Current Research in Food Science 6 (2023) 100506

Contents lists available at ScienceDirect

Current Research in Food Science

journal homepage: www.sciencedirect.com/journal/current-research-in-food-science

Grape-derived pectic polysaccharides alter the tannin and pigment composition of Cabernet Sauvignon red wines

Ingrid Weilack, Lea Mehren, Andreas Schieber, Fabian Weber *

Institute of Nutritional and Food Sciences, Molecular Food Technology, University of Bonn, Friedrich-Hirzebruch-Allee 7, D, 53115, Bonn, Germany

ARTICLE INFO

Handling Editor: Professor A.G. Marangoni

Keywords:
Pectic polysaccharides
Tannins
Anthocyanins
Pigments
Interactions
Precipitability

ABSTRACT

Tannins, anthocyanins, and polymeric pigments are essential phenolic constituents of red wine because they provide color, color stability, and mouthfeel properties like astringency. The behavior of these compounds is significantly affected by pectic polysaccharides, whereby the extent of their influence on red wine quality depends on their structural features and their interactions with the polyphenols. In the present study, the composition of the pectic polysaccharides of commercially available Cabernet Sauvignon wines and their impact on anthocyanin, tannin, and polymeric pigment analyses was characterized. This was accomplished by preparation of polysaccharide deprived wines and comparison of the polyphenolic composition of both, the wines and their corresponding polysaccharide-free counterparts. The results show that the cell wall fragments enhance the spectral absorbance of anthocyanins by facilitating anthocyanin self-association, leading to a co-pigmentation-like effect. Low molecular weight pectins like rhamnogalacturonan II and polygalacturonic acids with a low degree of esterification are assumed to form soluble complexes with anthocyanins and also prevent protein precipitation of tannins, which was reduced by 6–13%. High molecular weight pectins with a high degree of esterification lead to the increased precipitability of pigments and tannins by a factor of 1.3 to 32.4 and 1.1 to 1.9, respectively, seemingly impairing the incorporation of anthocyanins in tannins to form precipitable polymeric pigments that are responsible for the longevity of red wine color. The increased precipitability of the pigments due to the interactions with the polysaccharides may indicate the formation of pigmented yet non-covalent aggregates that show comparable properties to the covalently formed precipitable pigments. The formation of those non-covalent structures may affect red wine color stability and astringency.

1. Introduction

While the presence and composition of polyphenols in red wine are undisputed of prime importance for red wine quality, it has become evident that polysaccharides play a modulating role for color and color stability but also for mouthfeel properties like astringency. During fermentation, these compounds are extracted from the grapes to the must or wine, whereby their extractability depends on numerous intrinsic and extrinsic factors like the class of polyphenols, fermentation protocols, and grape maturity. The very polar anthocyanins are readily extracted at the beginning of the fermentation, whereas tannins or procyanidins are extracted later due to the increasing alcohol content. Besides the changes in solubility, the constant desorption and adsorption of phenolic compounds on grape cell materials are of at least the same importance (Hensen et al., 2022). The ripening process of grapes is accompanied by the softening of the grape skin, which is associated with the enzymatic degradation of the polysaccharides in the cell walls like hemicellulose, cellulose, and in particular pectic compounds. This degradation process encompasses overall depolymerization, the loss of arabinogalactans, and the decrease of the degree of methylation, and consequently to an increased solubility of the pectin molecules (Nunan et al., 1998). The change of polysaccharide composition influences the potential interactions with the polyphenols, which increases the extractability of tannins and anthocyanins (Hanlin et al., 2010; Hernández-Hierro et al., 2012). Previous studies (Guadalupe et al., 2007; Ducasse et al., 2010) showed that this can have both positive and negative effects on red wine quality. Wines treated with enzymes displayed higher color intensity and color stability but also higher tannin

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

* Corresponding author.
E-mail addresses: weilack@uni-bonn.de (I. Weilack), lmehren@uni-bonn.de (L. Mehren), schieber@uni-bonn.de (A. Schieber), fabian.weber@uni-bonn.de (F. Weber).

https://doi.org/10.1016/j.crfs.2023.100506
Received 30 January 2023; Received in revised form 11 April 2023; Accepted 20 April 2023
Available online 23 April 2023

concentrations. These tannins presented a higher degree of polymerization and galloylation, which was shown to elicit a coarser astringency (Vidal et al., 2003a).

While all these processes occur during maceration, they vastly modify the composition of the resulting wine as most of the de-esterified and de-polymerized pectin fragments find their way into the wine (Gao et al., 2015), where they can interact with the wine polyphenols. These interactions are driven by various mechanisms like hydrophobic interactions, hydrogen bonding, and electrostatic forces, resulting in the formation of non-covalent binding and complexation (Weber, 2022). As a result, the interactions between polysaccharides and polyphenols influence red wine quality characteristics like color and mouthfeel. Several authors (Mazzaracchio et al., 2004; Padayachee et al., 2012; Fernandes et al., 2020) observed that the binding of anthocyanins and polysaccharides results in an intensified red color due to a co-pigmentation-like effect, which can increase the anthocyanin stability. The protein precipitability of tannins can be enhanced or impaired by polysaccharides depending on their structure. De Freitas et al. (2003) showed a disrupting effect for both neutral and acidic polysaccharides, whereas others (Carvalho et al., 2006; Watrelot et al., 2017) reported an increased protein precipitation in the presence of arabinogalactan proteins and rhamnogalacturonan II (RG-II) molecules. Consequently, the changed tannin precipitability alters the astringency perception (Luck et al., 1994; Vidal et al., 2004). Because of the high complexity of red wines, these studies used model experiments to investigate the mechanisms of the underlying interactions between certain pectin fragments and polyphenolic compounds. However, it is necessary to understand which of the before-mentioned interactions actually occur in finished red wines to understand which structural features of pectic polysaccharides are desirable in red wines. This will help to make informed decisions during the winemaking to ensure red wine quality with the potential for ageing. The present study addresses this lack of knowledge by characterizing pectic polysaccharides in commercially available Cabernet Sauvignon wines and investigating their influences on the polyphenolic composition. This was achieved by composing polysaccharide-free counterparts from the wines and comparing the phenolic composition of wines and the corresponding reconstituted wines.

2. Materials and methods

2.1. Materials

Acetic acid, hydrochloric acid (HCl), ethanol, and potassium bisulfite were obtained from VWR International GmbH (Darmstadt, Germany). Urea, bovine serum albumin fraction V, and (+)-catechin were sourced from Carl Roth (Karlsruhe, Germany). Sodium hydroxide and sodium nitrate were acquired from Honeywell Fluka (Offenbach, Germany) and Acros Organics (Geel, Belgium), respectively. Sodium chloride, sulfuric acid, and methanol (HPLC grade) were purchased from Th. Geyer GmbH & Co. KG (Renningen, Deutschland). Maleic acid, ferric chloride, triethanolamine (TEA), and tartaric acid were obtained from Alfa Aesar (Kandel, Germany). Propionic acid, *n*-propanol, and sodium azide were acquired from Merck KGaA (Darmstadt, Germany). Food-grade sodium hydroxide, ethanol, and acetic acid were sourced from Emprove Essential (Merck KGaA, Darmstadt, Germany), Brennerei Kessler (Bad Peterstal-Griesbach, Deutschland), 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).

2.2. Wine samples

Experiments were carried out with six different commercially available Cabernet Sauvignon wines of the 2018 vintage from three wine-growing regions. The wines were from 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 vintage that the wines were made of assured enough time for initial polymeric pigment formation while still holding an ageing potential. When the experiments were conducted, the wines were three years old. The general composition of the 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.

2.3. Separation of wine polyphenols and polysaccharides by using solid phase extraction

To obtain polysaccharide-free phenolic extracts from the wines, solid phase extraction using a food-grade adsorbent resin and food-grade chemicals was performed following the protocol published by Weber et al. (2013) with a few modifications as follows. Each wine sample (750 mL) was diluted with water (3:5) and was loaded onto a column filled with Resinex AD3300 (65 mm × 450 mm; 1.5 L bed volume), which was previously washed with 250 mL of a 0.1% (w/v) sodium hydroxide solution and preconditioned with 2 L of water. The loaded column was washed with 2 L of water (1.3 fold of the bed volume) to remove sugars and organic acids. The polyphenols were eluted with approximately 3 L of ethanol acidified with acetic acid (29:1 v/v) at a gravity flow rate of approximately 10 mL/min. The collected extracts were concentrated using a rotary evaporator and consecutively lyophilized. Extractions were conducted in duplicate, and yields were determined gravimetrically. Prior to further chemical analyses, the lyophilized extracts were pooled and 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).

2.4. Polyphenol characterization of the wine and polyphenolic extracts

Anthocyanins were analyzed following the protocol reported by Harbertson et al. (2009). Protein precipitation was combined with bisulfite bleaching to determine tannins and polymeric pigments (Harbertson et al., 2002, 2003) using a reformulated resuspension buffer (urea 8.3 M, 5% TEA, pH 7 adjusted with HCl) as published by Harbertson et al. (2015). To quantify total iron reactive phenolics, an aliquot of the sample was diluted with the previously mentioned resuspension buffer to a total volume of 875 μL and incubated for 10 min. Absorbance at 510 nm was measured before and after the addition of 125 μL of ferric chloride solution using the Jasco V-730 double-beam spectrophotometer (JASCO Deutschland GmbH, Pfungstadt, Germany). Tannins and total iron reactive phenolics were expressed as catechin equivalents (CE) according to an external calibration curve.

2.5. Precipitation of total soluble polysaccharides

The total soluble polysaccharides (TSP) were extracted from red wines and polyphenol-rich extracts by ethanolic precipitation according to Watrelot et al. (2017) with some modifications as follows. Ethanol was evaporated from 180 mL of wine and wine was concentrated to dryness by lyophilization. The residue was dissolved in 18 mL of water, obtaining a 10-fold concentration of the wine, and 90 mL of cold ethanol acidified with hydrochloric acid (0.1 M) was added. Samples were kept at 4 °C for 18 h on an orbital shaker at a speed of 150 rpm. Subsequently, the samples were centrifuged at 4816*g* for 20 min. Pellets were washed three times with 80% ethanol, then dissolved in water, and finally lyophilized. To precipitate the TSP from the extract samples, 300 mg of

Table 1
General composition of red wine samples determined by Fourier-transform mid-infrared (FT-IR) spectroscopy and titration for total and free SO_2.

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	8.4	2.4	4.7	1.9	1.4	3.7	32	n.d.[b]
Beringer	9.5	9.2	4.9	1.3	1.0	3.8	111	25
Bundschuh	9.4	5.4	5.3	1.3	2.1	3.8	90	12
Canyon Road	9.1	11.1	4.4	1.3	0.9	3.9	70	3
Las Mulas	9.5	1.9	4.6	1.3	1.4	3.8	64	6
Weinbiet	10.8	2.8	4.5	1.2	1.1	3.9	78	24

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

extracts was dissolved in 7.5 mL of water and 37.5 mL of cold acidified ethanol (0.1 M) was added. After the precipitation process at the conditions described before, the samples were centrifuged at 10 947*g* for 20 min. Pellets were washed and lyophilized as described before. The extraction was conducted in duplicate, and yields were determined gravimetrically.

2.6. Characterization of the soluble polysaccharides

2.6.1. Determination of the degree of methylation and the degree of acetylation

The degree of methylation (DM) and the degree of acetylation (DA) were determined according to Larsen et al. (2019) using headspace solid-phase dynamic extraction gas chromatography (HS-SPDE-GC) with flame ionization detection (FID) after saponification. The SPDE equipment (Chromtech, Idstein, Germany) was installed in a CTC-Combi-PAL-Autosampler (Bender and Hobein, Zurich, Switzerland) to a GC FID system (Agilent Technologies model 6890). DM and DA were calculated as mol of methyl/acetyl groups per 100 mol of galacturonic acid (GalAc) as described earlier (Levigne et al., 2002) and are given in percentage.

2.6.2. Quantification of galacturonic acid, L-rhamnose, and L-fucose

The monomer composition of the soluble polysaccharides was analyzed following the protocol of Larsen et al. (2019). Hydrolysis of the samples was carried out according to 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. Specific monosaccharides were analyzed in the supernatant after centrifugation at 10 947*g* for 10 min. Absorbance was measured at 340 nm.

2.6.3. Determination of the molecular weight distribution of soluble polysaccharides

High-performance size exclusion chromatography (HPSEC) on a Smartline HPLC system with a RI detector 2300 (Knauer, Berlin, Germany) equipped with two SEC-Diol columns (300 and 120 Å, 3 μm; YMC, Kyoto, Japan) was used to determine the molecular weight (MW) distribution of the soluble polysaccharides as described by Larsen et al. (2019). Samples were dissolved in water (50 °C) and dialyzed against demineralized water (MWCO 12–14 kDa). Polysaccharides were eluted using water with 50 mM sodium nitrate and 0.25% sodium azide. MWs were calculated with eight pullulan standards ranging from 0.504 to 708 kDa (ReadyCal-Kit Pullulan, PSS- Polymer Standards, Mainz, Germany). The chromatograms were divided 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 the fractions relative to the total area were calculated. Raw data of the MW distribution determined with SEC is shown in table A1 in the supplemental data.

2.7. Statistical analysis

Statistical analysis of the results was performed using XLSTAT (Version 2019.1.1, Addinsoft Technologies, Paris, France). For pairwise comparisons, an ANOVA with a selected significance level of $p < 0.05$ was used.

3. Results and discussion

3.1. Solid phase extraction of polyphenols and gravimetric determination of total soluble polysaccharides (TSP)

The yields of the polysaccharide-free polyphenolic extracts obtained by solid-phase extraction are presented in the supplementary material (Table A2). To verify that the polysaccharides were successfully separated from the polyphenols, the concentrations of the TSP (Table A2) of the polyphenolic extracts were determined and referenced to the corresponding wine concentrations. As the precipitation of the TSP entails the co-precipitation of proteins and polyphenols (Selvendran, 1975), the amounts of proteins and polyphenols in the precipitate were determined. The protein concentration ranged from 0.9 ± 0.1% for the Adentu wine to 6.3 ± 0.2% for the Beringer wine, and the proportions of iron reactive polyphenols ranged from 5.9 ± 1.0% to 16.8 ± 3.4% for the Las Mulas and Bundschuh wines, respectively, indicating that the majority of compounds in the precipitate were wine polysaccharides. The TSP yields of the polyphenolic extracts (Table A2) show that the extracts contain negligible amounts of TSP compared to the high values of the wines and that the wine polysaccharides were successfully removed.

3.2. Polyphenol characterization of the wines and polyphenolic extracts

Fig. 1 presents the results of the photometric assay including the anthocyanin, non-precipitable (np-PP) and precipitable polymeric pigments (p-PP), and tannin measurements of the wines and polyphenolic extracts, respectively. To ensure that no phenolic subclasses were discriminated by the extraction protocol, the total phenolic contents of the samples were determined. The total phenolic contents ranged from 2000 mg/L to 3085 mg/L catechin equivalents in the Las Mulas and Bundschuh wines and from 1667 mg/L to 3217 mg/L catechin equivalents in the Las Mulas and Bundschuh extracts, respectively. The total phenolics of the wines were not significantly different ($p \leq 0.05$) from the corresponding extracts. As expected, there are obvious differences in the composition of the polyphenols between the wine samples that can be caused by regional and enological differences. Since the subject of this study was the evaluation of the interactions between wine polyphenols and polysaccharides, only the differences between the wine samples and the corresponding polysaccharide-free extracts will be discussed in depth. The Weinbiet wine and extract contain the highest concentrations of anthocyanins and show the highest difference between wine and extract (Fig. 1). The differences in the other samples are comparatively low. The Adentu, Las Mulas, and Weinbiet extracts contain fewer tannins than the corresponding wines, whereas the tannin

Table 2
Characterization of the pectic polysaccharides of the wine samples including the distribution of the high and medium molecular weight (MW) fractions, the degree of methylation (DM) and acetylation (DA) and the concentrations of the sugar moieties galacturonic acid (GalAc), rhamnose (Rha), and fucose (Fuc). Means having the same letters are not significantly different at $p \leq 0.05$. Means presented with standard deviation; n = 3.

Sample	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	63.9 ± 0.3 A	36.1 ± 0.3 F	27.2 ± 0.5 C	1.8 ± 0.1 C	16.0 ± 0.6 D	5.5 ± 0.8 B	1.3 ± 0.1 C
Beringer	41.9 ± 1.5 E	58.1 ± 1.5 B	12.3 ± 0.1 D	2.9 ± 1.6 B,C	27.3 ± 1.2 C	11.0 ± 0.4 A	1.4 ± 0.1 B,C
Bundschuh	49.0 ± 0.3 C	51.0 ± 0.3 D	38.4 ± 1.5 B	2.8 ± 0.2 B,C	47.6 ± 0.6 A	2.8 ± 0.1 C,D	1.5 ± 0.1 A,B
Canyon Road	37.3 ± 0.7 F	62.7 ± 0.7 A	17.8 ± 0.1 D	9.2 ± 0.7 A	26.7 ± 1.2 C	3.3 ± 0.1 C	1.4 ± 0.1 B,C
Las Mulas	45.4 ± 1.4 D	54.6 ± 1.4 C	34.6 ± 2.7 B	4.3 ± 0.4 B	25.4 ± 1.5 C	2.3 ± 0.1 C,D	1.3 ± 0.1 B,C
Weinbiet	54.7 ± 0.3 B	45.3 ± 0.3 E	53.1 ± 2.4 A	3.8 ± 0.3 B,C	38.4 ± 1.7 B	2.2 ± 0.1 D	1.3 ± 0.1 C

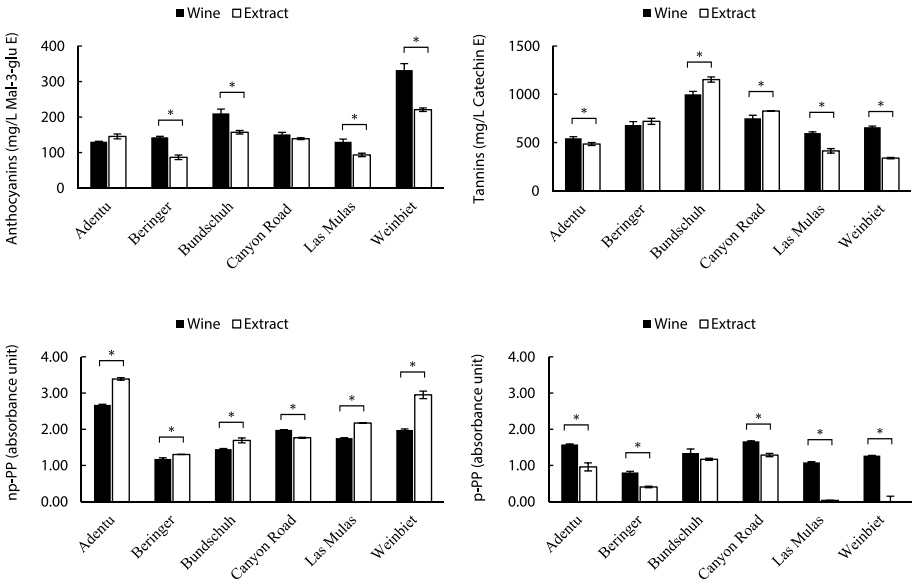

Fig. 1. Phenolic composition including total anthocyanins, non-precipitable polymeric pigments (np-PP), precipitable polymeric pigments (p-PP), and total tannins of the wine and extracts. Results obtained by photometric assays (Harbertson et al., 2002, 2003, 2009, 2015). Means having an asterisk (*) show a significant difference ($p \leq 0.05$) between wine and extract samples.

concentrations of the other extracts are higher than the ones in the wines. The latter was already observed in an earlier study (Weilack et al., 2021). Accordingly, the wines can be classified into two groups, with one group that comprises more tannins in the wines, whereas the other group shows more tannins in the extracts. Interestingly, in most samples, the removal of the polysaccharides results in an increase in np-PP and a concomitantly decrease in the p-PP, with the Canyon Road samples being the only exceptions. The Adentu, Las Mulas, and Weinbiet present the highest differences in polymeric pigment composition between wines and extracts, whereby the Las Mulas and Weinbiet extracts seem to have no p-PP at all. The differences in p-PP proportion and tannin concentrations between wines and extracts correlate statistically significant (Pearson correlation with $\alpha = 0.05$; $R^2 = 0.981$) supporting the classification of the wines into these two groups.

3.3. Composition of the total soluble polysaccharides

To characterize the soluble polysaccharides of the wines, different parameters including molecular weight (MW) distribution, monomeric sugar composition, and degrees of methylation and acetylation (DM and DA) of the pectin remnants were determined using size exclusion chromatography (SEC), photometric assays, and GC-FID (Table 2 and Fig. 2). The molecular weights of the soluble polysaccharides show a broad distribution across all samples with characteristic peaks at around 150, 50, 10, and 4.6 kDa (Fig. 2). According to the literature (Ayestarán et al., 2004; Guadalupe and Ayestarán, 2007; Gao et al., 2015), the high MW fraction (>15 kDa) contains arabinogalactans (AG), arabinogalactan-proteins (AGP), mannans, mannoproteins (MP), and in small amounts homo- (HG) and rhamnogalacturonan I (RG-I), whereas the main constituent of the medium (15–5.5 kDa) and low MW fractions (<5.5 kDa) are rhamnogalacturonan II (RG-II) dimers and monomers (~10 kDa and ~4.6 kDa; Pellerin et al., 1996), respectively. Besides the RG-II molecules, these MW fractions also include low molecular weight fragments of homogalacturonan (HG), rhamnogalacturonan I (RG-I), AGP, and MP (Guadalupe and Ayestarán, 2007). The Adentu and Weinbiet wines present the highest proportions of the high MW fraction (Table 2), indicating high proportions of AG, AGP, and MP. Accordingly, these wines have the lowest proportions of the medium MW fraction. The Beringer and Canyon Road wines show the opposite ratio, indicating high proportions of RG-II. Although the Bundschuh wine contains statistical significantly more high MW polysaccharides than the Las Mulas wine, the differences between the two wines of the high and medium MW polysaccharides are relatively low. The chromatograms in Fig. 2 indicate that the Las Mulas wine has a similar polysaccharide profile as the Weinbiet wine, which is more accentuated in the high MW fraction. Besides the Adentu wine, all wines possess a considerable amount of low MW fraction of the pectic polysaccharides, whereby the Bundschuh wine shows the most pronounced peak followed by the Beringer and Canyon Road wines, which indicates the presence of RG-II monomers and other low MW fragments.

To determine the relative proportions of the pectic structures in the wines, the monosaccharide composition including galacturonic acid (GalAc), rhamnose (Rha), and fucose (Fuc) was analyzed (Table 2). Due to their well-defined occurrence in the pectin structure, this allows for

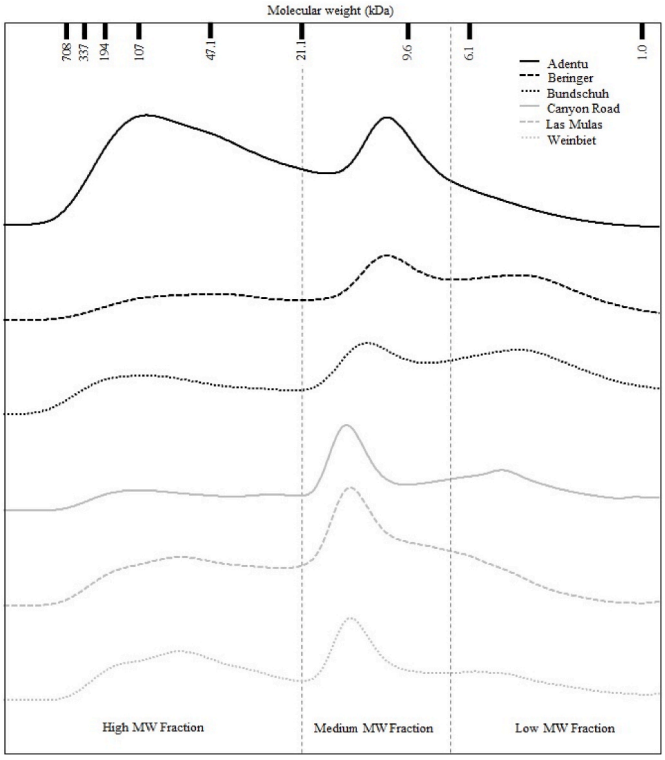

Fig. 2. Size exclusion chromatograms of the pectic polysaccharides of the wine samples including the high (>15 kDa), medium (15–5.5 kDa) and low (<5.5 kDa) molecular weight (MW) fractions. Column was calibrated with standards illustrating the peak molecular weight distribution.

correlating the GalAc, Rha, and Fuc concentrations to the relative amount of HG, RG-I, and RG-II molecules, respectively (Larsen et al., 2019). The ratio of Rha to GalAc provides information on the proportion of the RG-I main chain, and hence, on the relative amount of GalAc that is incorporated in the RG-I backbone (Ma et al., 2016). The GalAc concentrations vary widely from 16.0 ± 0.6 mg/g for the Adentu wine to 47.6 ± 0.6 mg/g for the Bundschuh wine. The Adentu wine has the highest proportion of high MW polysaccharides, whereas the Bundschuh wine possesses less high MW molecules and a pronounced low MW fraction (Fig. 2), suggesting that the HG chain was degraded to a different extent during ripening and winemaking. This can be assigned to different polygalacturonase activities, which in turn depend on grape maturity and/or the use of pectolytic enzymes (Vidal et al., 2001). The Rha concentrations were generally lower than the GalAc concentrations and ranged from 2.2 ± 0.1 mg/g for the Weinbiet wine to 11.0 ± 0.4 mg/g for the Beringer wine (Table 2), showing that the wines contain small proportions of RG-I. This finding is supported by Vidal et al. (2003b), who stated that RG-I is a minor component of wine pectic polysaccharides and determined it to be of around 4%, which may be due to its poor solubility or its fragmentation by glycanases during winemaking (Vidal et al., 2001). However, the pectin fragments of the Adentu and Beringer wines still comprise higher ratios of Rha to GalAc, indicating that 34.6% and 40.4% of the GalAc found in the wines, respectively, derive from the RG-I backbone. This is supported by a characteristic peak in the SEC chromatograms at around 50 kDa, which may be assigned to RG-I (Vidal et al., 2003b).

RG-II is the most abundant pectic polysaccharide in wines (Guadalupe et al., 2014), thus, the Fuc concentrations of the wines are quantified to characterize their RG-II content. While the Adentu, Las Mulas, and Weinbiet wines have the lowest Fuc concentration, therefore proposedly the lowest RG-II proportion, the Bundschuh wine exhibits the highest RG-II proportion. This coincides only partially with the size distribution of the polysaccharides that assigned the highest proportion of RG-II to the Canyon Road wine. However, as reported earlier, some wines still contain high GalAc and Rha contents, indicating the presence of HG and RG-I fragments, which can also be found in the medium MW fraction.

The degree of esterification of the pectic polysaccharides, being the degree of methylation (DM), describes the proportion of GalAc units that is esterified with methanol. Besides that, GalAc and Rha can be bound to acetyl residues, which are determined by the degree of acetylation (DA). Accordingly, the remaining GalAc monomers carry a free carboxy and hydroxy group, whereby the first one can be dissociated depending on the pH of the wine, both of which have a large impact on polarity and hydrophobicity of the pectic polysaccharides. The DMs range from 12.3 ± 0.1% for the Beringer wine to 53.1 ± 2.4% for the Weinbiet wine, whereas the DAs of the wines are smaller and range from 1.8 ± 0.1% for the Adentu wine and 9.2 ± 0.7% for the Canyon Road wine (Table 2). Since a higher degree of esterification increases the hydrophobicity of the pectic molecules, the pectin fragments found in the Weinbiet wine can be considered more hydrophobic, whereas the polysaccharides of the Beringer wine are more polar. During the ripening process of grapes, several enzymes play a role in the degradation of the cell wall polysaccharides and the accompanied softening of the grape skin. The main acting enzymes of this process are polygalacturonase (PG), pectin methylesterase (PME), and β-galactosidase. They are responsible for the cleavage of unesterified polygalacturonans, the de-esterification of methylated galacturonic acid units, and the de-polymerization of (1 → 4)-β-galactan constituents of pectic polysaccharides, respectively (Nunan et al., 1998; Minic and Jouanin, 2006). Nunan et al. (1998) stated that at the end of the maturation process, the degree of esterification of pectin is at around 48% and that the polygalacturonase can only cleave bonds of de-esterified GalAc units, making their preceding de-esterification inevitable. Together with the high amount of high MW pectic polysaccharides and a degree of esterification of 53.1 ± 2.4% of the Weinbiet sample, this gives rise to the assumption that the grapes of this wine, in contrast to the other wines, may have been harvested earlier and not treated with any macerating enzymes.

3.4. Interactions between wine polysaccharides and polyphenols

Due to the comparison of the wines with their corresponding extracts, the differences observed in the polyphenolic composition between them (Fig. 1) can be attributed to the presence or absence of the polysaccharides in wine or extracts, respectively. These polysaccharides can interact directly with the wine polyphenols and/or with the BSA that is used to precipitate polymeric pigments and tannins during analysis (de Freitas et al., 2003; Mateus et al., 2004). Both interactions, also in synergy, can lead to differences in the assay readings.

In most samples, the presence of the polysaccharides increase the anthocyanin readings in the wines compared to the extracts. Since the determination of the anthocyanins is a photometric measurement, this enhancing effect may be assigned to the adsorption of the anthocyanin molecules to the pectin fragments followed by the self-association of other anthocyanin molecules (Padayachee et al., 2012). This anthocyanin stacking results in the bathochromic and hyperchromic shift of the absorbance spectra of the anthocyanins, which occurs in the presence of certain polysaccharide structures (Mazzaracchio et al., 2004; Fernandes et al., 2020). Due to their pH dependent structural equilibria, part of the anthocyanins exists as the flavylium cation at wine pH, which allows ionic interactions between the anthocyanins and dissociated GalAc units (Holzwarth et al., 2012). The free hydroxy groups of the HG and RG backbone can form hydrogen bonds with the hydroxy groups of polyphenols. However, as the major anthocyanin malvidin-3-glucoside carries two methoxy groups on its B-ring, hydrophobic interactions with methylated GalAc units also play a role in the stabilization of anthocyanins (Mazzaracchio et al., 2004; Fernandes et al., 2020). The Weinbiet, Las Mulas, and Bundschuh wines show the highest degrees of esterification and the biggest differences in anthocyanin concentrations.

The higher hydrophobicity of these high methoxylated pectic polysaccharides may lead to stronger interactions with the predominant malvidin-3-glucoside. Despite the lower degree of esterification of the Beringer polysaccharides, they still cause an increase in the anthocyanin concentrations, indicating that other forces are also involved in the self-association of the anthocyanins.

The differences in tannin and polymeric pigment concentrations demonstrate that the pectic polysaccharides not only contribute to the stacking of anthocyanins but also alter the precipitability of other polyphenols. As described above, the Adentu, Weinbiet, and Las Mulas wines have higher proportions of the high MW polysaccharides AGPs, AGs, and MPs, whereas the Beringer, Bundschuh, and Canyon Road wines possess high proportions of smaller polysaccharides like RG-II and fragments of HG, RG-I, AGPs, and MPs. While the exclusion of these polysaccharides leads to a decrease in tannin concentrations of the Adentu, Las Mulas and Weinbiet wines by 11%, 31% and 49%, respectively, the tannin concentrations of the other wines increase by 6–13%, indicating that the respective pectic polysaccharides of the wines enhance or prevent tannin precipitation and accordingly lead to altered tannin readings. Attenuated tannin precipitation results in lower tannin readings, whereas enhanced precipitation will increase the apparent tannin concentration. A strong correlation of the differences in tannin concentrations with the Fuc concentrations ($R^2 = 0.834$) supports the theory that the precipitability of tannins is affected by the polysaccharide composition, more specifically by the proportion of RG-II. The disruptive effect of pectins on the aggregation of tannins and proteins has been shown to increase with the polarity of the polysaccharide (de Freitas et al., 2003; Mateus et al., 2004; Carvalho et al., 2006), whereby the polarity depends on the amount of GalAc units and the degree of esterification. According to Vernhet et al. (1996), RG-II has the highest charge density when compared to AGP fractions and may therefore be more effective in the prevention of aggregation. In this study, the Beringer, Bundschuh, and Canyon Road wines show the highest inhibiting effect, which may be assigned to their higher proportions of RG-II. Additionally, the pectins found in the Bundschuh wine have the highest amount of GalAc units and the most pronounced fraction of low MW polysaccharides, leading to the assumption that they contain a higher concentration of polygalacturonic acids that were shown to solubilize protein-tannin aggregates due to their ionic character (de Freitas et al., 2003). Altogether, this results in an effective prevention of precipitation in the Beringer, Bundschuh, and Canyon Road wines. In contrast, some AGPs and AGs are considered neutral carbohydrates that not only have a small or no preventive effect on protein-tannin aggregation but even enhance protein precipitation by co-aggregation of these polysaccharides with proteins and tannins, leading to even bigger complexes (Vernhet et al., 1996; Carvalho et al., 2006). As the Adentu, Las Mulas, and Weinbiet wines have high proportions of the high MW polysaccharides that consist of AGPs and AGs, besides others, this may explain the increased precipitability of the tannins found in the wines. Furthermore, the pectic polysaccharides of the Weinbiet wine are the highest esterified, which reduces their polarity and may lead to the strongest enhancement of the precipitation. This indicates that the tannins, that were additionally found, in the Adentu, Las Mulas, and Weinbiet wines when compared to the according extracts may not be actually present but are the result of the interactions and co-aggregation with wine polysaccharides.

The differences between the non-precipitable polymeric pigments (np-PP) and the precipitable polymeric pigments (p-PP) correlate strongly ($R^2 = -0.746$), which might be explained by an increased (1.3- to 32.4-fold) precipitability of polymeric pigments as a result of their interaction with polysaccharides. Additionally, the complexation of the anthocyanins by the polysaccharides may lead to the formation of precipitable pigments as shown by Sommer et al. (2016), which depends on the polysaccharide composition. The polysaccharides of the Beringer and Bundschuh wines exhibit a relatively strong co-pigmenting effect on anthocyanins, but small differences in the np-PP and p-PP proportions comparing wine and polysaccharide deprived extracts. The increased concentration of p-PP may be explained by the higher precipitability of parts of the np-PP due to their interactions with the relatively low proportions of AGPs and AGs. However, as anthocyanins can interact even more strongly with the high proportion of RG-II and polygalacturonic acids due to their cationic character and the high electron density of these polysaccharides, the complexes between the anthocyanins and polysaccharides appear to remain soluble, which is supported by earlier studies (Ducasse et al., 2010; Larsen et al., 2019). In the Adentu, Las Mulas, and Weinbiet wines, greater differences in anthocyanin and polymeric pigment concentrations were observed, suggesting that not only np-PP but also anthocyanins may form precipitable pigments with the large AGP and AG molecules of these wines. Guadalupe et al. (2007) reported that most of the neutral pectic polysaccharides, like AGPs and AGs, precipitated post maceration due to their size, but acidic pectic polysaccharides remained constant. This raises the question whether the increased amount of p-PP can be reasoned only by the enhanced protein precipitability of the pigments and the tannins, or whether these complexes would also precipitate without the addition of BSA during the centrifugation of the samples. Since no p-PP were found in the Las Mulas and Weinbiet wines, the complexation of the anthocyanins by the polysaccharides may have resulted in the formation of insoluble pigments, which may lead to an impaired incorporation of anthocyanins into tannin molecules. This is further supported by the fact that the sum of the polymeric pigments is higher in these wines than in their extracts. It was also observed for anthocyanin-polysaccharide complexes in previous studies that attributed this behavior to the size of the polysaccharides (Larsen et al., 2019; Hensen et al., 2022).

3.5. Implications for red wine quality

Despite the precipitation of some anthocyanins, the co-pigmentation of the anthocyanins by wine polysaccharides stabilizes the color of young wines and provides long-term stabilization of the anthocyanins against degradation (Cheynier et al., 2006; Holzwarth et al., 2012; Larsen et al., 2019). The complexes that are formed by non-covalent bonds between anthocyanins and neutral, high molecular weight polysaccharides appear to have the same properties as p-PP as they absorb light at 520 nm. Complexation limits discoloration by pH changes and SO_2-bleaching, suggesting that the polysaccharides prevent additions of sulfite and water probably by steric hindrance. Furthermore, these complexes are seemingly protein precipitable, hence, the resulting aggregates are measured as p-PP. This shows that they are colored complexes that may continue to contribute to the color of red wines. However, their structure differs from the original definition of polymeric pigments as tannins with incorporated anthocyanins (Somers, 1971). Unlike the pigmented tannins, which are based of covalent bonds, the formation of the polysaccharide-pigment complexes is largely reversible (Weber, 2022), which could lead to a lower long-term stability of the wine color. Moreover, depending on the polysaccharide composition, the complexes formed seem to impair the formation of p-PP in the original sense, being tannins with covalently incorporated anthocyanins, which could affect not only color stability but also red wine astringency.

Red wine astringency is the result of the interactions of tannins with saliva proteins, leading to the precipitation of the tannins and a loss of lubrication in the oral cavity (Charlton et al., 2002), whereby the extent of this reaction is influenced by tannin concentration and composition (Gawel, 1998). Additionally, the present study shows that the protein precipitability of tannins may be enhanced or inhibited in the presence of pectic polysaccharides depending on the polarity of the polysaccharides, which may result in a different astringency perception. The polarity of the tannins, in turn, may influence their interactions with the polysaccharides, and the pigmentation of tannins is believed to increase their polarity due to the cationic character of the anthocyanins (Salas

et al., 2003; Weber et al., 2013). Consequently, the formation of p-PP during red wine aging will affect the interactions with both the saliva proteins and the pectic polysaccharides. While the Beringer, Bundschuh, and Canyon Road wines contain p-PP that may further decrease tannin precipitation due to their enhanced interaction with the RG-II molecules, the lack of p-PP in the Las Mulas and Weinbiet wines may support the increased precipitation of tannins or the formation of bigger aggregates due to their hydrophobic interactions with the high MW pectic polysaccharides. Therefore, this study partly supports the previously stated hypothesis (Weilack et al., 2021) that a change in perceived astringency of aged wines may be due to increased interactions between pigmented tannins and wine polysaccharides, as precipitation of tannins was prevented in some wines. However, another study showed different behaviors of RG-II molecules depending on the structure of the protein that was used for the precipitation of tannins (Carvalho et al., 2006). This indicates that the effects of the pectic polysaccharides may differ comparing saliva proteins and BSA and accordingly red wine astringency, which is part of ongoing investigations.

4. Conclusions

The interactions between wine polyphenols and pectic polysaccharides were investigated by comparing their composition in red wines and corresponding polysaccharide-free extracts. The results show that the composition of the grape pectic polysaccharides, that are extracted and modified during fermentation, can alter the properties of tannins and pigments by stabilizing anthocyanins through co-pigmentation like effects and changing their precipitability. This precipitability is used as an analytical measure on the one hand but is also the driving force for the perception of astringency, which leads to the assumption that the observed effects can also change mouthfeel properties of the wines. The color of aged red wines is mainly attributed to the presence of polymeric pigments; however, this study reveals that polysaccharides can form complexes with anthocyanins and np-PP, which affect the formation and measurement of precipitable polymeric pigments as they have comparable properties. This leads to the conclusion that it is necessary to reconsider the established concept of polymeric pigments, which until now mainly refers to tannins with incorporated anthocyanins. As certain structural characteristics of the pectin molecules enhance or attenuate tannin precipitation, it may be very likely that they influence the perception of astringency and in particular the perception of astringency sub-qualities. Altogether, this study provides continued information on the impact of certain cell wall polysaccharides and their degradation products on the tannin and pigment composition of red wines. Since these hypotheses result from the study of Cabernet Sauvignon wines, it is plausible that the results of other wines may be different given the substantial varietal differences in composition of polysaccharides and polyphenols. This emphasizes the necessity of further research in this area.

Funding

This work was supported by the German Ministry of Economics and Technology (via AiF) and the FEI (Forschungskreis der Ernährungsindustrie e.V., Bonn). Project AiF 20024N.

CRediT authorship contribution statement

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

Acknowledgments

The authors thank Sandra Feifel from the Wein Campus Neustadt for supporting us with the data on the general composition of the wines.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.crfs.2023.100506.

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