Spain).

2.7.2. Analysis Conditions. Volatile compounds present in wines were analyzed by headspace solid-phase microextraction coupled to gas chromatography-mass spectrometry (HS-SPME-GC/MS) as previously described.³² Briefly, 250 μ L of each wine sample were placed in a 20 mL glass vial and the sample was incubated for 5 min at 45 $^{\circ}$ C under a stirring speed of 250 rpm, followed by volatile extraction using a divinylbenzene/carboxen/polydimethylsiloxane (DVB/CAR/PDMS) fiber (Supelco Inc., Bellefonte, Pennsylvania) for 30 min at the same temperature and stirring speed. Then, the analytes were desorbed into the GC injector over 6 min at 250 $^{\circ}$ C. The separation and detection of volatile compounds was accomplished using a 436-GC model (Bruker Daltonics, Bremen, Germany) coupled to a SCIION single quadrupole mass detector (SQ-MS) and a Bruker Daltonics MS workstation (version 8.2, Bruker Daltonics, Bremen, Germany). A fused silica Rxi-5Sil MS (30 m $\times$ 0.25 mm $\times$ 0.25 μ m; Restek Corporation, U.S., Bellefonte, Pennsylvania) was used, and the carrier gas was high-purity helium (Gasim, Porto, Portugal) at a constant flow of 1 mL/min. The column temperature was 40 $^{\circ}$ C kept for 1 min, increasing to 250 $^{\circ}$ C (5 min), and held for 5 min, followed by increasing to 300 $^{\circ}$ C (rate 5 $^{\circ}$ C/min). The SQ-MS was conducted in the electron ionization (EI) mode at 70 eV with temperatures of 250, 260, and 41 $^{\circ}$ C for the transfer line, ion source, and manifold, respectively. The data acquisition was performed in full scan mode considering a mass range of 40–400 m/z (500 ms scan time).

Standard compounds for volatile quantification were dissolved in a wine model solution (5 g/L tartaric acid, 12% ethanol, pH 3.2), and a range of known concentrations of each compound was analyzed under the same conditions as wine samples by HS-SPME-GC/MS. The calibration curves were computed by the respective area of the peak versus concentration for each volatile compound. The recovery of the standard volatile compounds was assessed by spiking the wine model solution with known concentrations of each compound at three points within the calibration range (low, mid, and high). The spiked samples were analyzed by the same HS-SPME-GC/MS method as that described for the wine samples. For reliable results, an acceptable recovery range of 80–120% was considered. The recoveries are summarized in Table S2. The recoveries were calculated using eq 3:

$$R (\%) = \frac{\text{measured concentration}}{\text{known concentration}} \times 100 \quad (3)$$

2.8. Statistical Analysis. Statistical analyses were performed using GraphPad Prism 8 software (version 8.0.1, San Diego, California; www.graphpad.com). One-way and two-way analysis of variance (ANOVA) were used in the variance analysis and the differences between means were calculated using Tukey's and Holm–Sidak multiple comparison tests. They were considered significant when p -values were below 0.05 ($p < 0.001$ and $p < 0.0001$). In assays of volatile composition, the differences were evaluated using an ordinary one-way analysis of variance (ANOVA) between the control and each condition under study using the GraphPad Prism 9 software (version, 9.3.0, San Diego, CA). The volatile concentrations were considered significantly different for p -values below 0.05.

3. RESULTS AND DISCUSSION

3.1. Effect of Centrifugation and Heat on Wine Turbidity. Solid particles suspended in the wine solution increase turbidity, giving the wine a cloudy and undesirable appearance. It was determined the turbidity of the control wine and the wine treated with bentonite and lignin to understand if the presence of this biopolymer contributed to the wine's clarification process. The values are shown in Figure 2. Turbidity was measured before (blue bars) and after (pink bars) the centrifugation step because some particles accumulated spontaneously even without centrifugation.

The results for the 7-day lignin treatment indicated variations from the wine control. During this time, some solid particles were deposited by gravity; however, the addition of KL and OL significantly reduced the turbidity of the wine even without the centrifugation step (Figure 2A blue bars). On the other hand, as bentonite, LS at both concentrations (25 and 100 g/hL), causes an increase in turbidity, most likely due to the occurrence of adsorption phenomena with suspended particles that do not settle due to gravity (Figure 2A blue bars). After centrifugation, the bentonite showed a significant decrease in wine turbidity, even though the centrifugation procedure alone helped to clarify the wine (Figure 2A pink bars). KL outperforms LS and OL in reducing turbidity due to its higher molecular weight (69 000 g/mol⁴⁹) and ability to deposit due to its hydrophobic nature and drag suspended

particles more effectively. The 30-day treatment resulted in a decrease in turbidity in the samples, including the control wine, even without the centrifugation process, as the samples had more time to settle the colloidal particles; however, the centrifugation step helped to remove suspended particles that caused turbidity, resulting in a reduction in turbidity (Figure 2B pink bars versus blue bars). Regardless of the centrifugation procedure, KL reduced the turbidity of wine samples throughout 30 days.

3.1.1. Thermal Stability of Wines. The heat test is widely used in the wine industry to estimate the haze-forming potential and protein instability. It is considered to be the most reliable tool for predicting sediment development in bottles during storage. Protein haze is a visual problem that results from the precipitation of grape proteins that are extracted during the winemaking process. This phenomenon can occur at any temperature but tends to form more quickly if the wine is stored at high temperatures. Turbidity lower than 2 NTU, calculated by the difference between a heated (Figure 2 brown bars) and a nonheated sample (Figure 2 pink bars), indicates a stable wine. The results for the 7-day treatment revealed that the control wine tends to be stable because, after the treatment with temperature, the turbidity difference was less than 2 NTU. As expected, bentonite improved the stability by eliminating the colloidal content with no variation in turbidity before and after the temperature experiment. Except for the OL at 100 g/hL, all types of lignin help in the stability of wine since the difference between the heated and nonheated samples is below 2 NTU and lower than the wine control. LS at both concentrations and KL at 100 g/hL had the best performance since the turbidity after the heat test was lower (around 40% for LS) than the turbidity before the temperature treatment which means that this type of lignin prevents protein haze formation (Figure 2A brown bars). The same trend was observed for 30 days of treatment. The results suggest that LS at both concentrations and KL at 25 g/hL can help prevent turbidity increase after heating the samples (Figure 2B, brown bars are smaller than pink bars). At higher concentrations and temperatures, OL may interact differently with the wine matrix, possibly increasing the aggregation and precipitation of colloidal particles.

3.2. Determination of Protein Levels. The protein levels for all wine samples are summarized in Table 2. Traditionally,

wineries use fining chemicals, such as bentonite, to remove unstable proteins. The purpose is to use the lowest number of fining agents while simultaneously reducing the unstable protein concentration to a level that prevents precipitation in the bottle. Bentonite is an absorbent clay that is widely utilized in the wine industry. As expected, it promoted a significant decrease (ca. 80%) in protein amount compared to the control wine. The type and concentration of lignin determined its effectiveness in removing proteins from wine samples. In the 7-day experiment, KL showed the highest potential for protein removal, decreasing protein content by 13% at 25 g/hL and 37% at 100 g/hL, compared to the control wine (67 mg/L BSA equiv). LS also significantly reduced the protein content, decreasing 16% at 25 g/hL and 90% at 100 g/hL from day 7 to day 30. For OL at 100 g/hL, the protein content decreased by approximately 50% after aging from day 7 to day 30. As reported in the literature,^{33,34} proteins can adsorb onto the surface of lignin, leading to their precipitation and a consequent decrease in its levels.

Compared with the results in Figure 2, LS showed a lower tendency to develop protein turbidity (the brown bar is lower than the pink bar in Figure 2), likely due to the reduction in protein content. For KL at 25 g/hL, the difference in NTU associated with turbidity was relatively similar to that of the control wine. However, at 100 g/hL, the difference between the heated and unheated samples was smaller than that of the control wine, suggesting that lignin helped control the increase in turbidity, which may also be associated with the decrease in protein over 7 days. For OL, despite the reduction in protein content, no decrease in turbidity was observed from 7 to 30 days. This indicates that other molecules or colloids may have precipitated, suggesting a different interaction between the OL and the wine matrix.

3.3. Evaluation of the Phenolic Compound Content. The phenolic compounds in wines were quantified using the TPC index and the Folin–Ciocalteu assay. The results obtained for wines that undergo different treatments are presented in Figure 3.

The total phenolic compound index was determined based on the ability of the aromatic rings to absorb in the ultraviolet region, with a maximum wavelength of 280 nm. As seen in Figure 3A (blue bars), after 7 days of contact with the different lignin there was a significant increase in the total phenolic compounds index in wines that were in contact with the different types of lignin. This increase was more pronounced for concentrations of 100 g/hL, although the increase was also significant at lower concentrations. On the other hand, after 30 days, only the samples in contact with KL and LS at 100 g/hL presented values slightly higher than those of the control wine. Moreover, there was a decrease in the TPC index for all wine samples treated with lignin compared to the 7-day process. These results evidence that the presence of lignin for the 7 days of contact has some influence on the values since lignin is a phenolic biopolymer composed of aromatic rings that can absorb at 280 nm, which may contribute to higher values when compared to the control wine. Bentonite allowed a slight decrease in the total phenolic index (around 5%) when compared with the control wine.

In contrast to the results described in the total phenolic compounds index, there were no significant variations in the TPC (using the Folin–Ciocalteu method) after 7 days for the samples treated with lignin (Figure 3A brown bars). Based on the results obtained, it seems that the treatment with lignin

Table 2. Quantification of Protein Amounts in Wine Samples by the BCA Method^a

samples	7 days (mg/L BSA equiv)	30 days (mg/L BSA equiv)
wine control	67.1 ± 2.8	22.7 ± 1.4
wine—bentonite 40 g/hL	13.5 ± 0.3 ^d	9.9 ± 1.6 ^d
wine—OL 25 g/hL	63.8 ± 3.2	21.6 ± 0.5
wine—OL 100 g/hL	62.8 ± 3.9	30.7 ± 0.9
wine—KL 25 g/hL	58.3 ± 2.3 ^a	25.8 ± 1.9
wine—KL 100 g/hL	42.2 ± 2.4 ^d	23.3 ± 1.1
wine—LS 25 g/hL	56.3 ± 4.0 ^b	19.8 ± 1.8
wine—LS 100 g/hL	75.3 ± 5.8	5.6 ± 1.4 ^d

^aStatistical differences were achieved by one-way ANOVA and represented by different letters: a (*), b (**), c (***), and d (****). The values are represented by the mean ± standard deviation (*n* = 9). Asterisks correspond to significant differences compared to the wine control. (**p* ≤ 0.05, ***p* ≤ 0.01, ****p* ≤ 0.001, *****p* ≤ 0.0001). nd, not determined.

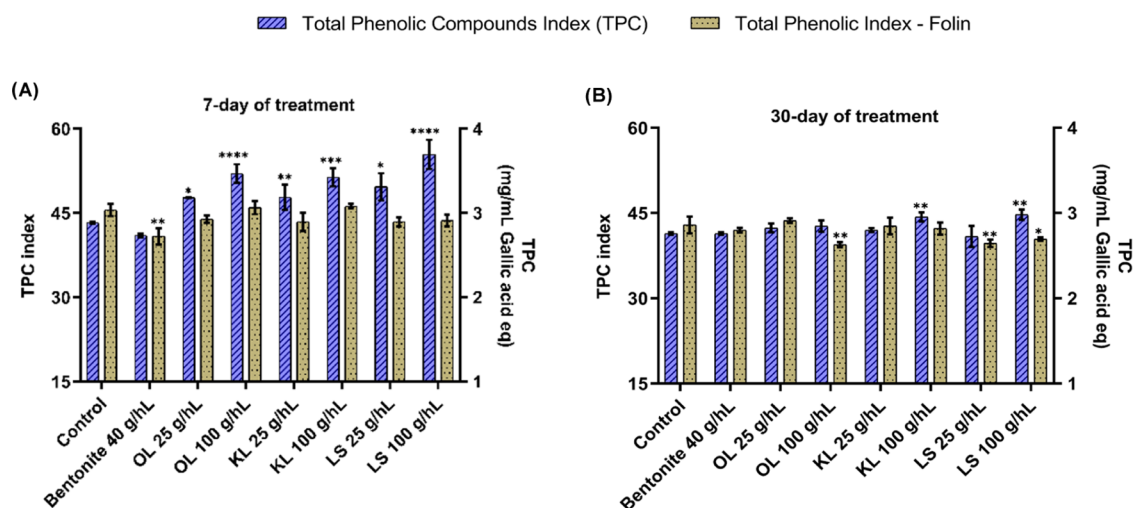


Figure 3. Determination of the phenolic content by the total phenolic content index (blue bars) and the Folin method (brown bars) for the wine control and wine samples treated with different fining agents (bentonite, OL, KL, and LS) at two concentrations. Statistical analysis was carried out one-way ANOVA. Asterisks (*) indicate a statistical difference (* $p \leq 0.05$, ** $p \leq 0.01$, *** $p \leq 0.001$, **** $p \leq 0.0001$) relative to the control wine. Bars represent the mean $\pm$ standard deviation ($n = 9$).

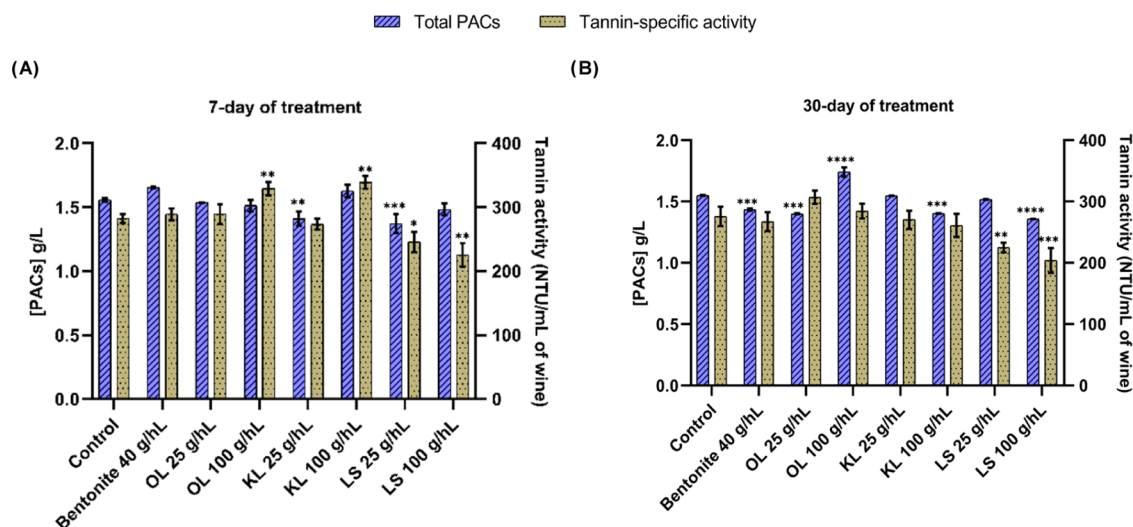


Figure 4. Quantification of total proanthocyanidins (condensed tannins) present in control wine and wine treated with bentonite or lignin (blue bars) and determination of the tannin activity analyzed by nephelometry (brown bars). Statistical analyses were performed by one-way ANOVA, and the asterisk indicates significant differences between samples and wine control (* $p \leq 0.05$, ** $p \leq 0.01$, *** $p \leq 0.001$, **** $p \leq 0.0001$). Bars represent the mean $\pm$ standard deviation ($n = 9$).

does not interfere with the Folin–Ciocalteu reagent. The results obtained using the Folin–Ciocalteu method with bentonite are consistent with those previously determined for the total phenolic compounds index. When the results obtained after 7 and 30 days are compared, a decrease in phenolic compound content is observed, consistent with the reduction noted in the total phenolic index. Prolonging the contact time between wines and the biopolymer may increase the adsorption and subsequent deposition of phenolic compounds, leading to their reduction over time. It is also important to remark that LS at 25 g/hL and OL at 100 g/hL allowed a slight decrease in the total phenolic compound content, which may be a positive point since some colloidal phenolic compounds are involved in oxidation, excess astringency, and bitterness that alter the organoleptic characteristics of wines.

3.4. Quantification of Total Proanthocyanidins. The quantification of the total proanthocyanidins (condensed tannins) and the tannin activity measured by nephelometry are reported in Figure 4.

The content of PACs in all samples showed some differences when compared to the control wine, ranging from 1.3 to 1.8 g/L (Figure 4 blue bars). After 7 days of interaction, the wine samples treated with different types of lignin showed a decrease in proanthocyanidins, with a significant 12% reduction observed for KL and LS at 25 g/hL (Figure 4A). Regarding wines in contact with lignin for 30 days (Figure 4B), the content of PACs in all wines, especially those treated with LS at both concentrations and KL at 25 g/hL, was reduced (maximum of 13%) compared to the control wine. This reduction may be attributed to interactions between lignin and tannins, which could lead to tannin condensation reactions and subsequent precipitation. Bentonite also reduced the level of

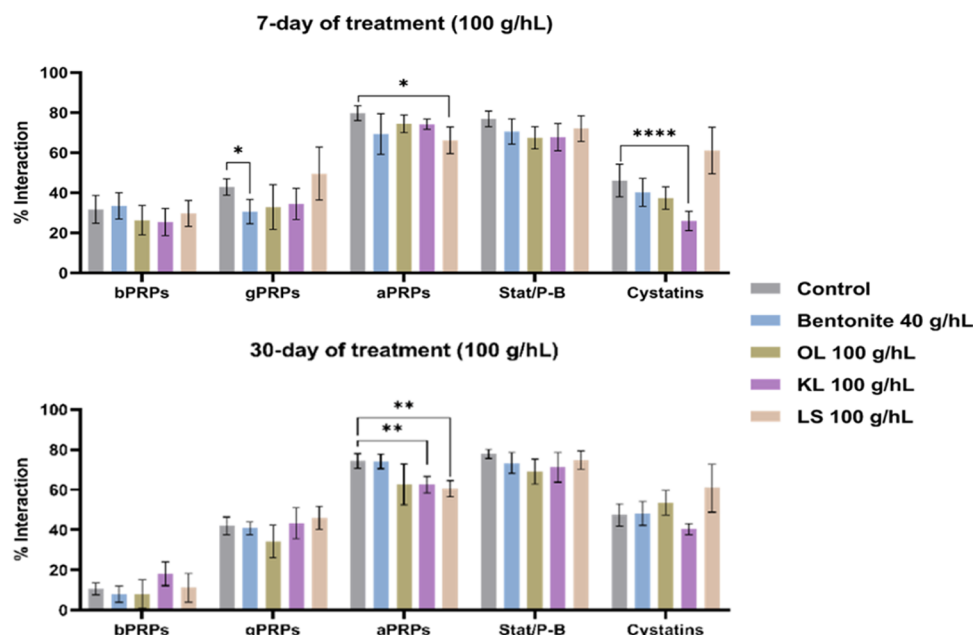


Figure 5. Percentage of the interaction of the different SP families with each treated wine (bentonite at 40 g/hL and OL, KL, and LS at 100 g/hL) for both 7 and 30 days of treatment. Control conditions refer to the wine without modulating agents. Statistical analysis was made by two-way ANOVA. Asterisks (*) indicate statistical differences ($*p \leq 0.05$, $**p \leq 0.01$, $****p \leq 0.0001$) relative to the control wine. Bars represent the mean $\pm$ standard deviation ($n = 6$).

proanthocyanidins by 3%. OL (100 g/hL) exhibited the opposite impact, significantly increasing the content of the PACs. However, the lignin samples contain only negligible levels of PACs and, hence, do not contribute to the observed increases.

3.4.1. Tannin-Specific Activity. Tannins are compounds that confer structure and complexity to wines. These compounds can interact with salivary proteins (SP), forming insoluble complexes associated with astringency. The tannin-specific activity was evaluated by using nephelometry and the BSA protein (bovine serum albumin) as a model. This methodology aims to explore the putative interaction of the compounds present in wine and proteins, which increase the turbidity of the solution. The results obtained for all the samples are displayed in Figure 4 (brown bars). The type and concentration of lignin have an impact on the tannin-specific activity. OL and KL at 100 g/hL showed a significant increase in the turbidity of wines in the presence of BSA (7 days of contact; Figure 4A brown bars). In the case of KL, the increase can be attributed to a rise in PAC content. For OL at 100 g/hL, the increase may result from the interaction between BSA and the lignin surface, particularly since a decrease in condensed tannin content was observed.^{33–35} On the other hand, complex interactions between lignin and tannins may enhance tannin reactivity through selective binding, structural modifications, or the formation of more active tannin complexes, which could explain this increase. More interesting was that LS resulted in a considerable decrease of turbidity for both concentrations (25 and 100 g/hL), which may be the first indication that this biopolymer can modulate the astringency of wines. This result is a consequence of the decrease in PAC concentration previously reported in Figure 4 (blue bars). This is a relevant feature since higher amounts of tannins in wine give rough or abrasive sensory characteristics. Also, many wineries try to achieve a balanced level of astringency because it is one of the most recognized sensory properties in wine

associated with quality. The decrease in the turbidity of wines in the presence of BSA is more pronounced after 30 days of contact with LS (Figure 4B, brown bars), particularly at 100 g/hL.

3.5. Effect of Lignin on the Interaction between Wine Phenolic Compounds and Salivary Proteins. The typical human saliva profile has been previously characterized by proteomic analysis.²⁵ The chromatogram (Figure S3) is divided into different families of SP that have been widely associated with astringency perception, such as basic proline-rich proteins (bPRPs), glycosylated PRPs (gPRPs), acidic PRPs (aPRPs), statherin, P–B peptide, and cystatins.⁸ Since statherin and the P–B peptide are coeluted, they were analyzed together. The effect of the addition of bentonite and lignin to wines on the SP interaction and precipitation was evaluated. After centrifugation, the insoluble aggregates were removed, and the remaining part (SP that did not interact) was analyzed (Figure 5). The results of SP were obtained by subtracting the area of the chromatographic peak corresponding to each SP family remaining in the solution after interaction from the initial concentration. Then, to evaluate and compare the reactivity toward different wines (with or without modulating agents), this latter value was divided by the initial concentration to obtain the final interaction value (%).

Overall, the results demonstrated that certain types of lignin were effective in reducing the interaction between phenolic compounds and specific families of SP, consequently leading to a decrease in the level of SP precipitation. This effect seems to be specifically associated with the type, concentration, and days of treatment with lignin. Among the several types of lignin, LS showed the highest ability to reduce the interaction between aPRPs and wine phenolic compounds, one of the most important SP families involved in astringency perception. This effect was observed for the highest concentration (100 g/hL) for both 7 and 30 days of treatment. These results agree with

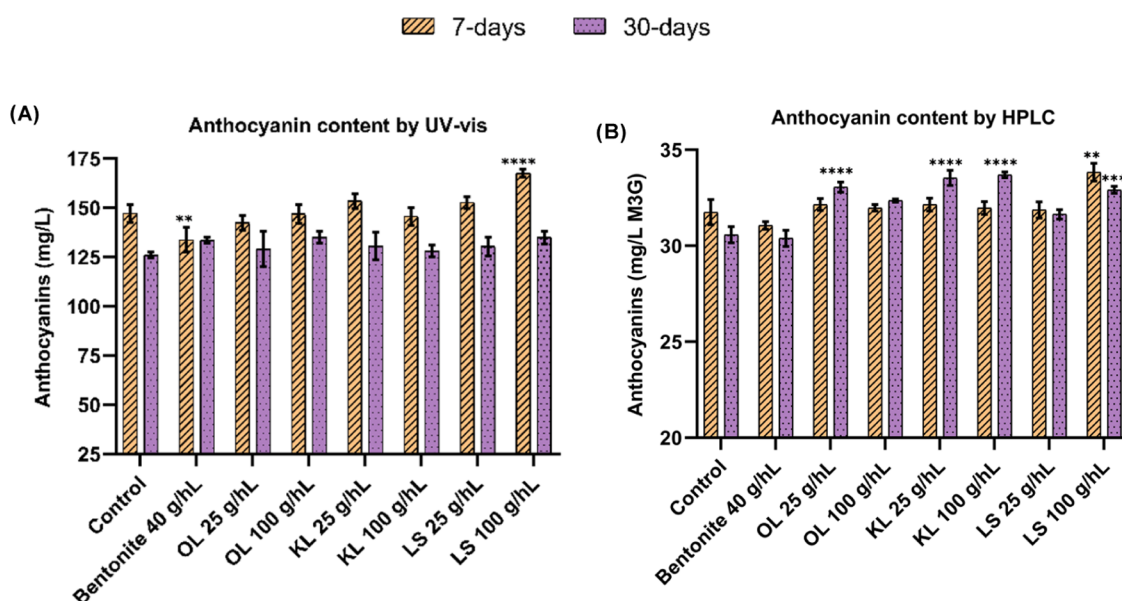


Figure 6. Quantification of free anthocyanins by the reaction with bisulfite (A) and by HPLC (B) after 7 and 30 days is represented by the orange and pink bars, respectively. Statistical differences were achieved by one-way ANOVA. Asterisks correspond to significant differences compared to the wine control (* $p \leq 0.05$, ** $p \leq 0.01$, *** $p \leq 0.001$, **** $p \leq 0.0001$). Bars represent the mean $\pm$ standard deviation ($n = 9$).

the decrease of the PAC concentration and tannin-specific activity demonstrated in Figure 4, making it a promising candidate for use as a modulating agent to balance wine astringency. On the other hand, KL at 100 g/hL also significantly reduced the interaction between aPRPs (significant only for 30 days of treatment) and cystatins with wine phenolic compounds. Comparing the days of treatment and concentration, the latter seems to be the most important factor affecting the higher efficiency of KL. Although the higher effect is observed at the highest lignin concentration (100 g/hL), a significant effect of OL 25 g/hL on reducing the interaction of gPRPs and wine compounds was also noted (Figure S4). On the other hand, bentonite, a common fining agent, can reduce the interaction of gPRPs with wine polyphenols only for the 7-day treatment (Figure 5).

Fining agents are crucial for softening or reducing wine astringency and bitterness as well as for stabilizing haze formation through physical or chemical reactions with wine compounds. However, fining can also potentially remove positive flavor characteristics. Therefore, new fining agents should avoid inducing drastic changes in wine compounds to mitigate the risk of overfining. In this regard, LS and KL showed interesting results in reducing SP interaction and precipitation at a moderate level, turning them into suitable candidates for improving the overall wine quality.

3.6. Anthocyanins. The anthocyanin content was quantified by UV-visible spectroscopy using the bleaching bisulfite method and by HPLC-DAD. The results obtained are presented in Figure 6.

As shown in Figure 6, the presence of lignin influenced the total amount of free anthocyanins. The results obtained by UV-vis spectroscopy after 7 days (Figure 6A orange bars) had a greater variation compared to the control. LS at 100 g/hL exhibited an increase in free anthocyanin levels (around 12%). This can be due to the ability of LS to disaggregate anthocyanin aggregates formed during the production and aging, yielding the increase of solubilized free anthocyanins available to be decolorized by bisulfite. After 30 days of contact

(Figure 6A pink bars), the values did not show significant differences compared with the control wine. Moreover, comparing the results obtained after 7 and 30 days of contact, it can be observed that over time the content of anthocyanins decreases with a reduction of approximately 14%. However, in the presence of lignin, this decrease was less pronounced indicating that this biopolymer favors the stabilization of anthocyanins and thus the color of red wine. On the other hand, bentonite decreased in the concentration of free anthocyanins, which agrees with the significant decrease observed in the phenolic content previously discussed.

During red wine aging, anthocyanins undergo several reactions that result in the formation of new pigments. The quantification of anthocyanins and the identification of the anthocyanin-derived compounds formed during aging can be performed by HPLC-DAD coupled with mass spectrometry. Figure 6B contains the results obtained in the quantification of anthocyanins by HPLC. As can be observed, there is a slight increase in the concentration of anthocyanins in wines that were in contact with the different types of lignin, similar to what was described previously in the bisulfite method, which can be due to the disaggregation of polymeric anthocyanin complexes. The increase was more pronounced after 30 days at lignin concentrations of 100 g/hL. However, in the case of LS, a significant increase in anthocyanin content was observed within just 7 days. Comparing the methods shown in Figure 6, the concentration of these pigments was notably higher when using the bisulfite method compared to HPLC-DAD. This discrepancy is likely due to the bisulfite method's sensitivity to other colored pigments, which are also affected by bisulfite, making it less accurate for quantifying anthocyanins.

Regarding the chromatographic profile of the wine samples treated with bentonite or lignin, it can be observed that this was identical to the chromatographic profile of the wine without any clarification. The identity of each compound was achieved by the LC-MS considering the data reported in the literature (Table S2). Different phenolic compounds, especially anthocyanins and their derivatives were observed in all

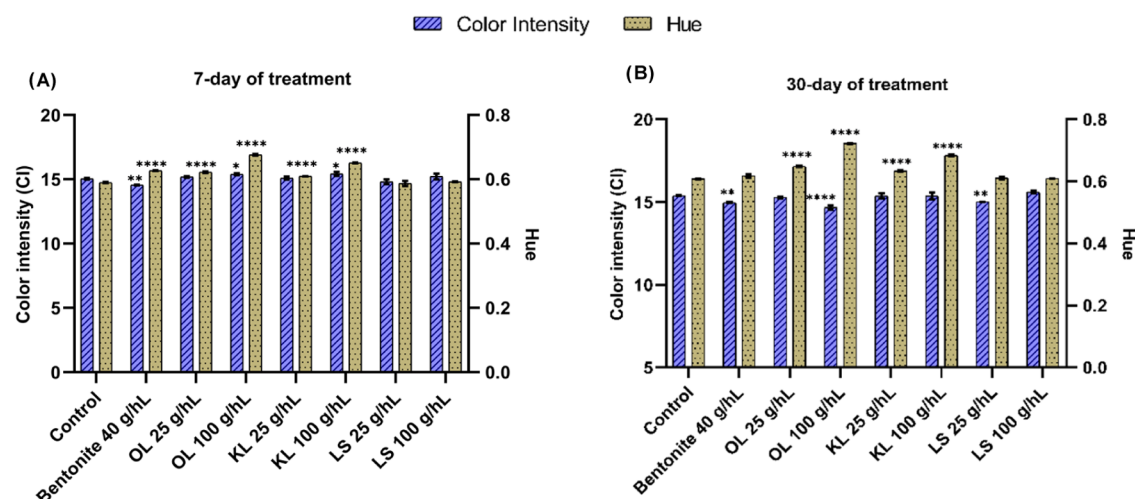


Figure 7. Color intensity (CI) of wine samples treated with bentonite and different types of lignin at different contact times (blue bars) and determination of the hue for all samples (brown bars). Statistical analysis was made by one-way ANOVA. Asterisks (*) indicate a statistical difference (* $p \leq 0.05$, ** $p \leq 0.01$, *** $p \leq 0.001$, **** $p \leq 0.0001$) relative to the control wine. Bars represent the mean $\pm$ standard deviation ($n = 9$).

samples. Malvidin-3-*O*-glucoside and Malvidin-3-*O*-coumaroylglucoside were the most abundant anthocyanins detected; however, peonidin-3-*O*-glucoside and petunidin-3-*O*-glucoside were also observed. Regarding the increase in anthocyanin content, malvidin-3-*O*-coumaroylglucoside exhibited a slight increase of 4–7% across all three types of lignin. Malvidin-3-*O*-glucoside increased by approximately 4% only with lignosulfonates at a concentration of 100 g/hL. Petunidin-3-*O*-glucoside levels increased in wine samples treated with both kraft lignin and lignosulfonates at 100 g/hL. Pyranoanthocyanins, such as A-type and B-type vitisins, were also identified in wine samples. These compounds are described to increase the color stability of red wines due to their higher stability toward hydration reactions and bisulfite bleaching. Other anthocyanin-derived compounds were also detected in all wine samples resulting from the reaction of anthocyanins with other phenolic compounds, such as catechin, vinylphenol, or vinylcatechol. Hydroxybenzoic acids, catechins, epigallocatechins, and procyanidin trimers were also detected in the wine samples, with coumaric and cinnamic acids being the predominant phenolic compounds. Treatment with KL led to an increase in coumaric acid, while aging with OL resulted in higher cinnamic acid levels. Conversely, treatment with LS caused a slight decrease in the number of identified phenolic compounds. These results suggest that the content of each phenolic compound is influenced by the type and amount of lignin used.

3.7. Chromatic Properties. The chromatic properties of red wines are associated with the presence of anthocyanins and all their derivatives, which are directly correlated with wine quality. The chromatic properties of wines were evaluated by determining the color intensity (CI) and CIELAB color coordinates.

3.7.1. Color Intensity (CI). The CI of all wine samples was determined by the sum of the absorbances at 420, 520, and 620 nm. The results are presented in Figure 7 (blue bars). The yellow tones of lignin are one of the major drawbacks associated with the use of this biopolymer, as its addition to some matrices can change the color of the finished product. Depending on the extraction method and purification degree, lignin can range from pale yellow to brown.

The results obtained demonstrate that the presence of lignin has an impact on the color coordinates of the wine. Regarding the wines that were clarified with lignin for 7 days, for KL and OL at both concentrations (Figure 7A blue bars), it was observed an increase in the color intensity related to the intensification of the absorbance at 420 nm which can be due to the contribution of the yellow color of lignin. Bentonite yielded a decrease in the wine color intensity due to the reduction of the absorbance at 520 and 620 nm. These results are correlated with the decrease in the anthocyanin concentration as these pigments absorb at 520 nm. Regarding the wines that were in contact with lignin for 30 days (Figure 7B blue bars), two conditions yielded a significant decrease in CI, the wine treated with OL at 100 g/hL and LS at 25 g/hL. In the case of OL, the reduction was due to the decrease in the absorption at 620 nm. In the case of LS, the decrease was due to the reduction of the absorption at 420 and 620 nm.

The hue of the wine samples was assessed and compared to the control wine. This hue is calculated as the ratio between the absorbances at 420 and 520 nm (Figure 7 brown bars). Except for the LS, the clarification of wines with OL and KL yields the increase of the yellow character of the final wine, due to the darker color of these lignin samples, which remained brown in the aqueous solution, and caused the increase of color intensity for the 7 days experiment, as previously described. The same was observed during the 30 day experiment (Figure 7B), with higher values, most likely due to the longer period in contact with wine. In the case of bentonite, an increase in the hue of the wine was observed after 7 days of treatment due to the decrease in absorbance at 520 nm as a result of the decrease in anthocyanins concentration as already discussed above.

3.7.2. CIELAB Color Coordinates. The CIELAB coordinates of the wine samples were determined to evaluate the color change of wine during the treatment with the different types of lignins for 7 and 30 days (Table 3).

According to the results presented in Table 3, the lightness (*L*) values for the 7 days were similar for all samples, indicating that lignin does not interfere with the sample transparency. After 30 days, there was an increase of *L* in wine samples treated with OL at 100 g/hL. Regarding the colorimetric

Table 3. CIELAB Coordinates for the Wine Control and Wine Clarified with Bentonite and Lignin for 7 and 30 Days^a

samples	CIELAB color coordinates 7 days			
	<i>L</i> *	<i>a</i> *	<i>b</i> *	ΔE
control	63.3 $\pm$ 0.1	38.6 $\pm$ 0.1	5.31 $\pm$ 0.09	
bentonite 40 g/hL	63.06 $\pm$ 0.08	38.44 $\pm$ 0.08	8.68 $\pm$ 0.08 ^d	3.39
OL 25 g/hL	63.34 $\pm$ 0.08	37.80 $\pm$ 0.07 ^b	6.1 $\pm$ 0.1 ^a	1.08
OL 100 g/hL	63.4 $\pm$ 0.1	36.68 $\pm$ 0.03 ^d	7.5 $\pm$ 0.1 ^d	2.92
KL 25 g/hL	63.56 $\pm$ 0.04	37.9 $\pm$ 0.2 ^a	5.88 $\pm$ 0.07	0.89
KL 100 g/hL	63.2 $\pm$ 0.3	37.1 $\pm$ 0.3 ^d	7.4 $\pm$ 0.1 ^d	2.56
LS 25 g/hL	63.4 $\pm$ 0.2	38.5 $\pm$ 0.3	5.03 $\pm$ 0.02	0.42
LS 100 g/hL	63.0 $\pm$ 0.6	38.8 $\pm$ 0.7	4.9 $\pm$ 0.2	0.93
	CIELAB color coordinates 30 days			
	<i>L</i> *	<i>a</i> *	<i>b</i> *	ΔE
wine control	61.79 $\pm$ 0.07	39.81 $\pm$ 0.06	7.92 $\pm$ 0.02	
bentonite 40 g/hL	62.54 $\pm$ 0.05 ^a	38.87 $\pm$ 0.08 ^c	7.70 $\pm$ 0.04 ^a	1.22
OL 25 g/hL	62.3 $\pm$ 0.1 ^a	38.5 $\pm$ 0.1 ^c	8.89 $\pm$ 0.07 ^d	1.15
OL 100 g/hL	64.2 $\pm$ 0.2 ^d	35.6 $\pm$ 0.2 ^d	10.5 $\pm$ 0.1 ^d	1.62
KL 25 g/hL	62.1 $\pm$ 0.3	38.8 $\pm$ 0.3 ^c	8.8 $\pm$ 0.1 ^d	4.74
KL 100 g/hL	62.6 $\pm$ 0.4 ^b	37.5 $\pm$ 0.3 ^d	10.6 $\pm$ 0.2 ^d	4.60
LS 25 g/hL	62.43 $\pm$ 0.04 ^a	39.3 $\pm$ 0.3	7.64 $\pm$ 0.09 ^b	0.94
LS 100 g/hL	61.2 $\pm$ 0.1 ^a	40.3 $\pm$ 0.2	7.5 $\pm$ 0.1 ^c	1.07

^aStatistical differences were assessed concerning the wine control and evaluated by one-way ANOVA reported using different letters: a (*), b (**), c (***), and d (****). The values are represented by the mean $\pm$ standard deviation ($n = 9$). Asterisks indicate significant differences from the wine control (* $p \leq 0.05$, ** $p \leq 0.01$, *** $p \leq 0.001$, **** $p \leq 0.0001$).

coordinates *a** and *b**, it was observed that for the * parameter there is a decrease for KL and OL (independently of the contact time) when compared to the control wine, indicating a decrease in the red color contribution. Considering the *b** values, the samples in contact with OL and KL showed higher values of this color coordinate compared with the control wine. This indicates a higher contribution of the yellow color to the overall color of wine due to the yellowish color of lignin. This has already been discussed above in the analysis of the red wine hue (Figure 7). This effect was directly correlated to the concentration of lignin. On the other hand, the light brownish-yellow tone of LS has a low contribution to the wine color change, and the decrease of values of the *b** coordinate shifted to the blue axis agrees with the increase of the absorbance at 620 nm. Bentonite also demonstrated a strong influence on wine color, particularly for the yellow-blue coordinate. Regarding the ΔE values, a dependence on the type and quantity of lignin was observed. After 7 days of treatment, OL and KL at 100 g/hL had ΔE values of approximately 2.5 and 2.9, respectively, suggesting that an inexperienced observer could detect these differences. However, bentonite resulted in a greater color difference compared to that of lignin. After 30 days, only kraft lignin showed an ΔE value of around 4.5, indicating a noticeable color difference that could be clearly detected by observers.

3.8. Dialysis Index. The dialysis index determines the structural complexity and molecular weight of the chemicals found in wine. Based on the results obtained (Figure S5), the clarification process does not affect the complexity of the wine, except when LS is used at 100 g/hL during 7 days of contact

time, yielding a significant decrease in the wine complexity. This decrease could be related to the decrease in the PAC content, as previously described. Overall, after 30 days of contact with the different types of lignin, there are no significant differences in the complexity of the wine compounds compared with the control wine.

3.9. Quantification of Volatile Compounds by HS-SPME-GC/MS. Volatile compounds, including terpenes, phenols, alcohols, esters, aldehydes, ketones, and lactones, play an important role in the sensory characteristics of wines. They can be generated during the fermentation and aging processes or can come directly from the grape berries. This work evaluated the volatile profile of the control wine and the impact of lignin during a 7-day clarification process. The levels of 23 volatile compounds were determined in all samples (Table S3), of which 6 of them showed significant differences, Figure 8.

The presence of some volatile compounds might have a negative connotation since some of them confer the wine's unwanted odors and flavors. Isoamyl alcohol is an example, it is associated with descriptors like cheese and fuse.³⁶ In this study, the Port wine employed as a control had a high concentration of isoamyl alcohol (approximately 350 mg/L) which is above the olfactory perception threshold (30 mg/L).^{37,38} The application of lignin as a clarifying agent during the 7 days was very positive since there was a decrease of up to 27% in the case of LS at 100 g/hL. For LS concentration of 25 g/hL or using KL, it was possible to observe significant differences with decreases between 13 and 16% of this volatile compound. The presence of 1-hexanol confers odors that evoke newly cut grass or hay, as well as resin,³⁶ and has a negative connotation as it usually refers to grapes that have not reached an optimal level of maturity before being harvested. The results obtained for the samples analyzed in this study were lower than the olfactory perception threshold (8 mg/L);^{37,38} nonetheless, the three types of lignin allowed for a significant decrease in these volatile compounds following wine clarification. Furfural belongs to the aldehyde class, and it is usually found in red wine at levels below their odor thresholds (14 mg/L).^{37,38} This compound may be involved in polymerization reactions with polyphenols, and it is associated with descriptors like almonds, bread, caramelization products, and sometimes dried wood.³⁶ The results of this work demonstrated that furfural concentrations were much lower compared to the odor threshold, but for the wines treated with lignosulfonates, it was observed a significant increase in furfural concentration. The sulfite liquors that produce the lignosulfonates contain some traces of pentoses, an undesirable constituent that could be the furfural source. The LS used in this work underwent an ultrafiltration process, which can be insufficient to remove traces of furfural, which may lead to the increase shown in Figure 8. Still, the concentration values obtained are much lower than its olfactory perception threshold. Esters are chemical molecules found in wine that contribute to its fruity aroma. The diethyl succinate and ethyl decanoate concentrations found in the control wine were relatively low compared to the odor thresholds of 200 mg/L and 200 μ g/L,³⁸ respectively. In the first case, there was a decrease in KL and LS at 100 g/hL compared with the control wine. Regarding the ethyl decanoate, in which its presence is associated with brandy and fruit descriptors,³⁶ there was a significant increase in levels of approximately 64%. Finally, the concentration observed in control wine for ethyl octanoate was

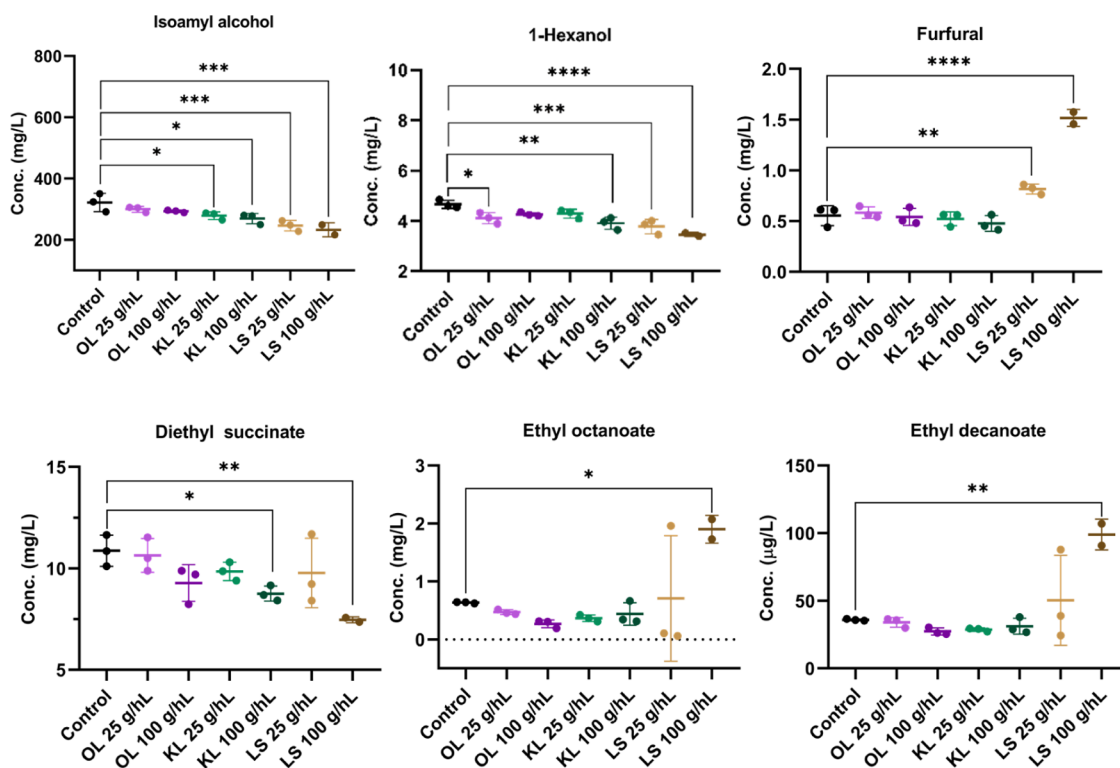


Figure 8. Volatile compounds with significant differences in the groups under study compared to the control group. Statistical analysis was made by one-way ANOVA. Asterisks (*) indicate a statistical difference ($*p \leq 0.05$, $**p \leq 0.01$, $***p \leq 0.001$, $****p \leq 0.0001$) relative to the control wine. Bars represent the mean $\pm$ standard deviation ($n = 3$). Sample LS 100 g/hL is representative of an $n = 2$, as the third triplicate had to be excluded due to a low chromatogram signal.

near to, and in some cases, higher than, the odor threshold (0.6 mg/L).^{37,38} The wine treated with LS at 100 g/hL showed the greatest rise in ethyl octanoate compared with the other types of lignin. This increase may result in a sweeter aroma directed toward the pineapple flavor as these are the descriptors associated with this volatile compound.

In summary, this study is the first to explore the effects of various types of lignin on the composition of Port wine. Lignosulfonate (LS) emerged as the most promising, effectively reducing tannin content and minimizing the interaction of salivary proteins with polyphenols, which can help manage astringency. Additionally, LS decreased undesirable aromas (isoamyl alcohol and 1-hexanol) and protein levels after 30 days, while increasing anthocyanin content, suggesting its role in depolymerizing aggregated anthocyanin fractions. Overall, lignin did not significantly alter the final color of the wine ($\Delta E < 5$), despite its yellow hue, which is often seen as a limitation. This innovative approach highlights lignin, a sustainable byproduct, as a valuable tool for enhancing wine quality, emphasizing circularity, and exploring new applications in the wine industry.

■ ASSOCIATED CONTENT

SI Supporting Information

The Supporting Information is available free of charge at <https://pubs.acs.org/doi/10.1021/acs.jafc.4c05442>.

Information about the pH values of wine samples, results of the salivary protein interaction for lignin concentrations of 25 g/hL, identification of the phenolic compounds in wine samples by LC/DAD-MS, results for

the dialysis index, and quantification of the volatile levels in wine samples by HS-SPME-GC/MS (PDF)

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Notes

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