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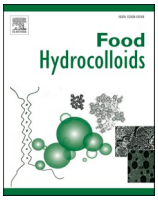

Pectic polysaccharides modulate colloidal stability and astringency perception of bottle aged Cabernet Sauvignon wines

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ABSTRACT

Anthocyanins, tannins, and polymeric pigments, which are formed during red wine aging, are arguably the most important phenolic constituents of red wine, because they provide color, color stability, and mouthfeel properties like astringency. The extraction of polyphenols is accompanied with the extraction of grape-derived pectic polysaccharides leading to interactions between the two compound classes during fermentation and wine aging affecting tannin and pigment precipitability. Thereby, the implications for red wine quality are dependent on the structural features of the polysaccharides. In this study, polyphenolic and pectic polysaccharide composition of commercially available Cabernet Sauvignon wines, which were subjected to forced aging, were characterized to investigate the effect of red wine aging on the interactions between polysaccharides and polyphenols. This was accomplished by removing polysaccharides from the wines and comparing the polyphenolic composition of the wines and the reconstituted polysaccharide-free wines. Descriptive sensory analysis including "overall astringency", "unripe" and "dry" was conducted on all samples to examine the implications of polysaccharide-polyphenol interactions on the astringency perception of red wines. The analyzed wines can be classified into two groups based on their colloidal stability, whereby large, neutral, and highly esterified polysaccharides promoted tannin and pigment protein precipitability and impaired the age-related formation of pigmented tannins. Small, acidic, and polar pectin fragments show a preventive effect on the precipitation of polyphenols. Sensory analysis revealed that the perception of the astringency sub-qualities "unripe" and "dry" are related to the interactions between pectic polysaccharides and polyphenols. Depending on the polysaccharide composition, the astringency of the wines increased or decreased during aging.

1. Introduction

It is generally accepted that anthocyanins and tannins are two of the major red wine components which determine product quality by providing color and mouthfeel to red wines. Anthocyanins and tannins are extracted from the grape berry to the must during winemaking, but the extractability of these compounds is strongly influenced by various inherent and external factors like the type of polyphenol, winemaking techniques, and grape maturity (Hanlin, Hrmova, Harbertson, & Downey, 2010; Hensen, Hoening, Weilack, Damm, & Weber, 2022; Hernández-Hierro, Quijada-Morín, Rivas-Gonzalo, & Escribano-Bailón, 2012). The latter also determines the composition of cell wall polysaccharides, as the ripening process of grapes is associated with the a balance between biosynthesis and enzymatic degradation of polysaccharides like hemicellulose, cellulose, and in particular pectin (Nunan, Sims, Bacic, Robinson, & Fincher, 1998). This includes the de-esterification of methylated galacturonic acid units followed by the breakdown of unesterified polygalacturonans and (1 → 4)-β-galactans of pectic polysaccharides (Minic & Jouanin, 2006; Nunan et al., 1998). Because the strength of interactions between polysaccharides and polyphenols depends on structural features like branching complexity and degree of esterification, the alterations in polysaccharide composition affect the extractability of tannins and anthocyanins as they constantly adsorb and de-adsorb on grape cell material (Hanlin et al., 2010; Liu, Le Bourvellec, & Renard, 2020; Medina-Plaza et al., 2020). Together with polyphenols, pectin fragments end up in the wine where interactions driven by hydrogen bonding, hydrophobic and electrostatic forces take place (Gao, Fangel, Willats, Vivier, & Moore, 2015; Weber,

Abbreviations: p-PP, precipitable polymeric pigments; np-PP, non-precipitable polymeric pigments.

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2022).

The interactions with polyphenols impact their protein precipitability, whereby prevention and enhancement of protein precipitation were both reported depending on the type of pectic polysaccharide (Carvalho et al., 2006; de Freitas & Mateus, 2001; Watrelot, Schulz, & Kennedy, 2017). Polysaccharides with the ability to stabilize wine color, prevent tannin (self-) aggregation and precipitation are referred to as protective/stable colloids (Alcalde-Eon, García-Estévez, Puente, Rivas-Gonzalo, & Escribano-Bailón, 2014; Guadalupe, Palacios, & Ayestarán, 2007; Osete-Alcaraz, Bautista-Ortín, & Gómez-Plaza, 2020). The enhanced precipitability of tannins and pigments in the presence of certain polysaccharides is due to the formation of unstable colloids, which consist of pigmented protein precipitable polysaccharide-polyphenol complexes (Alcalde-Eon et al., 2014; Guadalupe et al., 2007; Weilack, Mehren, Schieber, & Weber, 2023). This phenomenon was reported to impair the covalent incorporation of anthocyanins into tannins to form pigmented tannins during red wine aging (Weilack et al., 2023). Earlier research (Vidal, Francis, Noble, et al., 2004; Weber, Greve, Durner, Fischer, & Winterhalter, 2013; Weilack, Schmitz, Harbertson, & Weber, 2021) suggests that the formation of pigmented tannins plays a role in the attenuation and softening of astringency associated with wine aging due to changes in physico-chemical properties; hence, the impaired formation of pigmented tannins could possibly impact red wine astringency perception. This highlights the necessity to examine the relationship between the structural features of pectic polysaccharides and the colloidal stability of wines.

In general, polysaccharides can alter red wine astringency in the finished wines either by changing its texture or interacting with polyphenols and/or salivary proteins (Brandão et al., 2017; Luck et al., 1994; Vidal, Francis, Williams, et al., 2004). According to Gawel, Oberholster, and Francis (2000), red wines induce not only the perception of overall astringency, but also astringency related sensations, which can be classified as astringency sub-qualities. Due to the heterogeneous and complex composition of red wines, the exact triggers of different sub-qualities are difficult to unravel, but have received increased attention in recent years (Ferrero-del-Teso et al., 2024; Sáenz-Navajas, Ferrero-del-Teso, Jeffery, Ferreira, & Fernández-Zurbano, 2020; Wang, Olarte Mantilla, Smith, Stokes, & Smyth, 2020). However, most studies are based on model wines and isolated reactions between polyphenols, polysaccharides, and proteins (González-Muñoz et al., 2022).

The present work is a follow-up of a previous study (Weilack et al., 2023), which investigated the implications of the interactions between pectic polysaccharides and polyphenols on the composition of the latter in young wines. They showed that large and neutral pectin fragments led to an increased protein precipitability of tannins and pigments, which might have resulted in an impaired reaction between anthocyanins and tannins to form polymeric pigments. In contrast, small and polar pectin fragments protected polyphenols from precipitation (Weilack et al., 2023). The objective of the present study was to examine the influence of pectic polysaccharides on the composition of polyphenols in bottle aged red wines and the implications for red wine astringency, particularly considering the age-related attenuation of astringency. This was achieved by subjecting the same six commercially available Cabernet Sauvignon wines to forced aging to trigger age-related changes in the polyphenolic composition like the formation of polymeric pigments. Besides the characterization of pectic polysaccharides, polysaccharide-free reconstituted wines were composed, and polyphenols were determined and compared in all samples. Additionally, all samples were subjected to sensory profiling including taste attributes "sour" and "bitter" and mouthfeel attributes "overall astringency", "unripe" and "dry" to examine the influence of pectic polysaccharides on the astringency perception and the changes thereof during aging.

2. Materials and methods

2.1. Materials

Maleic acid, ferric chloride, triethanolamine (TEA), and tartaric acid were sourced from Alfa Aesar (Kandel, Germany). Urea, bovine serum albumin fraction V, and (+)-catechin were acquired from Carl Roth (Karlsruhe, Germany). Acetic acid, hydrochloric acid (HCl), ethanol, and potassium bisulfite were sourced from VWR International GmbH (Darmstadt, Germany). Sodium hydroxide and sodium nitrate were obtained from Honeywell Fluka (Offenbach, Germany) and Acros Organics (Geel, Belgium), respectively. Propionic acid, *n*-propanol, and sodium azide were purchased from Merck KGaA (Darmstadt, Germany). Sodium chloride, sulfuric acid, and methanol (HPLC grade) were acquired from Th. Geyer GmbH & Co. KG (Renningen, Deutschland). Food-grade sodium hydroxide, ethanol, and acetic acid were obtained from Emprove Essential (Merck KGaA, Darmstadt, Germany), Brennerei Kessler (Bad Peterstal-Griesbach, Germany), and Macron Fine Chemicals (VWR International GmbH, Darmstadt, Germany), respectively. Food grade adsorbent resin (Resinex AD3300) was provided by Jacobi Carbons Group (Frankfurt am Main, Germany). For the model wines, food-grade sucrose (Südzucker AG, Mannheim, Germany), tartaric acid (Otto Fischar GmbH, Saarbrücken, Germany), potassium bitartrate (Natuurlijk, Ede, Netherlands) and lactic acid (Otto Fischar GmbH, Saarbrücken, Germany) were used.

2.2. Wine samples

This study was carried out on commercially available Cabernet Sauvignon wines, which were subjected to forced aging. This was achieved by storing the wines in a heated incubator at 35 °C for 10 weeks. Originally, the six different Cabernet Sauvignon wines were of the 2018 vintage from three wine-growing regions. At the time of the study, the wines were 3 years old. The wines were made in the following wineries and regions: Weinbiet (14 % v/v ethanol) and Emil Bauer (Bundschuh, 13.5 % v/v ethanol) from the Palatinate region in Germany, Adentu and Las Mulas (each 13.5 % v/v ethanol) from Central Valley, Chile, and Beringer and Canyon Road (each 13 % v/v ethanol) from California, USA. The wines were chosen to reflect a broad variability of geographical origins. The sample coding references the respective winery with the prefixes "fresh reference" wines for the reference wines, which were kept at 10 °C for 10 weeks, hence, did not undergo aging, and "aged" for the aged wines. All samples were analyzed after 10 weeks of storage.

The general composition of the "aged" wines was assessed by Fourier-transform mid-infrared (FT-IR) spectroscopy, including the appropriate calibration method (WineScan FT120 Basic, Foss, Hilleroed, Denmark) (Table 1). Free and total SO_2 contents were determined by titration and are included in Table 1. All bottles were closed with screw caps. The "fresh reference" wines were object of the previous study (Weilack et al., 2023), in which their general, phenolic, and pectic polysaccharide composition have been investigated.

2.3. Removal of wine polysaccharides by using solid phase extraction

Solid phase extraction was performed to obtain polysaccharide-free phenolic extracts of the "aged" red wines using a food-grade adsorbent resin and food-grade chemicals. The applied extraction protocol was published by Weber et al. (2013) with a few modifications as described by Weilack et al. (2023). The extracts of two bottles of each wine were combined, concentrated under vacuum, consecutively lyophilized, and yields were determined gravimetrically.

2.4. Polyphenol composition of the wines and polyphenolic extracts

For polyphenol analyses, the lyophilized extracts were combined and

Table 1
General composition of the "aged" Cabernet Sauvignon samples after storage at 35 °C for 10 weeks determined by Fourier-transform mid-infrared (FT-IR) spectroscopy and titration for total and free SO_2. Due to method robustness, analysis was conducted in single determination.

Wine	Glycerol [g/L]	Residual sugars [g/L]	Titratable acidity [g/L TAE[a]]	Tartaric acid [g/L]	Lactic acid [g/L]	pH	Total SO_2 [mg/L]	Free SO_2 [mg/L]
Adentu (CHL)	8.4	2.4	4.7	1.7	1.4	3.7	56	n.d.[b]
Beringer (USA)	9.5	8.0	4.9	1.2	0.9	3.8	99	5
Bundschuh (GER)	9.3	5.7	5.3	1.3	2.0	3.8	90	4
Canyon Road (USA)	9.1	11.2	4.6	1.8	0.9	3.9	72	7
Las Mulas (CHL)	9.5	1.8	4.5	1.2	1.4	3.8	69	5
Weinbiet (GER)	10.5	2.9	4.4	1.1	1.0	3.9	73	23

[a] Titratable acidity is expressed in g/L tartaric acid equivalents (TAE).
[b] n.d. = not detected.

dissolved at concentrations of 2 g/L in a wine-like solution (12% ethanol by volume, 5 g/L tartaric acid, pH 3.3 adjusted with NaOH). The determined phenolic characteristics of the "fresh reference" and "aged" wines and corresponding extracts included total anthocyanins, non-precipitable polymeric pigments (np-PP), precipitable polymeric pigments (p-PP), and were assessed following the photometric assays described by Harbertson, Picciotto, and Adams (2003, 2009, 2015) using a Jasco V-730 double-beam spectrophotometer (JASCO Deutschland GmbH, Pfungstadt, Germany). Anthocyanins are expressed as malvidin-3-glucoside equivalents (Mal-3-glu equiv.) based on an empirical factor and tannins were expressed as catechin equivalents according to an external calibration curve. The "fresh reference" wines and corresponding extracts have been analyzed after 10 weeks of storage at 10 °C and results have been published (Weilack et al., 2023). The analyses were conducted in triplicate.

2.5. Precipitation of total soluble polysaccharides

Total soluble polysaccharides (TSP) were obtained from red wines and polyphenol-rich extracts using ethanolic precipitation following the protocol published by Watrelot et al. (2017) with some adjustments outlined by Weilack et al. (2023). The extraction process was carried out twice and yields were determined through gravimetric measurements.

2.6. Characterization of total soluble polysaccharides

2.6.1. Degree of methylation (DM) and degree of acetylation (DA)

According to Larsen, Buerschaper, Schieber, and Weber (2019), the determination of the degree of methylation (DM) and the degree of acetylation (DA) employed headspace solid-phase dynamic extraction gas chromatography (HS-SPDE-GC) with flame ionization detection (FID) after saponification. The SPDE equipment (Chromtech, Idstein, Germany) was integrated in a CTC-Combi-PAL-Autosampler (Bender and Hobein, Zurich, Switzerland) and connected to a GC FID system (Agilent Technologies model 6890). DM and DA were quantified as mol of methyl/acetyl groups per 100 mol of galacturonic acid (GalAc), as previously outlined (Levigne, Thomas, Ralet, Quemener, & Thibault, 2002), and are expressed in percentage. The analysis was conducted in triplicate.

2.6.2. Determination of monosaccharide composition (galacturonic acid, L-rhamnose, and L-fucose)

The quantification of the monomer composition of the total soluble polysaccharides was conducted using the methodology established by Larsen et al. (2019). Sample hydrolysis was carried out as indicated in the enzyme kits from Megazyme (Wicklow, Ireland) using sulfuric acid (2 M) at 100 °C (6 h) for the determination of GalAc and hydrochloric acid (2.4 M) at 100 °C (1 h) for contents of rhamnose and fucose, respectively. After centrifugation at 10947*g* for 10 min, the specific monosaccharides were measured in the supernatant by absorbance readings at 340 nm. The analysis was conducted in triplicate.

2.6.3. Molecular weight distribution of total soluble polysaccharides

The molecular weight (MW) distribution was assessed through high-performance size exclusion chromatography (HPSEC) equipped with a Smartline HPLC system with a RI detector 2300 (Knauer, Berlin, Germany) and two SEC-Diol columns (300 and 120 Å, 3 μm; YMC, Kyoto, Japan) following the method published by Larsen et al. (2019). Samples were dissolved in water (50 °C) and dialyzed against demineralized water (MWCO 12–14 kDa). Elution of polysaccharides was achieved using water containing 50 mM sodium nitrate and 0.25% sodium azide. MWs were determined using eight pullulan standards ranging from 0.504 to 708 kDa (ReadyCal-Kit Pullulan, PSS- Polymer Standards, Mainz, Germany). Chromatograms were segmented into three representative fractions: high molecular weight fraction (15–708 kDa), medium molecular weight fraction (5.5–15 kDa), and low molecular weight fraction (<5.5 kDa). The proportions of each fraction were calculated relative to the total area. The analysis was conducted in triplicate.

2.6.4. Protein content

The proportion of proteins of the total soluble polysaccharide (TSP) precipitates was quantified using the combustion method on a Euro EA - CHNSO Elemental Analyzer (HEKAtech GmbH, Wegberg, Germany) following the instructions of the manufacturer. Samples (2 mg) were weighed into a sample cup and directly analyzed. Acetanilide was used as external standard. Using 6.25 as nitrogen to protein conversion factor, the percentage of proteins was calculated. The analysis was conducted in duplicate.

2.7. Sensory profiling of samples

To determine the effects of interactions between polysaccharides and polyphenols on red wine astringency, the "fresh reference" and "aged" wines and their polysaccharide-free extracts were evaluated by panel tasting. The protocol of the sensory profiling was oriented on the optimized descriptive profiling (ODP) established by Rita de Cássia dos Santos Navarro da et al., 2012, which was shown to be suitable for the correlation of sensory and instrumental measurements. The wines were subjected to a bench tasting to identify the most important attributes and astringency sub-qualities of the wines. The astringency sub-qualities were selected based on the mouth-feel wheel by Gawel et al. (2000). The attributes that were agreed upon were "sour" and "bitter" as taste attributes and "astringency", "unripe" and "dry" as mouthfeel attributes. Gawel et al. (2000) defined the astringency sub-qualities "unripe" and "dry" as "a negative hedonic grouping consisting of an astringent feel associated with excessive acidity and associated green flavour notes" and "feelings of lack of lubrication or desiccation in the mouth", respectively. These definitions were presented to the panel during the tastings. After the pre-selection of the judges, the sensory panel consisted of 19 judges, of which nine were female and 10 were male with ages ranging from 23 to 60 years (mean 41 years). Panelists were recruited from the professional and private environment of the study leader on a strict voluntary basis. The pre-selection of the panel was

composed of two sessions. During the first session panelists were familiarized with the attributes and their differentiation. Solutions of caffeine (1.5 g/L; Siegfried Pharma Chemikalien, Minden, Germany), tartaric acid (2.5 g/L; Otto Fischar GmbH, Saarbrücken, Germany), aluminum sulfate (2.5 g/L; Euro OTC Pharma GmbH, Bönen, Germany), catechin (3 g/L; Sigma Aldrich Chemie GmbH, Steinheim, Germany) and tannic acid (2 g/L; Omikron GmbH, Neckarwestheim, Germany) were presented in a Pinot noir wine from 2018 used as basic wine to train "bitter", "sour", "astringency", "unripe" and "dry", respectively. This was achieved by advising the panel to assign the attributes to the corresponding solutions, whereby the matching had to be 80% correct. The second session was dedicated to the recognition of various concentrations of these solutions. Solutions of the reference materials were presented containing zero, 33%, 66%, and 100% of the standard concentrations used in the first session, whereby only two of the consecutive samples were allowed to be mixed up. Afterwards, the judges were familiarized with the intensity scale of the final tasting, which was a continuous scale from 0 to 10 for "very low intensity" and "very high intensity", respectively, and reference material. Two differently concentrated reference solutions were presented and their position on the scale was discussed. Trial tastings were held in two sessions, each presenting four wines and one reference solution. For the profiling tasting, the extract samples were dissolved in model wine solutions prepared for each wine sample according to their general composition determined by FT-IR (Table 1; compare Weilack et al. (2023) for composition of "fresh reference" wines) except for the SO_2 addition and their yield after extraction (Table A1). Wine samples were presented in a balanced random order in clear, coded glasses and were tasted in duplicate. One reference solution for each attribute was provided in every session. The panelists were advised to taste 10 mL of the samples, keep them in the mouth for 10 s and rank intensities after spitting. They were advised to neutralize the oral cavity with water and bread and to wait 3 min before tasting the following sample. All sessions were conducted as home use tests using the sensory tool RedJade 2021 (RedJade Sensory Solutions, LLC., Martinez, CA).

The study design was submitted to the Ethics Committee of the Rheinische Friedrich-Wilhelms-Universität Bonn, which approved this study (reference number 460/20). Informed consent was obtained from each panelist as part of the submitted request.

2.8. Statistical analysis

The results were statistically analyzed using XLSTAT (Version 2019.1.1, Addinsoft Technologies, Paris, France). Two-way ANOVA with a selected significance level of $\alpha = 0.05$ followed by pairwise comparison (Tukey (HSD)) was applied to analyze protein concentrations and polyphenol compositions. The phenolic composition of the "fresh reference" wines and corresponding extracts, which were previously published by Weilack et al. (2023), were statistically reanalyzed considering the comparison with the results of the "aged" wines and corresponding extracts. Three-way ANOVA with a selected significance level of $\alpha = 0.05$ followed by pairwise comparison (Tukey (HSD)) was applied to analyze the TSP concentrations. Principal Component Analysis (PCA) was performed on all attributes of the sensory profiling to investigate the effect of pectic polysaccharides and aging of wines on their sensory perception. Pearson's correlation analysis was conducted to examine the relationship between analytical parameters and sensory profiling. An agglomerative hierarchic cluster analysis (AHC), which examines the dissimilarity of the "aged" samples, was performed based on the tannin concentrations and polymeric pigment compositions (Ward method).

3. Results and discussion

3.1. Removal of polysaccharides and precipitation of total soluble polysaccharides (TSP)

Table A1 (supplemental data) presents the yields of the polyphenolic extracts obtained by solid phase extraction, the total soluble polysaccharides (TSP) concentrations of the wines and the extracts, and the protein concentrations of the alcohol insoluble precipitates. The results for the "fresh reference" wines, which were stored at 10 °C for 10 weeks, have been published earlier, but are included for convenience (Weilack et al., 2023). The TSP concentrations of the polyphenolic extracts were determined by referencing to the corresponding extract concentration in the wine. The results (Table A1) show that the polyphenolic extracts contain only negligible amounts of TSP compared to the high yields of the wines; hence, the wine polysaccharides were successfully removed ensuring that the differences seen between wines and polysaccharide-free extracts can be assigned to the presence and absence, respectively, of soluble polysaccharides.

Because the alcoholic precipitation of TSP causes the co-precipitation of proteins (Selvendran, 1975), the protein concentrations in the precipitates were determined to rule out that the differences in polyphenol composition of the Cabernet Sauvignon wines and corresponding extracts are due to interactions between polyphenols and proteins. The protein concentrations ranged from 0.9 ± 0.1 % for the "fresh reference" Adentu wine to 6.3 ± 0.2% for the "fresh reference" Beringer wine (Table A1) indicating that the majority of compounds in the precipitates were wine polysaccharides. Moreover, parts of the protein content derive from the protein residues of polysaccharides like arabinogalactan-proteins and mannoproteins. However, after the aging, the TSP yields of the "aged" wines, except for the Weinbiet and Bundschuh wines, are significantly higher than their "fresh reference" counterparts. The higher TSP yields after the accelerated aging are hardly correlated with an increased co-precipitation of proteins ($r = 0.47$; Table A1), which becomes evident by the fact that the "aged" Canyon Road, Beringer, and Bundschuh wines show no difference or even lower protein concentrations compared to the corresponding "fresh reference" wines. Earlier research reported that the structural features of pectic polysaccharides like degree of methylation (DM), proportions of galacturonic acid, and molecular weight highly impact their alcohol solubility (Guo et al., 2016; Karnik, Jung, Hawking, & Wicker, 2016). Because TSP were obtained through alcoholic precipitation, the data hints that the polysaccharides underwent structural changes during the aging that led to an increased alcohol insolubility and higher TSP yields.

3.2. Polyphenol composition of the wines and the corresponding polysaccharide-free counterparts

Anthocyanins, tannins, and the ratio of protein precipitable polymeric pigments (p-PP) and non-precipitable polymeric pigments (np-PP) of the "fresh reference" and "aged" wines and corresponding polysaccharide-free counterparts were determined (Fig. 1). To display and discuss the compositional changes the wines underwent during aging and the removal of polysaccharides, the results of the "fresh reference" wines and their corresponding extracts, which have been published earlier (Weilack et al., 2023), are included in Fig. 1.

While Table 1 shows that the general composition of the wines was not significantly affected by the accelerated aging (data of the "fresh reference" wines is not shown), the polyphenolic composition of the wines changed due to age-related reactions indicating that the "aged" samples can be described as distinct wines (Fig. 1). During aging, anthocyanin concentrations generally decreased in wine and extract samples, which was expected due to degradation, conversion, and incorporation of anthocyanins into polymeric pigments (McRae et al., 2012). Besides the anthocyanins of the "aged" Weinbiet wine, which declined by 33%, the other "aged" wines show a decline of around 50%

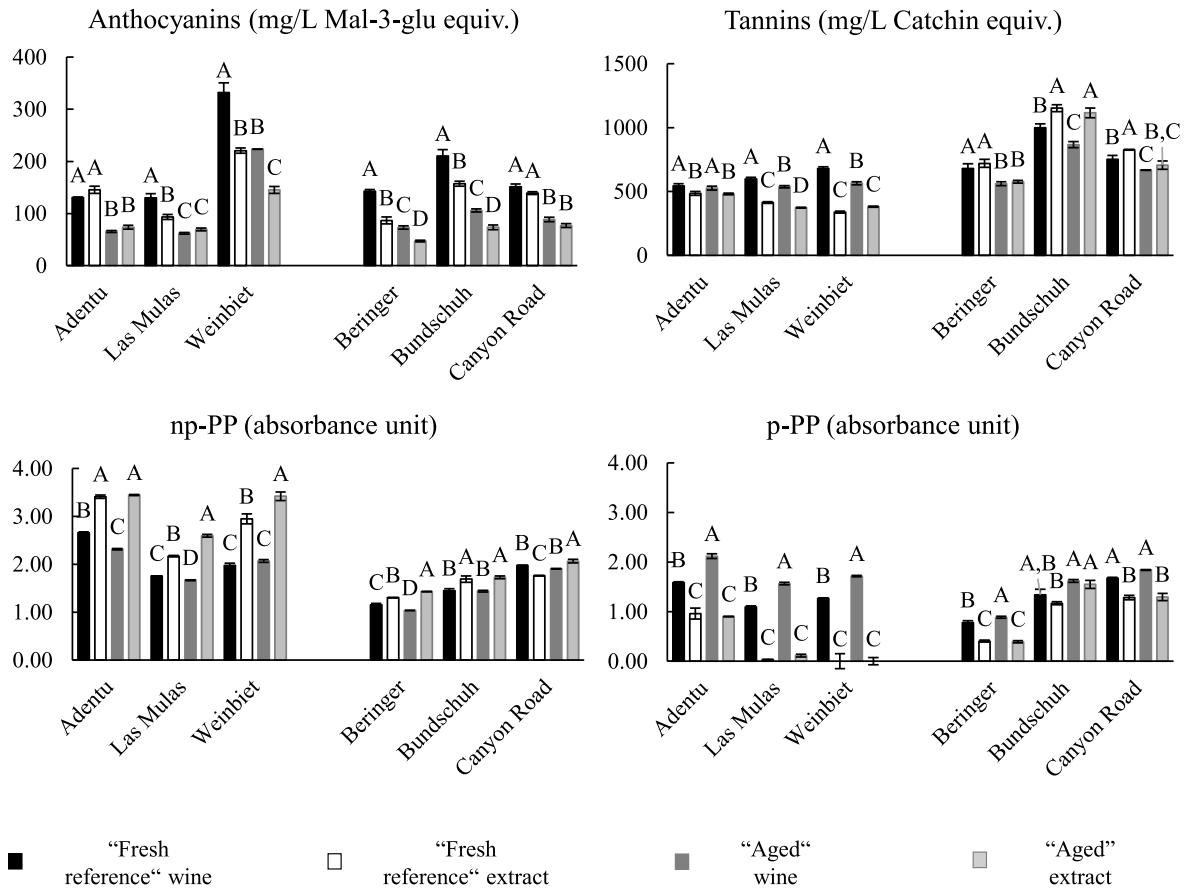

Fig. 1. Phenolic composition of commercially available Cabernet Sauvignon wines, which were subjected to forced aging (35 °C for 10 weeks; "aged"), "fresh reference" wines (10 °C for 10 weeks), and their corresponding polysaccharide-free extracts. Analyses included total anthocyanins, non-precipitable polymeric pigments (np-PP), precipitable polymeric pigments (p-PP), and total tannins and were obtained by photometric assays (Harbertson et al., 2003, 2009, 2015). Weilack et al. (2023) have previously published the results of the "fresh reference" wines and extracts. The gap between the Weinbiet and Beringer samples is implemented to differentiate the two groups according to whether protein precipitation of tannins is enhanced or reduced as mentioned in the text. Means having different letters show a significant difference ($\alpha = 0.05$) within each wine; n = 3.

in anthocyanins. Comparing the results of the "fresh reference" extracts with the "aged" extracts reveals that the actual decline of Las Mulas and Weinbiet anthocyanins amounts to 26% and 34%, respectively, while the decline in the other extract samples stays at around 50%. This indicates that the anthocyanins found in these two wines were less affected by the age-related changes in polyphenol composition. The "aged" Beringer, Bundschuh and Weinbiet wines show higher anthocyanin concentrations than their corresponding extracts probably due to co-pigmentation-like effects resulting from the interactions between wine polysaccharides and anthocyanins (Padayachee et al., 2012). These effects lead to hyperchromic shifts in the absorbance spectra of anthocyanins (Fernandes et al., 2020) and to seemingly higher anthocyanin concentrations in the wines, as assessed by the photometric assay. However, data shows that these co-pigmentation-like effects became less pronounced during the aging. The co-pigmentation-like effect is based on the adsorption of anthocyanins on pectins, followed by the self-aggregation and parallel stacking of anthocyanins (Padayachee et al., 2012). As anthocyanins generally decreased during the aging, this led to a reduced number of anthocyanins that could take part in the stacking possibly leading the diminished copigmentation.

Tannin concentrations decreased during aging in all wine samples but the Adentu wine, which shows no changes in tannin concentrations. In contrast, the tannin readings of the polysaccharide-free extracts with the exception of Beringer and Canyon Road show negligible changes in tannin concentrations. According to Weilack et al. (2023) the differing tannin readings, which are based on the protein precipitation of tannins, result from the modulation of tannin precipitability by pectic polysaccharides. Thereby, the composition of polysaccharides determines whether tannin precipitability is enhanced or prevented. Small, more polar and acidic polysaccharides, like RG-II and polygalacturonic acids, prevent tannin precipitability, whereas large, more neutral and esterified polysaccharides, like AGP, AG and HG fragments, increase tannin precipitability (Carvalho et al., 2006; de Freitas, Carvalho, & Mateus, 2003; Mateus, Carvalho, Luís, & de Freitas, 2004; Weilack et al., 2023). As seen earlier (Weilack et al., 2023), this allowed the categorization of the "fresh reference" wines into two distinct groups, according to the direction of the effect. Comparing the tannin concentrations of the "aged" wines and corresponding "aged" extracts, it appears that the "aged" samples still divide into the same two groups. Group 1 includes the Adentu, Las Mulas and Weinbiet wines, which show an increased protein precipitability, whereas in group 2 (Beringer, Bundschuh and Canyon Road wines) tannin precipitability decreases in the presence of the respective polysaccharides. This is supported by the cluster analysis of the tannin concentrations and polymeric pigment composition of the "aged" samples, which shows that the wines can be clustered into two main groups, with the Bundschuh and Weinbiet wines forming a separate group based on (pigmented) tannins and np-PP, respectively (Fig. 2).

The aging process led to a slight decline in non-precipitable polymeric pigments (np-PP) in the "aged" Adentu and Beringer wines, while they stagnated in the other wines. On the other hand, precipitable polymeric pigments (p-PP) increased significantly in the "aged" group 1 wines. This is supported by previous publications (Merrell, Larsen, & Harbertson, 2018; Weilack et al., 2021), which postulated that the

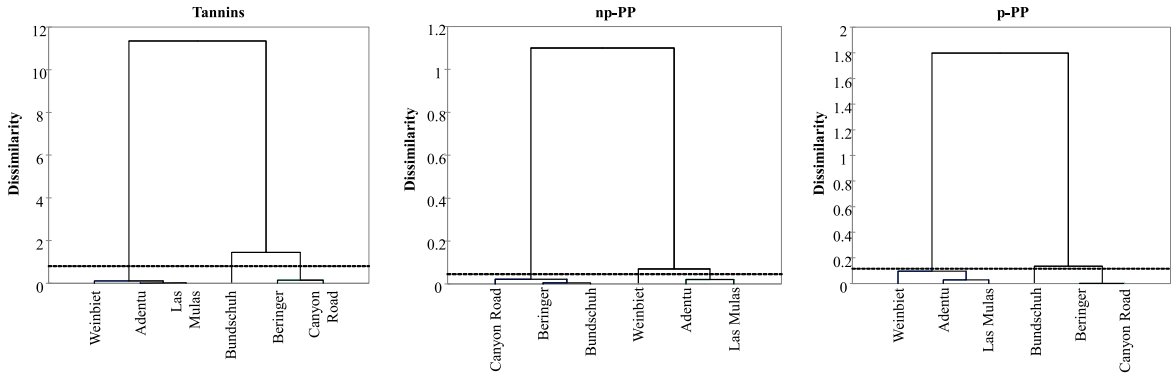

Fig. 2. Results of the agglomerative hierarchic cluster analysis (AHC; Ward method) based on the dissimilarities of the tannin concentrations and polymeric pigment compositions of the "aged" samples. Tannins and polymeric pigments were determined according to Harbertson et al. (2003, 2015) and are presented in Fig. 1.

formation of np-PP would reach a plateau during red wine aging either due to balanced formation and degradation of np-PP or favored formation of p-PP. However, this study shows that np-PP increased in the "aged" polysaccharide-free extracts while p-PP stagnate in all "aged" extracts but the Bundschuh extract, which shows an increase in p-PP. In general, the "aged" wines contain less np-PP and more p-PP than the "aged" polysaccharide-free counterparts, whereas the "aged" polysaccharide-free extracts show higher np-PP proportions and lower p-PP proportions. Thereby, group 1 wines show the bigger differences; hence, in the presence of pectic polysaccharides np-PP are apparently measured as p-PP indicating a higher protein precipitability (Weilack et al., 2023). Ultimately, this leads to the conclusion that certain structural features of pectic polysaccharides result in the formation of protein precipitable pigments, which are measured as p-PP, but are composed of pigmented polysaccharide-polyphenol complexes (Weilack et al., 2023). Altogether, this extends the findings of Graves and Sommer (2021), who showed that polymeric pigment determination using the protein precipitation assay was affected by polysaccharides depending on their concentration they were added to the wines. The authors attributed this effect to the reduced bisulfite bleachability of complexed anthocyanins, which was similarly shown for a decreased discoloration of anthocyanins due to hydration (Fernandes et al., 2021).

The differences in polymeric pigment proportions between wines and extracts become more pronounced with the aging of the wines suggesting the ongoing formation or pigmentation of these protein precipitable pigments. The "fresh reference" Las Mulas and Weinbiet extracts did not contain p-PP, indicating that their specific polysaccharides might have prevented the formation of p-PP (Weilack et al., 2023). The present study shows that the "aged" Las Mulas and Weinbiet extracts contain very few to no p-PP. Together with the fact that anthocyanins were seemingly less affected by age-related reactions, this reinforces the assumption that the aggregation of polysaccharides and pigments could even impair the incorporation of anthocyanins into tannins hindering the formation of conventional, covalently bound p-PP. In contrast, the increase in p-PP of the "aged" Bundschuh polysaccharide-free extract suggests that conventional p-PP were actually formed in this wine during aging. The proceeding incorporation of anthocyanins into tannins renders the tannins more polar, which may lead to an increased interaction with negatively charged RG-II molecules (Dufrechou, Doco, Poncet-Legrand, Sauvage, & Vernhet, 2015). This may result in an enhanced protective effect of RG-II towards the precipitation of tannins in the "aged" Bundschuh wine (Watrelot et al., 2017). It cannot be completely ruled out that storage at 35 °C during the accelerated aging of the wines could have triggered reactions that are not observed during typical aging of red wines at lower temperatures.

Earlier research (Alcalde-Eon et al., 2014; Guadalupe et al., 2007; Osete-Alcaraz et al., 2020; Siebert, Carrasco, & Lynn, 1996) reported that some polysaccharides may act as protective colloids in red wine, stabilizing anthocyanins, anthocyanin-derived pigments, and tannins preventing them from aggregating with proteins and self-aggregation. At the same time, Siebert et al. (1996) and Guadalupe et al. (2007) observed the precipitation of unstable colloidal polyphenol-protein-polysaccharide ternary complexes. Therefore, the altered protein precipitability of tannins and pigments due to polysaccharides can be assigned to the presence and formation of protective/stable or unstable colloids, respectively. Accordingly, the categorization of the wines into two groups can be based on the stability of the colloidal system, being unstable for group 1 (Adentu, Las Mulas and Weinbiet) and stable for group 2 (Beringer, Bundschuh and Canyon Road). In the following, total soluble polysaccharides will be characterized to investigate age-related changes of the structural features of (un-)stable colloids.

3.3. Total soluble polysaccharides (TSP) composition

To characterize the soluble polysaccharides of the "aged" wines, the molecular weight (MW) distribution, the monomeric sugar composition, and the degree of methylation (DM) and acetylation (DA) were determined using size exclusion chromatography (SEC), photometric assays, and GC-FID (Table 2 and Fig. 3). Because all the wines showed an increased precipitation of TSP (Table A1), which may be due to the structural changes of the wine pectic polysaccharides, the composition of TSP may partly be assigned to the composition of the additionally precipitated polysaccharides. The polysaccharide composition of the Cabernet Sauvignon wines that were not subjected to accelerated aging but stored for 10 weeks at 10 °C, following referred to as "fresh reference" wines, was subject of an earlier work and is described by Weilack et al. (2023).

Fig. 1 shows the MW distribution of the polysaccharides of the "aged" wines, which were stored at elevated temperatures for 10 weeks to simulate red wine aging. The polysaccharides show a high polydispersity across all samples and are split into three fractions: the high MW fraction (>15 kDa) comprising substances such as arabinogalactans (AG), arabinogalactan-proteins (AGP), mannans, mannoproteins (MP), as well as small quantities of homo- (HG) and rhamnogalacturonan I (RG-I) (Ayestarán, Guadalupe, & León, 2004; Gao et al., 2015; Guadalupe & Ayestarán, 2007); the medium MW fraction (5.5–15 kDa) including rhamnogalacturonan II (RG-II) dimers (~10 kDa; Pellerin et al., 1996) and medium MW fragments of homogalacturonan (HG), rhamnogalacturonan I (RG-I), AGP, and MP; the low MW fraction (<5.5 kDa) containing rhamnogalacturonan II (RG-II) monomers (~4.6 kDa; Pellerin et al., 1996) and HG, RG-I, AGP, and MP fragments with low molecular weights (Guadalupe & Ayestarán, 2007). With the exception of the "aged" Bundschuh and Beringer wines, the MW distributions of the "aged" wines shift slightly from the high MW to the medium and small MW fractions (Table 2 and Fig. 3). All "aged" wines show a distinct

Table 2
Composition of the pectic polysaccharides of the "aged" Cabernet Sauvignon wine samples after storage at 35 °C for 10 weeks including the proportions of the high and medium molecular weight (MW) fractions, the degree of methylation (DM) and acetylation (DA) and the monosaccharide composition including galacturonic acid (GalAc), rhamnose (Rha), and fucose (Fuc). Means having the same letters are not significantly different at $\alpha = 0.05$. Means presented with standard deviation; n = 3.

Wine	High MW fraction (15–708 kDa) [%]	Medium MW fraction (5.5–15 kDa) [%]	DM [%]	DA [%]	GalAc [mg/g]	Rha [mg/g]	Fuc [mg/g]
Adentu	55.3 ± 0.6 A	44.7 ± 0.6 D	8.3 ± 0.1 E	0.1 ± 0.1 D	37.0 ± 1.5 A	9.1 ± 0.5 A	1.7 ± 0.1 A
Beringer	47.4 ± 2.4 B	52.6 ± 2.4 C	24.3 ± 1.1 C	8.2 ± 0.1 A	32.9 ± 3.1 A	3.2 ± 0.1 B	1.3 ± 0.1 B
Bundschuh	49.5 ± 0.6 B	50.5 ± 0.6 C	30.7 ± 0.8 B	3.2 ± 0.6 B,C	35.2 ± 0.6 A	2.8 ± 0.1 B,C	1.7 ± 0.1 A
Canyon Raod	34.4 ± 0.6 D	65.6 ± 0.6 A	13.4 ± 0.1 E	3.5 ± 0.2 B,C	33.0 ± 1.8 A	3.3 ± 0.1 B	1.4 ± 0.1 B
Las Mulas	39.7 ± 0.5 C	60.3 ± 0.5 B	19.3 ± 1.0 D	1.9 ± 0.4 C,D	31.1 ± 0.1 A	2.4 ± 0.1 C	1.3 ± 0.1 B
Weinbiet	50.6 ± 1.6 B	49.4 ± 1.6 C	48.5 ± 2.6 A	4.3 ± 0.4 B	34.9 ± 1.0 A	2.3 ± 0.1 C	1.4 ± 0.1 B

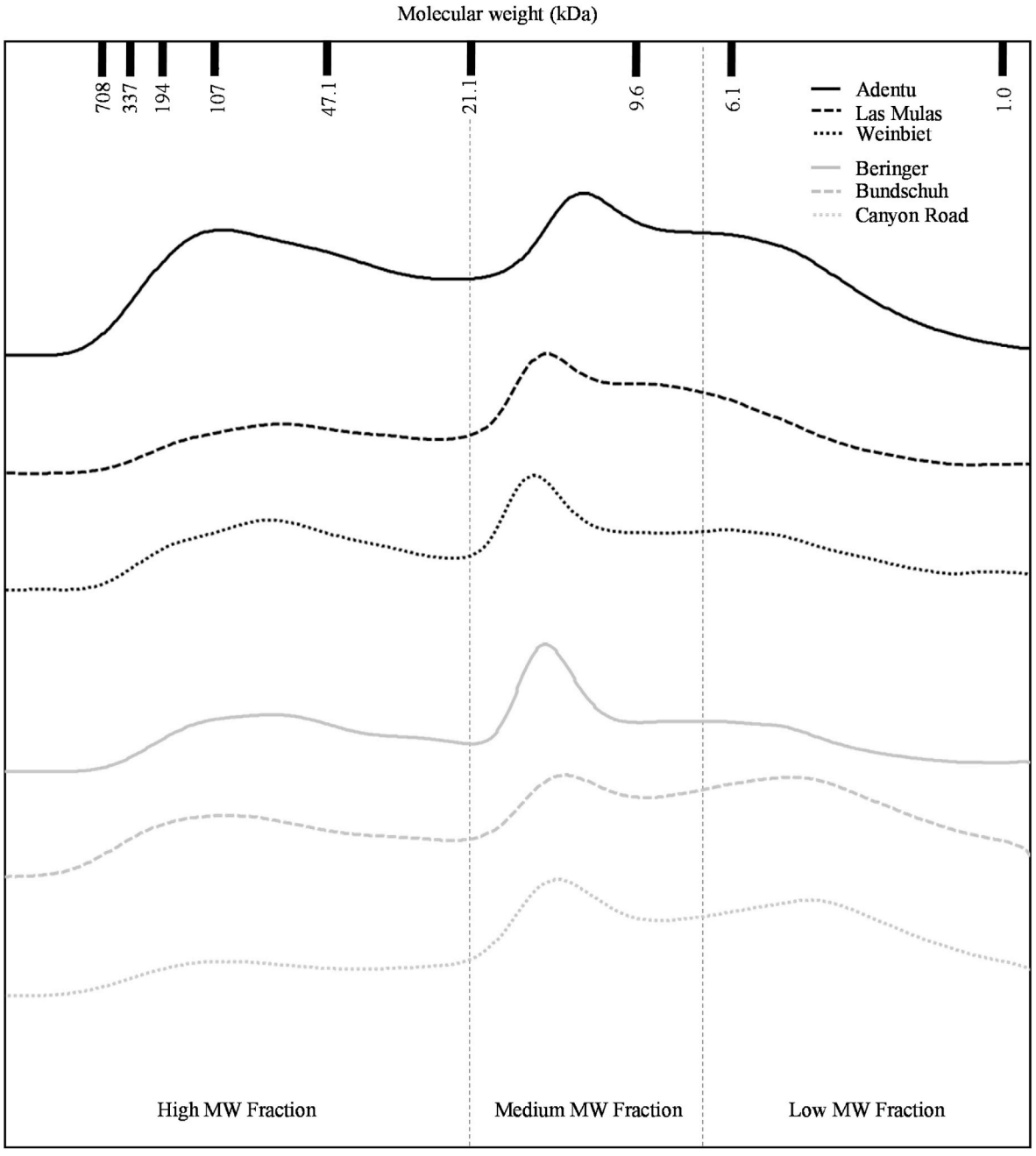

Fig. 3. Size exclusion chromatograms of the pectic polysaccharides of the "aged" Cabernet Sauvignon wine samples after storage at 35 °C for 10 weeks including the high (>15 kDa), medium (15–5.5 kDa) and low (<5.5 kDa) molecular weight (MW) fractions. Chromatograms are overlayed and offset. Chromatograms of the "fresh reference" wine samples were presented earlier (Weilack et al., 2023). MW calculation was calibrated with standards illustrating the peak molecular weight distribution.

peak in the low MW fraction at around 4.6 kDa, suggesting the presence of RG-II monomers and low MW fragments of other pectic polysaccharides, like polygalacturonic acids. While the proportions of fractions are constant for the Bundschuh wines, the "aged" Beringer wine shows a higher proportion of high MW and a lower proportion of low MW polysaccharides after the aging of the wine. Like the "fresh reference" wines (Weilack et al., 2023), the "aged" Adentu and Weinbiet wines show the highest proportions of the high MW fraction (Table 2) suggesting high proportions of AG, AGP, MP, HG and RG-I. In contrast, the "aged" Bundschuh, Beringer, Canyon Road and Las Mulas wines show high proportions of the medium MW fraction, hence, considerable amounts of RG-II molecules and other low MW fragments.

To further characterize the composition of pectic polysaccharides, galacturonic acid (GalAc), rhamnose (Rha) and fucose (Fuc) concentrations were determined (Table 2), which can be correlated with the relative proportions of HG, RG-I and RG-II fragments, respectively, due to their well-defined occurrence in the pectin structure (Larsen et al., 2019). While the "fresh reference" wines showed a wide range of GalAc concentrations (16.0 ± 0.6 mg/g for the Adentu wine to 47.6 ± 0.6 mg/g for the Bundschuh wine; Weilack et al., 2023), the aging of the wines led to the GalAc concentrations approaching a similar level and ranging from 31.1 ± 0.1 to 37.0 ± 1.5 mg/g for the "aged" Las Mulas and Adentu wines, respectively. In contrast to the "aged" Bundschuh and Weinbiet wines, which showed lower GalAc concentrations after aging, the GalAc concentrations of the other wines increased. The shift of the MW distribution of the polysaccharides towards smaller molecules, together with an increased TSP yield (Table A1) indicates the degradation of larger HG chains to smaller oligomers during the simulated aging, which resulted in an enhanced alcohol precipitability as published by Guo et al. (2016) and Karnik et al. (2016).

Data presented in Table 2 and Fig. 3 shows that the polysaccharide composition of the wines became more similar to each other during the accelerated aging, which is particularly evident in the concentrations of the monomeric sugar concentrations. The structural changes observed in this study were similarly reported by Doco, Quellec, Moutounet, and Pellerin (1999), Guadalupe and Ayestarán (2007), and Quijada-Morín, Williams, Rivas-Gonzalo, Doco, and Escribano-Bailón (2014), who showed that various periods of storage of red wines led the polysaccharide composition to shift towards smaller pectin fragments. While these authors stated that the amount of TSP remained unchanged or declined, Guo et al. (2016), and Karnik et al. (2016) showed that lower degrees of esterification, less neutral sugars, higher GalAc concentrations and less RG-I fragments would lead to an increased alcohol insolubility. As the TSP composition of the "aged" wines is characterized by these features, this might explain the increased TSP yields.

The number of free hydroxy groups and in particular the number of free and dissociated carboxylic acid groups, which is represented by the degree of esterification, significantly influences the polarity and hydrophobic nature of pectic polysaccharides. Moreover, the strength of interactions between polysaccharides and polyphenols depend on structural features like the degree of esterification (Liu et al., 2020). During the aging of the wines, the DM and DA decreased in all samples apart from the "aged" Beringer wine, in which the esterification increased. The decrease of DM and DA together with the increase in GalAc concentrations and the shift to lower MW render the resulting polysaccharides more polar, which impacts the interactions with polyphenols and proteins. As the changes of the polysaccharide composition led to smaller proportions of the large, neutral polysaccharides in the "aged" Adentu, Las Mulas and Weinbiet wines and an increase in smaller, less esterified, thus more acidic, pectin fragments, the differences of tannin concentrations between "aged" wines and corresponding polysaccharide-free extracts become less pronounced. This indicates that the structural changes of pectic polysaccharides result in a slightly less enhanced protein precipitation of tannins, thus, less unstable colloids. On the other hand, the "aged" Bundschuh wine shows a higher RG-II concentration than the "fresh reference" wine, which had a Fuc concentration of 1.5 ± 0.1 mg/g (Weilack et al., 2023), thus the Bundschuh polysaccharides show a higher masking effect for tannins.

Overall, as described earlier, the "aged" wines can still be categorized into two groups according to the precipitability of tannins and pigments, thus, the colloidal stability (Fig. 2). Group 1 wines mainly contained large, neutral, and more esterified, HG and RG-I fragments, which formed unstable colloids with polyphenols and proteins, whereas group 2 polysaccharides appear to be protective colloids, which were composed of a larger proportion of small, acidic, more polar, and less esterified pectin fragments including RG-II. This indicates that the polysaccharide composition of the wines at the beginning of the aging process might be decisive for the development of wine polyphenols and particularly for essential compounds like polymeric pigments.

3.4. Sensory analysis

3.4.1. Implications of polyphenol and polysaccharide interactions on the astringency perception of "fresh reference" Cabernet Sauvignon wines

Astringency and bitter perception of red wines are associated with tannin concentrations and their composition, which includes the degree of polymerization, galloylation and the number of trihydroxylated monomers (de Freitas & Mateus, 2001; Noble, 1998; Vidal et al., 2003). To ensure that the results of the sensory analysis can be reasoned by the presence or the lack of polysaccharides rather than tannin composition in the samples, the polysaccharide-free polyphenolic extracts were dissolved in model wines that were composed according to their yields and the general composition of the corresponding wines. This way, differences caused by ethanol content, residual sugar, acidity, and pH value are eliminated.

Fig. 4 shows the principal component analysis (PCA) of the sensory profiling of all Cabernet Sauvignon samples, the wines and the corresponding polysaccharide-free extracts, including the attributes "sour", "bitter", "astringency", "unripe", and "dry". The detailed ratings of the attributes for the wines and extracts are presented in Figure A1. The attribute "sour" correlates negatively with the attributes "bitter", ($r = -0.515$; sign. with $p = 0.010$), "unripe" ($r = -0.414$; sign. with $p = 0.044$), and "dry" ($r = -0.180$) while "astringency" hardly correlates with any of the other attributes ("bitter": $r = 0.075$; "sour": $r = 0.289$; "unripe": $r = 0.049$; "dry": $r = 0.300$). The samples are split into two groups along the first PC showing that the wines tend to be rated more sour than the extracts, whereas the extracts appear to be more bitter, unripe and dry; hence, the samples can be differentiated by whether or not they contain polysaccharides. The sourness of the "fresh reference" wines correlates with the GalAc concentration ($r = 0.792$); thus, the sourness perception may be related to the proportion of HG fragments, which were extracted into the wine. Therefore, the wines taste more sour in the presence of acidic pectic polysaccharides. This is supported by Chong, Cleary, Dokoozlian, Ford, and Fincher (2019), who reported that sourness of Cabernet Sauvignon wines was related to HG concentrations.

While the astringency sub-qualities, "unripe" and "dry", are related to the polysaccharide content of the samples, the overall "astringency" appears to be correlated rather to the aging of the samples, as the "fresh reference" and "aged" samples are spread along the second PC. Therefore, overall astringency and sub-qualities seem to be perceived independently from each other which was also observed by Wang et al. (2020). The PCA shows a higher explained variance (56%) between the wines and their polysaccharide-free extracts than between the "fresh reference" and "aged" samples (23%). According to Sáenz-Navajas et al. (2020), tannin concentrations were correlated to the general dryness and dryness on palate perception of red wines, whereas tannin activity, which is driven by hydrophobic interactions with proteins, was solely related to the dryness on palate. However, the authors could not link other mouthfeel perceptions to the studied chemical parameters highlighting the need of extending the investigations beyond tannin composition (Sáenz-Navajas et al., 2020). Since hydrophobic

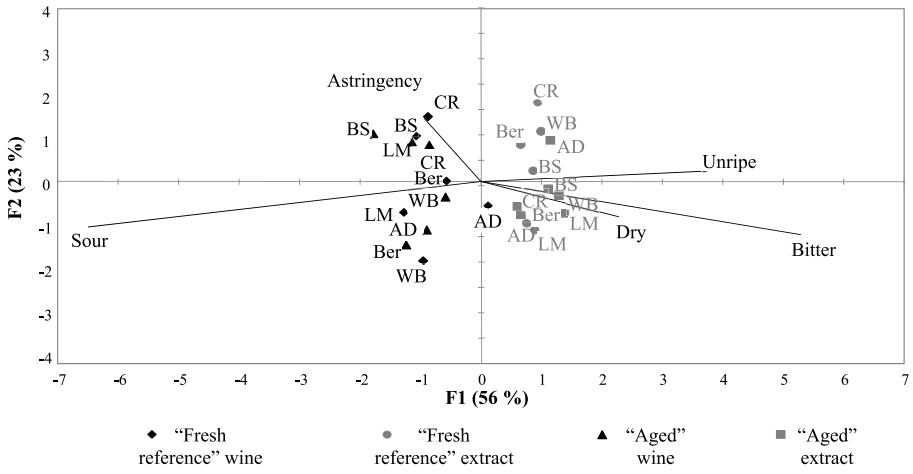

Fig. 4. Principal component analysis (PCA) of the sensory profiling of all Cabernet Sauvignon wines (AD: Adentu, Ber: Beringer, BS: Bundschuh, CR: Canyon Road, LM: Las Mulas, WB: Weinbiet) and corresponding polysaccharide-free extracts, including the attributes "sour", "bitter", "astringency", "unripe", and "dry".

interactions play a role in the interactions of tannins and polysaccharides, mouthfeel perceptions are very likely to be also related to polysaccharide composition.

The "fresh reference" Adentu, Canyon Road and Weinbiet wines are more astringent than their polysaccharide-free extracts, whereas the "fresh reference" Bundschuh wine shows lower "astringency" ratings than the extract (Figure A1). This is more or less in line with the tannin concentrations (Fig. 1), as the Adentu and Weinbiet wines show higher and the Bundschuh wine lower tannin readings than their polysaccharide-free counterparts. However, the higher tannin readings of the Adentu and Weinbiet wines result from an increased tannin precipitability due to interactions between tannins and larger, neutral polysaccharides with a high DM (Weilack et al., 2023). This leads to the assumption, that such tannin-polysaccharide aggregates, which are measured as tannins, contribute to the astringency perception of the wines. Furthermore, not only tannins show a higher precipitability, but also precipitable polymeric pigments are more precipitable. Previous studies (Watrelot et al., 2017; Weber et al., 2013; Weilack et al., 2021) suggested that the formation of pigmented tannins, which are determined as precipitable polymeric pigments, play a role in the attenuation of red wine astringency. The comparison of the "fresh reference" Adentu and Weinbiet wines with their corresponding polysaccharide-free extracts shows that in these wines little to no p-PP were formed (Fig. 1), which may also be adding to the increased astringency perception of the wines.

In contrast, the Bundschuh wine contains a higher proportion of small, acidic polysaccharides and a high concentration of RG-II molecules that lead to the prevention of tannin precipitability (Weilack et al., 2023) and consequently to a lowered astringency perception in the wine when compared to the polysaccharide-free extract (Figure A1). The Canyon Road wine appears to be out of line because the "fresh reference" wine is rated more astringent than the corresponding extract (Figure A1) while having a lower tannin precipitability due to its polysaccharide composition (Weilack et al., 2023). According to Carvalho et al. (2006), RG-II molecules can have different effects on the protein-tannin aggregation depending on the type of protein. In the presence of the globular protein α-amylase, RG-II appeared to prevent tannin precipitability while in the presence of IB8c, a representative of the proline-rich proteins (PRPs), RG-II increased protein-tannin aggregation (Carvalho et al., 2006). Since BSA used for tannin precipitation is a globular protein, the lower tannin readings of the "fresh reference" Bundschuh and Canyon Road wines (Fig. 1) cannot simply be related to the astringency perception that is assigned to the interactions between tannins and PRPs (Charlton et al., 2002). However, Vidal, Francis, Noble, et al. (2004) showed that acidic and neutral polysaccharides alone can decrease the astringency perception of a model wine indicating that they can interact with salivary proteins. Besides the highest RG-II concentration, the "fresh reference" Bundschuh wine contains the highest amount of GalAc and the second highest DM indicating the presence of a high proportion of methylated HG fragments (Table 2). Due to the methylation, these fragments are more hydrophobic and therefore they may take part in hydrophobic interactions with salivary PRPs comparable to the first step of astringency elucidation (Charlton et al., 2002). Consequently, this interaction may lead to a lack of PRPs that could precipitate tannins, thus, to a lower astringency perception of the "fresh reference" Bundschuh wine than of the polysaccharide-free counterpart (Figure A1).

In the "fresh reference" wines and the corresponding extracts, the "unripe" and "dry" perceptions of the wines are significantly correlated with Rha concentrations ("unripe": $r = 0.625$, "dry": $r = 0.634$), GalAc ("unripe": $r = -0.725$) concentrations and the Rha/GalAc ("unripe": $r = 0.817$) ratio indicating a relationship between these mouthfeel attributes and the HG and RG-I proportions of the wine polysaccharides. Wang et al. (2020) hypothesized that polysaccharides could lower saliva precipitation by competing with polyphenols in aggregating saliva proteins indicating that polysaccharides can reduce the amount of saliva proteins that could precipitate polyphenols. Comparable to the maltodextrin used in said study, HG is a linear chain of repeating units, in this case GalAc units, which are also partly methylated leading to a higher hydrophobicity enabling it to interact directly with the saliva proteins (Einhorn-Stoll, Archut, Eichhorn, & Kastner, 2021; Rodrigues et al., 2021). This may lead to a protective effect of HG fragments against an unripe and dry perception of red wine. RG-I, on the other hand, is a branched polysaccharide that may be sterically hindered to align and interact with the salivary proteins. A high number of RG-I fragments in the wines would therefore lead to the loss of the protective effect of HG fragments, resulting in an increased unripe and dry astringency, which was observed in the "fresh reference" Adentu and Beringer wines (Figure A1).

3.4.2. Implications of polyphenol and polysaccharide interactions on the astringency perception of bottle aged Cabernet Sauvignon wines

As mentioned before, the PCA of the sensory analysis (Fig. 4) showed a clear separation of the sample types, wine and polysaccharide-free wine, according to their polysaccharide content. The explained variance between the samples based on aging is not as pronounced. In contrast to the "fresh reference" wines, the "aged" Adentu, Las Mulas and Weinbiet wines (group 1) are less "astringent", "unripe" and "dry" than their polysaccharide-free counterparts (Figure A1). This is accompanied with decreasing tannin concentrations, while the proportions of

p-PP increase during aging (Fig. 1). As described before, these changes in polyphenol composition are not reflected in the results of the corresponding polysaccharide-free extracts, which stagnate at lower concentrations for both parameters; hence, while still having an increased tannin precipitability, tannins of the "aged" group1 wines are less precipitable after aging. Therefore, the prevented tannin precipitability together with the higher proportions of pigmented polysaccharide aggregates formed during aging may have a positive effect on the astringency development of the wines. Considering that these pigmented polysaccharide aggregates are measured as p-PP because of their protein precipitability and ability to absorb light at 520 nm, this supports the hypothesis that the formation of p-PP during red wine aging may be beneficial for astringency perception and quality. Yet, this study shows that this may not only be due to pigmented tannins, but also non-covalently bound polysaccharide-polyphenol-pigments may contribute to the attenuated astringency of aged red wines.

In contrast, the "aged" group 2 wines (Beringer, Bundschuh and Canyon Road) show higher means in the astringency-associated attributes than the "fresh reference" wines, and the "aged" wines are perceived as more astringent than their polysaccharide-free counterparts suggesting an opposing astringency development to the one described before (Figure A1). Contrary to the expectation that astringency would decrease in the presence of pigmented tannins (Vidal, Francis, Noble, et al., 2004; Weber et al., 2013; Weilack et al., 2021), the "aged" Bundschuh wine shows an increased astringency after aging despite the formation of p-PP (Fig. 1). As the "aged" Bundschuh wine contains the highest proportion of RG-II (Table 2) and pigmented tannins (Fig. 1), the increased astringency perception may be assigned to enhanced polar interactions between polysaccharides, pigmented tannins, and proteins. This suggests that the soluble complexes formed between the polymeric pigments, tannins, and the acidic, low molecular weight pectin fragments, including RG-II molecules, of the "aged" group 2 wines may still add to the astringency perception. This is supported by Brossard et al. (2021), who showed that both, soluble and insoluble, protein-tannin aggregates can modulate red wine astringency and sub-qualities.

Based on the polysaccharide composition of the wines, the Bundschuh wine appears to have undergone an enzyme treatment, while the Weinbiet wine is considered not treated (Weilack et al., 2023). Similar to the results presented in this study, Kuhlman, Hansen, Jørgensen, Du Toit, and Moore (2022) showed that Cabernet Sauvignon wines, which were not treated with enzymes, contained higher concentrations of large, neutral pectic polysaccharides and were described as being soft, fine, and velvety, whereas enzyme treated wines were more astringent with hard, chalky, grippy, grainy, and dry sub-qualities. The enzyme treatment was accompanied with higher amounts of polymerized and galloylated polyphenols (Ducasse et al., 2010; Kuhlman et al., 2022), which could lead to a coarser astringency perception (Vidal et al., 2003). Overall, astringency appears to emerge from the combination of the respective polysaccharide and polyphenol compositions, whereby the polysaccharide composition can be modulated by using pectolytic enzymes during the winemaking.

It should be mentioned that the astringency attributes are examined individually in this study, but it is unlikely that the reactions described are triggered independently of each other, since many different molecules and structures come together at the same time during red wine consumption (González-Muñoz et al., 2022).

4. Conclusion

The results of the sensory analysis together with the polyphenolic characterization of the wines and corresponding polysaccharide-free extracts show that the samples can be divided into two groups according to the polysaccharide composition of the "fresh reference" wines and their colloidal stability. On the one hand, the polysaccharides of the "fresh reference" Beringer, Bundschuh and Canyon Road wines consisted of a larger proportion of small, acidic, more polar, and less esterified, thus less hydrophobic pectin fragments, which formed stable colloids with polyphenols and prevented protein precipitation. On the other hand, the "fresh reference" Adentu, Las Mulas, and Weinbiet wines contained a greater portion of large, neutral, and more esterified, thus, more hydrophobic pectin fragments, which form unstable colloids and promote protein precipitation of polyphenols. Moreover, the latter appears to impair the formation of covalent protein precipitable polymeric pigments, which is associated with red wine aging due to the complexation of anthocyanins, tannins, and non-precipitable polymeric pigments and keeping them from further reactions. Instead, pigmented protein precipitable polysaccharide-polyphenol aggregates were formed during aging, which contribute to red wine astringency perception and possibly also to color stability. This suggests that the polysaccharide composition of the wines at the beginning of the aging process may be decisive for the development of the polyphenolic composition and astringency of aging wines. Using enzymes with certain pectolytic activities can modulate the composition of pectic polysaccharides during winemaking. This can influence wine style and its potential for aging, which is why further research on the influence of pectic polysaccharides on the polyphenolic composition and astringency perception of red wine is necessary.

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CRediT authorship contribution statement

Ingrid Weilack: Conceptualization, Methodology, Investigation, Formal analysis, Writing – original draft. **Lea Mehren:** Methodology, Investigation, Formal analysis. **Fabian Weber:** Conceptualization, Supervision, Writing – review & editing, Funding acquisition.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.foodhyd.2024.110402.

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