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Pectin forms polymeric pigments by complexing anthocyanins during red winemaking and ageing

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ABSTRACT

The long-term stability of red wine color depends on the formation of polymeric pigments from anthocyanins. Although there is still a lot of uncertainty about the specific structure of this diverse group of pigments, there is consensus that they are reaction products of anthocyanins and other polyphenols. Interactions between anthocyanins and pectic polysaccharides have been suggested to stabilize anthocyanins. This study explores the impact of such interactions by adding pectin during red winemaking.

The results demonstrate that these interactions induce the formation of additional polymeric pigments which enhance the pigment stability during fermentation and aging. While initial pigment formation is higher in wines with added pectin, a notable proportion of the complexes degrades in the later stages of fermentation. Presumably, tannins form insoluble complexes with pectin, reducing tannin concentration by more than 300 mg/L. Anthocyanin concentrations decrease by over 400 mg/L, and polymeric pigments double.

Anthocyanins that form polymeric pigments with pectic polysaccharides expand the range of pigments in red wines with possible consequences for the sensory properties of the wine. These findings highlight the complex interactions between pectin, anthocyanins, and tannins, and their influence on pigment formation and wine composition during fermentation and aging.

1. Introduction

Anthocyanins extracted from grape skins during winemaking are fundamentally responsible for the color of red wine. Beginning immediately after extraction and continuing during maturation of the wine, anthocyanins undergo numerous reactions with other grape constituents or microbial metabolites, which results in color changes and color stabilization (de Freitas, Fernandes, Oliveira, Teixeira, & Mateus, 2017; Weber & Schieber, 2023). One major route is the condensation with different other polyphenols like flavanols to form covalent polymeric pigments (Harbertson, Picciotto, & Adams, 2003). Despite their great impact on red wines, there is still considerable uncertainty regarding the structure of these compounds. A second but less investigated pigment-forming route is the interaction of anthocyanins with grape cell wall polysaccharides. They are extracted during maceration and degraded and modified during the whole winemaking process. Although polyphenol-polysaccharide interactions have extensively been investigated, especially in wines, their role in the formation of pigments has not yet been addressed (Bindon, Li, Kassara, & Smith, 2016; Bindon, Madani, Pendleton, Smith, & Kennedy, 2014; Bindon, Smith, Holt, & Kennedy, 2010; Bindon, Smith, & Kennedy, 2010; Hensen, Hoening, Weilack, Damm, & Weber, 2022; Le Bourvellec, Bouchet, & Renard, 2005; Le Bourvellec & Renard, 2012; Le Bourvellec, Watrelot, Ginies, Imberty, & Renard, 2012; Liu, Le Bourvellec, & Renard, 2020; Mekoue Nguela, Poncet-Legrand, Sieczkowski, & Vernhet, 2016; Osete-Alcaraz et al., 2020; Renard, Baron, Guyot, & Drilleau, 2001). Since anthocyanins exist in a pH-dependent equilibrium between the positively charged flavylium cation and their hemiketal form, the pH value of the medium determines the mechanisms and extent of interactions with polysaccharides, particularly with pectin. The latter, as the major polysaccharide involved, carries carboxylic acid groups which can be deprotonated at higher pH. Wines and musts commonly have pH values between 3.0 and 4.0, which leads to various interaction mechanisms based on ionic, hydrophobic, and hydrophilic forces (Celus, Kyomugasho, Van Loey, Grauwet, & Hendrickx, 2018; Fernandes et al., 2020). Additionally, the coiled structure of pectin molecules can enclose anthocyanins in hydrophilic or hydrophobic pockets (Le Bourvellec, Guyot, & Renard, 2004).

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All polymeric pigments show considerable light absorption around 520 nm and are more or less resistant against bisulfite bleaching and higher pH, which is why they are crucial for the long-term color stability of aging red wines. However, their structural diversity inhibits proper analytical characterization even with mass spectrometry or other advanced analytical methods (Cheynier et al., 2006). Quantification methods based on protein precipitation and bisulfite bleaching have been suggested as feasible alternative methods and are well-established in research and wineries (Harbertson, Kennedy, & Adams, 2002; Harbertson et al., 2003). These methods rely on common physico-chemical characteristics that are typical of (Weilack, Schmitz, Harbertson, & Weber, 2021) but not limited to polymeric pigments consisting only of polyphenols. They differentiate between protein-precipitable and non-precipitable polymeric pigments, so far referred to as large and small polymeric pigments, respectively. This designation is somewhat misleading, however, as the molecular size does not seem to be the decisive difference (Weilack, Schmitz, Harbertson, & Weber, 2021). Non-precipitable polymeric pigments are usually the only polymeric pigments found in musts and young wines. Only during the subsequent wine ageing, anthocyanins polymerize to form precipitable polymeric pigments. Although the protein precipitation method has been used to quantify wine pigments, its strength lies in detecting compounds with the same phenotype. Without identifying the full molecular structure, the measured pigments share similar characteristics and are grouped together as polymeric pigments.

Complexes formed by interactions between pectin and anthocyanins stabilize or precipitate anthocyanins depending on the various pectin structures (Larsen, Buerschaper, Schieber, & Weber, 2019). Stable anthocyanin-pectin complexes have been reported to have similar characteristics as polymeric pigments formed exclusively from polyphenols, as they absorb at 520 nm and are stable against bisulfite bleaching (Graves & Sommer, 2021). With similar color and stability characteristics, pectin-anthocyanin complexes might be as crucial for long-term color stability in red wines as covalent polymeric pigments.

It can be hypothesized that pectin facilitates the formation of stable pectin-anthocyanin complexes and that this effect can be revealed by comparing the development of pigments in red wines with additional pectin and non-treated wines. The investigation aims to assess the stability of these complexes throughout the aging of the wines, anticipating that their presence will contribute to enhanced color longevity.

2. Material and methods

2.1. Chemicals

Acetic acid, ethanol (100 %), hydrochloric acid (HCl) (37 %), potassium bisulfite (97.4 %), and acetonitrile (100 %) were purchased from VWR International GmbH (Darmstadt, Germany). Sodium hydroxide was obtained from Honeywell International Inc. (Seelze, Germany). Sodium chloride was acquired from neoFroxx GmbH (Einhausen, Germany). Triethanolamine (≥98 %), ferric chloride (97 %), L-(+)-tartaric acid, and maleic acid (98 %) were purchased from Alfa Aesar (Kandel, Germany). Gallic acid (>98 %) was bought from Fluka Chemie AG (Buchs, Switzerland). Urea (≥99.5 %), bovine serum albumin fraction V (≥98 %), and (+)-catechin ≥ 98 % were obtained from Carl Roth GmbH & Co. KG (Karlsruhe, Germany). Sodium azide (≥99 %) was obtained from Merck KGaA (Darmstadt, Germany). Formic acid (≥98 %) was sourced from Sigma Aldrich Chemie (Steinheim, Germany). Potassium pyrosulfate (food grade) was purchased from RWA Raiffeisen Ware Austria AG (Vienna, Austria). Potassium bicarbonate (food grade) was bought from Erbslöh Geisenheim AG (Geisenheim, Germany). Ultrapure water was obtained from a PURELAB flex 2 water purification system (ELGA 90LabWater, Paris, France).

2.2. Grape samples

'Pinot noir' and 'Cabernet Sauvignon' grapes for winemaking were sampled in 2020 with a low, medium, or high concentration of total soluble solids (TSS) measured with a digital refractometer (PAL-α ATAGO CO. LTD., Tokyo, Japan) (Table 1). The geodetic coordinates of the variety-specific vineyards in Neustadt an der Weinstrasse (Palatinate, Germany) were 49°23′53.3″N 8°11′02.0″E ('Cabernet Sauvignon') and 49°22′14.3″N 8°10′56.3″E ('Pinot noir'). 'Cabernet Sauvignon' (clone 1Gm on Binova 1Opp rootstock) was planted in 2008 with a vine by row spacing of 2.00 m × 1.20 m and 'Pinot noir' (clone Mariafeld on SO4 rootstock) was planted in1987 with a vine by row spacing of 1.88 m × 1.20 m. After picking, grapes were chilled instantly at 7 °C for 12 h until used for winemaking.

2.3. Winemaking

A standard winemaking protocol was used to prepare 'Cabernet Sauvignon' and 'Pinot noir' wines according to Hensen et al. (2022). The pH of the must from all grape samples was adjusted to 3.5 through the addition of potassium bicarbonate or tartaric acid. Commercial apple pectin (Classic AU 202, 69 % degree of esterification and 75 g/100 g galacturonic acid, kindly provided by Herbstreith & Fox GmbH & Co. KG, Neuenbürg, Germany) was added at a concentration of 0.5 % (w/w) to must. A control wine was made accordingly but without the addition of pectin. The potential alcohol of each wine was adjusted by the addition of sucrose.

'Cabernet Sauvignon' and 'Pinot noir' musts were fermented in 5 L cylindric plastic containers in 1.6 kg batches in triplicate. Fermentation containers were sealed with a lid equipped with a fermentation lock filled with potassium sulfate solution. Fermentation lasted for 12 days at 22 °C, during which containers were shaken three times daily. Samples (10 mL) were taken daily through a septum. Total soluble solids were measured with the refractometer PAL-α (ATAGO CO. LTD., Tokyo, Japan). Samples were centrifuged at 18,500*g* for 10 min and filtered through 0.2 μm syringe filters before further analysis.

After fermentation, wines were manually pressed through a mesh, filled in half-liter brown glass bottles with 200 mg/L sulfur dioxide, and stored for 12 months at 17 ± 1 °C.

2.4. Analysis of anthocyanins by UHPLC-DAD

Anthocyanins (delphinidin-*3-O*-glucoside, cyanidin-*3-O*-glucoside, peonidin-*3-O*-glucoside, petunidin-*3-O*-glucoside, malvidin-*3-O*-glucoside, delphinidin-*3-O*-acetylglucoside, cyanidin-*3-O*-acetylglucoside, peonidin-*3-O*-acetylglucoside, petunidin-*3-O*-acetylglucoside, malvidin-*3-O*-acetylglucoside, delphinidin-*3-O-p*-coumaroylglucoside, cyanidin-*3-O-p*-coumaroylglucoside, peonidin-*3-O-p*-coumaroylglucoside, petunidin-*3-O-p*-coumaroylglucoside, and malvidin-*3-O-p*-coumaroylglucoside) concentrations were measured according to Hensen et al. (2022) using a Nexera X2 UPLC system (Shimadzu Corporation, Kyoto, Japan) equipped with an autosampler SIL-30AC thermostatted to 10 °C, column oven CTO-20AC set at 40 °C equipped with a Kinetex C18 100 A, 1.7 μm, 150 × 2.1 mm column (Phenomenex Inc., Torrance, CA), and a DAD SPD-20A. water/formic acid (97/3; v/v) and acetonitrile/formic acid

Table 1
Total soluble solids (TSS) and titratable acid (TA) concentrations, and pH of grape samples used for winemaking.

Variety	TSS [°Bx]	TA [g/L]	pH	Harvest Date
'Cabernet Sauvignon' (CS)	17.1	10.5	3.3	09-01-2020
	20.9	7.3	3.6	09-15-2020
	21.8	6.6	3.7	09-28-2020
'Pinot noir' (PN)	16.7	13.3	3.0	08-18-2020
	19.7	8.4	3.3	09-01-2020
	21.6	7.3	3.4	09-15-2020

(97/3; v/v) were used as eluents A and B with a flow rate of 0.4 mL min^{-1} and the following gradient: 0 min, 4.0 % B; 2 min, 6.5 % B; 5 min, 10.0 % B; 6 min, 10.5 % B; 7 min, 11.0 % B; 11 min, 12.5 % B; 13 min, 14.0 % B; 15 min, 15.0 % B; 26 min, 26.0 % B; 26.1 min, 100.0 % B; 30 min, 100.0 % B; 30.1 min, 4.0 % B; 33 min, 4.0 % B. The injection volume was 5 μL. Anthocyanins were detected at 520 nm. Malvidin-*3-O*-glucoside chloride ≥ 95 % (PHYTOPLAN Diehm & Neuberger GmbH, Heidelberg, Germany) was used for quantification and identification. Analytes were identified by their elution order.

2.5. *Chemical characterization of tannin and polymeric pigment concentrations*

The method proposed by Harbertson et al. (2003) was used for tannins and polymeric pigments measurements with a modified resuspension buffer (urea 8.3 M, 5 % TEA, pH 7 adjusted with HCl) (Harbertson, Mireles, & Yu, 2015). Based on an external calibration curve, tannins were expressed as catechin equivalents (CE).

2.6. *Spectrophotometric analysis of color differences between samples*

The color of the young wines before bottling and after one year of aging was analyzed according to Weilack et al. (2021). Absorbance spectra of the wine samples were recorded between 300 and 800 nm in a 1 mm path-length glass cuvette (Hellma GmbH & Co. KG, Müllheim, Germany) with a Jasco V-730 double-beam spectrophotometer (JASCO Deutschland GmbH, Pfungstadt, Germany). L^*, a^*, and b^* were calculated with Spectra Manager Ver. 2.14G (JASCO Deutschland GmbH, Pfungstadt, Germany) after correcting the measurements to a 10 mm path length according to OIV suggestions (OIV, 2006).

2.7. *Statistical analysis*

R (Version 4.1.3) with R-studio (Version 2022.02.3) and packages dplyr (Version 2.1.1), ggplot2 (Version 3.3.5), lemon (Version 0.4.5), rstatix (Version 0.7.0), stat (Version 3.6.0), and tidyr (Version 1.0.2) were used for statistical analysis and graphical illustrations. For pairwise comparison, ANOVA and Tukey's post hoc tests with a significance level of $p < 0.05$ were used.

3. Results and discussion

As expected, only non-precipitable polymeric pigments were detected during fermentation in all samples. Formation of precipitable polymeric pigments usually begins with wine aging. They are typically not found in grapes and musts (Graves & Sommer, 2021). Concentrations of non-precipitable polymeric pigments differed largely between musts with added pectin and the corresponding control (Fig. 1). Pectin generally increased the concentration of non-precipitable polymeric pigments. Their highest concentrations were measured between days two and five of fermentation and declined afterwards. In contrast, anthocyanin concentrations were lower compared to the control during the first five days of fermentation due to the added pectin. The observed increase in non-precipitable polymeric pigment concentrations in all samples coincided with lower anthocyanin concentrations. This suggests a reciprocal relationship between the stability and extraction of anthocyanin (Hensen et al., 2022) and the formation of new pigments. It has been shown that anthocyanins and pectin interact and form rather stable and soluble complexes (Larsen et al., 2019), which would be considered as non-precipitable polymeric pigments in wine if the interaction prevented bisulfite bleaching. As a result of this complex formation, the concentration of free anthocyanins decreases. The increase in their concentration due to ongoing extraction compensates for the decline in anthocyanins, leading to a dynamic equilibrium between extraction, complexation, and derivatization. However, after five days of fermentation, pectin-enriched musts had similar concentrations of anthocyanins and non-precipitable polymeric pigments as the control samples. This suggests that most formed complexes were stable only under the initial conditions of fermentation including pH and ethanol concentration. During this stage, anthocyanins are typically extracted (Gao, Zietsman, Vivier, & Moore, 2019) simultaneously to cell wall polysaccharides and other polyphenols in the control. However, musts with added pectin contained an excess of pectin, which led to a substantial disparity in anthocyanin concentrations between control musts and pectin-enriched musts (Fig. 2). Furthermore, there was limited competition with other polyphenols, which further facilitated anthocyanin

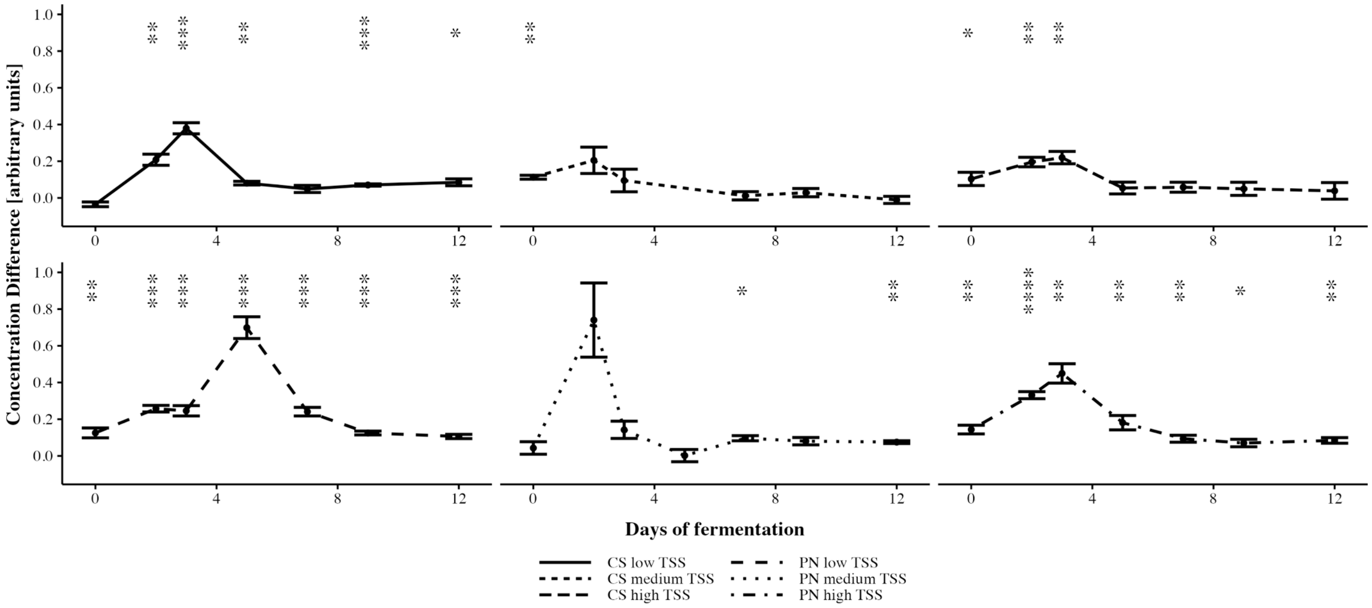

Fig. 1. Impact of pectin addition to fermenting musts on the concentration of non-precipitable polymeric pigments at different fermentation stages. A positive concentration difference shows an increase in non-precipitable polymeric pigment concentrations compared to fermenting musts without additional pectin. Means presented with standard error of means (n = 3). * indicates significant concentration differences between control and pectin-enriched musts [(*) = $p < 0.05$, (**) = $p < 0.01$, (***) = $p < 0.001$, (****) = $p < 0.0001$].

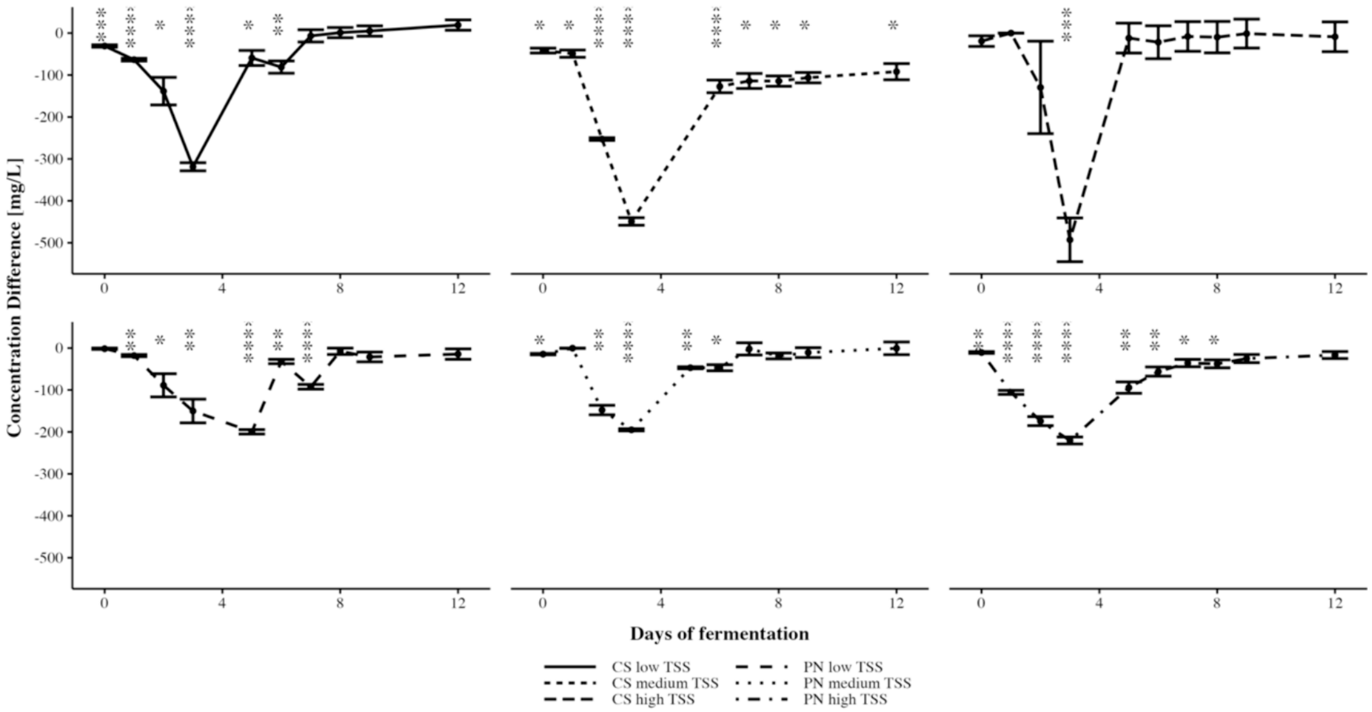

Fig. 2. Impact of pectin addition to fermenting musts on the concentration of anthocyanins at different fermentation stages. A negative concentration difference shows a decrease in anthocyanin concentrations compared to fermenting musts without additional pectin. Means presented with standard error of means (n = 3). * indicates significant concentration differences between control and pectin-enriched musts [(*) = $p < 0.05$, (**) = $p < 0.01$, (***) = $p < 0.001$, (****) = $p < 0.0001$].

interaction with pectin. After this initial stage, weaker complexes may be susceptible to breaking or precipitation as the ethanol concentration increased. Toward the end of fermentation, ethanol can attenuate the interactions between pectin and anthocyanins, resulting in the release of anthocyanins from the initially formed complexes, as demonstrated by Medina-Plaza et al. (2019). A small but still significant part of pigments formed by pectin interactions were resistant to further modifications or degradation until the end of fermentation, as their concentration persists throughout fermentation.

As reported by Graves and Sommer (2021), excess pectin in wines can increase the concentration of polymeric pigments. The researchers concluded that anthocyanins interact with pectins by forming stable aggregates resistant to bisulfite bleaching and protein precipitation. On the other hand, pectin can form insoluble complexes with anthocyanins, as shown by Larsen et al. (2019) depending on the pectin structure. The findings revealed that the changes in the concentrations of anthocyanins and non-precipitable polymeric pigments were generally similar, suggesting that the formation of soluble complexes between pectin and anthocyanins was likely more favored than precipitation in this specific experiment. However, the vastly changing concentrations of non-precipitable polymeric pigments during fermentation suggest diverse interactions between anthocyanins and pectin. These pigments include a range of complexes that vary in stability. However, only complexes that are stable can be attributed to polymeric pigments. As the results indicate, pectin forms stable complexes with anthocyanins, that share characteristics of polymeric pigments formed by the polymerization of polyphenols. Combining the present findings with those from recent publications (Graves & Sommer, 2021; Hensen et al., 2022; Weilack, Mehren, Schieber, & Weber, 2023), the established concept of polymeric pigments needs to be expanded to include polymeric pigments formed through pectin-anthocyanin interactions. As for now, the quantification of polymeric pigments combines pigments formed through pectin interactions with all other pigments. Whether there is a necessity to apply new methodologies that differentiate the different types of polymeric pigments needs to be assessed.

3.1. Interaction effects of pectin on anthocyanin concentrations

'Cabernet Sauvignon' and 'Pinot noir' wines contain 3-*O*-glucosides of cyanidin, delphinidin, malvidin, peonidin, and petunidin, whereas 'Cabernet Sauvignon' additionally contains the acetyl- and coumaroyl-glucosides. The added pectin significantly changed the concentrations of individual anthocyanins depending on the B-Ring substitution ($p < 0.05$) (supporting information Figure S2). Due to methoxy groups on the B-ring, anthocyanins interact with the added pectin via hydrophobic interactions (Larsen et al., 2019). Larsen et al. (2019) reported that hydrophobic interactions with pectin stabilize anthocyanins. Although the authors did not measure non-precipitable polymeric pigment concentrations, it is conceivable that stabilization is achieved through the formation of soluble pigments, as seen in the present study. The concentration of delphinidin-*3*-*O*-glucoside, bearing three hydroxy groups on the B-ring, was similarly reduced by pectin interactions. Previous studies indicated that anthocyanins featuring more hydroxy groups on the B-ring exhibit a stronger affinity for interactions with pectin, which could not be confirmed here (Buchweitz, Speth, Kammerer, & Carle, 2013; Fernandes, Bras, Mateus, & de Freitas, 2014; Larsen et al., 2019). Hydroxy groups located on the B-ring establish hydrogen bonds with pectin, resulting in the formation of stabilized complexes (Larsen et al., 2019). Moreover, the pH value of 3.35 ± 0.15 in the must facilitates the establishment of ionic forces between the positively charged flavylium cation and the negatively charged galacturonic acid. This adds another binding mechanism besides the mentioned hydrophobic and hydrophilic interactions.

Pectin interactions had a significantly lower effect on concentrations of acetylated and coumaroylated anthocyanins. The added pectin reduced coumaroylated anthocyanin concentrations the least. Potential steric hindrances prevented the interaction between the anthocyanin and pectin, while the additional coumaroyl group seemingly does not interact with pectin. Because the coumaroyl group only reduces the interactions between anthocyanins and pectin, the interactions mainly occur with the aglycone. Similarly, Larsen et al. (2019) reported that the

B-Ring substitution of the anthocyanin mostly determines the affinity for pectin interactions, rather than the sugar moiety, again showing that interactions might only occur between the aglycone and pectin.

Although pectin reduced individual anthocyanin concentrations differently during fermentation, the observed differences were small toward the end of fermentation. With ethanol concentrations increasing in the must and young wine, polar interactions between anthocyanins and pectin occur much less, reducing the effect of the differently substituted B-ring.

3.2. *Grape varietal influence on pectin-anthocyanin interactions and pigment formation*

Comparing both varieties, the additional pectin in the must had the same but differently pronounced effects on pigment formation and anthocyanin concentrations. The formation of non-precipitable polymeric pigments from pectin-anthocyanin interactions was slightly higher in 'Pinot noir' wines. Anthocyanin concentrations decreased more from pectin interactions in 'Cabernet Sauvignon' wines, particularly in wines made from medium mature 'Cabernet Sauvignon' grapes. Because this decrease was not accompanied by a comparable increase in non-precipitable polymeric pigment concentration, conditions in 'Cabernet Sauvignon' might favor the precipitation of anthocyanins from pectin interactions. Since the must contains all extracted compounds and fragments from the grape, these individual effects obviously reflect varietal differences. Since pH, ethanol, and temperature levels were nearly identical in all wine samples, extracted compounds like polyphenols and grape cell wall polysaccharides apparently interfered with the interaction of anthocyanins and the added pectin. This accelerated the increased precipitation of anthocyanins in 'Cabernet Sauvignon' samples.

Previous research has demonstrated that the interactions between polysaccharides and polyphenols are influenced by the specific composition given by the grape variety (Hensen et al., 2022). Different compounds like cell wall polysaccharides, grape- or yeast-derived proteins, as well as other polyphenols like tannins may affect these interactions and likely cause the different observations made here. The composition of 'Cabernet Sauvignon' must enhanced the precipitation of anthocyanin-pectin complexes, whereby the mechanisms causing the varietal-specific effects of anthocyanin-pectin interactions are unknown so far.

3.3. *Influence of pectin on tannin concentrations and precipitation during fermentation*

Tannins are generally extracted from grape berries with increasing ethanol concentrations during fermentation. After two days of fermentation, a notable increase in tannin concentrations was observed. The addition of pectin to the must reduced tannin concentrations (Fig. 3). Pectin binds tannins through hydrophobic interactions and hydrogen bonds, depending on the specific molecular structure and the degree of polymerization (Hanlin, Hrmova, Habertson, & Downey, 2010; Le Bourvellec et al., 2005; Ruiz-Garcia, Smith, & Bindon, 2014; Watrelot, Le Bourvellec, Imberty, & Renard, 2014). The formed complexes have been shown to either precipitate or potentially protect tannins (Bindon, Smith, & Kennedy, 2010; Osete-Alcaraz, Bautista-Ortin, & Gomez-Plaza, 2019). Tannins with a higher degree of polymerization have a greater affinity for pectin interactions and tend to precipitate, as shown by Bindon et al. (2016) in model experiments.

The reduced tannin concentration in wines with added pectin suggests that a larger proportion of pectin-tannin complexes precipitated in the presented experiments. After one week of fermentation, a similar increase in tannin concentrations was observed in the control and the pectin-enriched musts. In this final fermentation stage, the tannin concentration gap between control and pectin-enriched musts remained constant. The findings indicate that predominantly those tannins precipitate that are extracted early in the fermentation process. Subsequently, tannin concentrations in all wines changed similarly. During fermentation, skin tannins are typically extracted slightly before seed tannins (González-Manzano, Rivas-Gonzalo, & Santos-Buelga, 2004). Additionally, skin tannins have the highest degree of polymerization in the berry, facilitating the formation of insoluble complexes with pectin (Downey, Harvey, & Robinson, 2003; Souquet, Labarbe, Le Guerneve, Cheynier, & Moutounet, 2000). Both aspects suggest that the gap between tannin concentrations in pectin-enriched wines and control wines is caused by the precipitation of skin tannins through pectin interactions. Therefore, pectin-enriched wines might contain fewer skin tannins than control wines in the experiments.

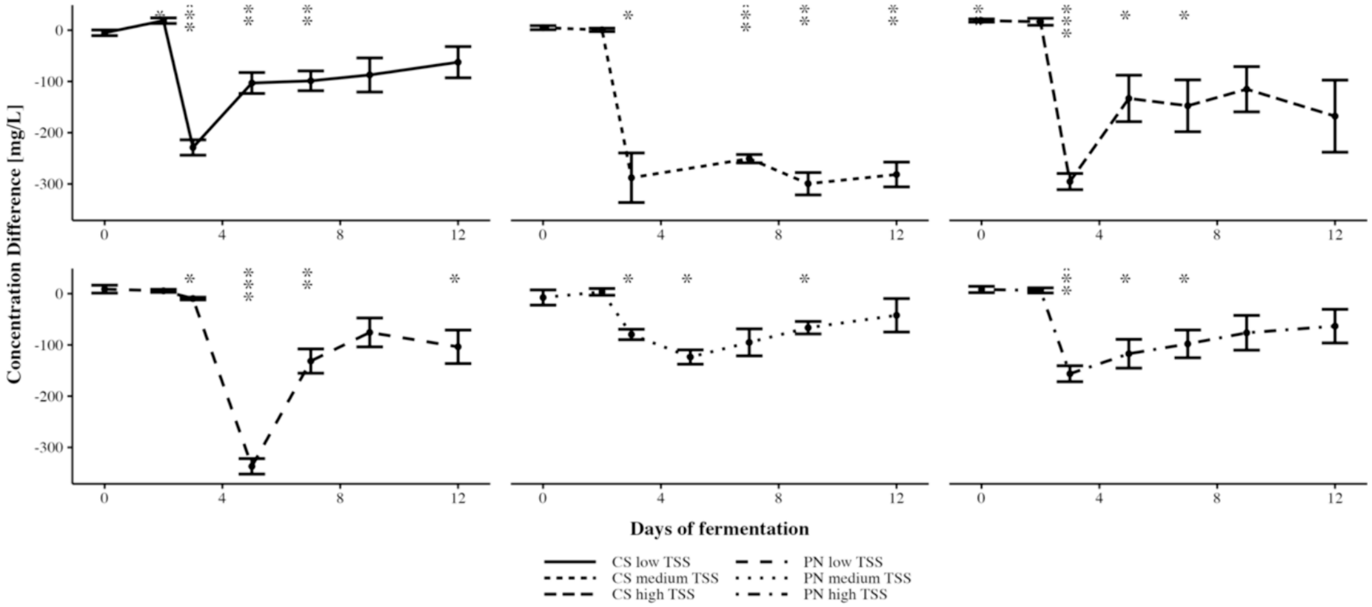

Fig. 3. Impact of pectin addition to fermenting musts on the concentration of tannins at different fermentation stages. A negative concentration difference shows a decrease in tannin concentrations compared to fermenting musts without additional pectin. Means presented with standard error of means (n = 3). * indicates significant concentration differences between control and pectin-enriched musts [(*) = $p < 0.05$, (**) = $p < 0.01$, (***) = $p < 0.001$, (****) = $p < 0.0001$].

Tannins extracted in the final stage of fermentation either formed soluble complexes or could not interact with the added pectin due to its limited availability as it is already occupied by anthocyanins or earlier extracted tannins. As seen for all analytes, differences in the phenolic composition between pectin-enriched and control musts were more pronounced during the first six days of fermentation. Afterward, the impact of pectin interactions was mitigated, likely due to a large proportion of the added pectin being already precipitated or bound in stable soluble complexes. Multiple publications have shown that cell wall polysaccharides can form complexes with tannins, which prevent tannin precipitation during winemaking (Hensen et al., 2022; Osete-Alcaraz et al., 2019; Watrelot, Schulz, & Kennedy, 2017). These effects mostly derive from mechanisms preventing polymerization and interactions with proteins that would otherwise precipitate tannins. However, in the present study, pectin causes tannin precipitation rather than preventing it.

3.4. Evolution of pectin-polyphenol interactions during wine aging

During one year of aging, tannin and anthocyanin concentrations decreased, and precipitable and non-precipitable polymeric pigment concentrations increased in most wines. The impact of pectin

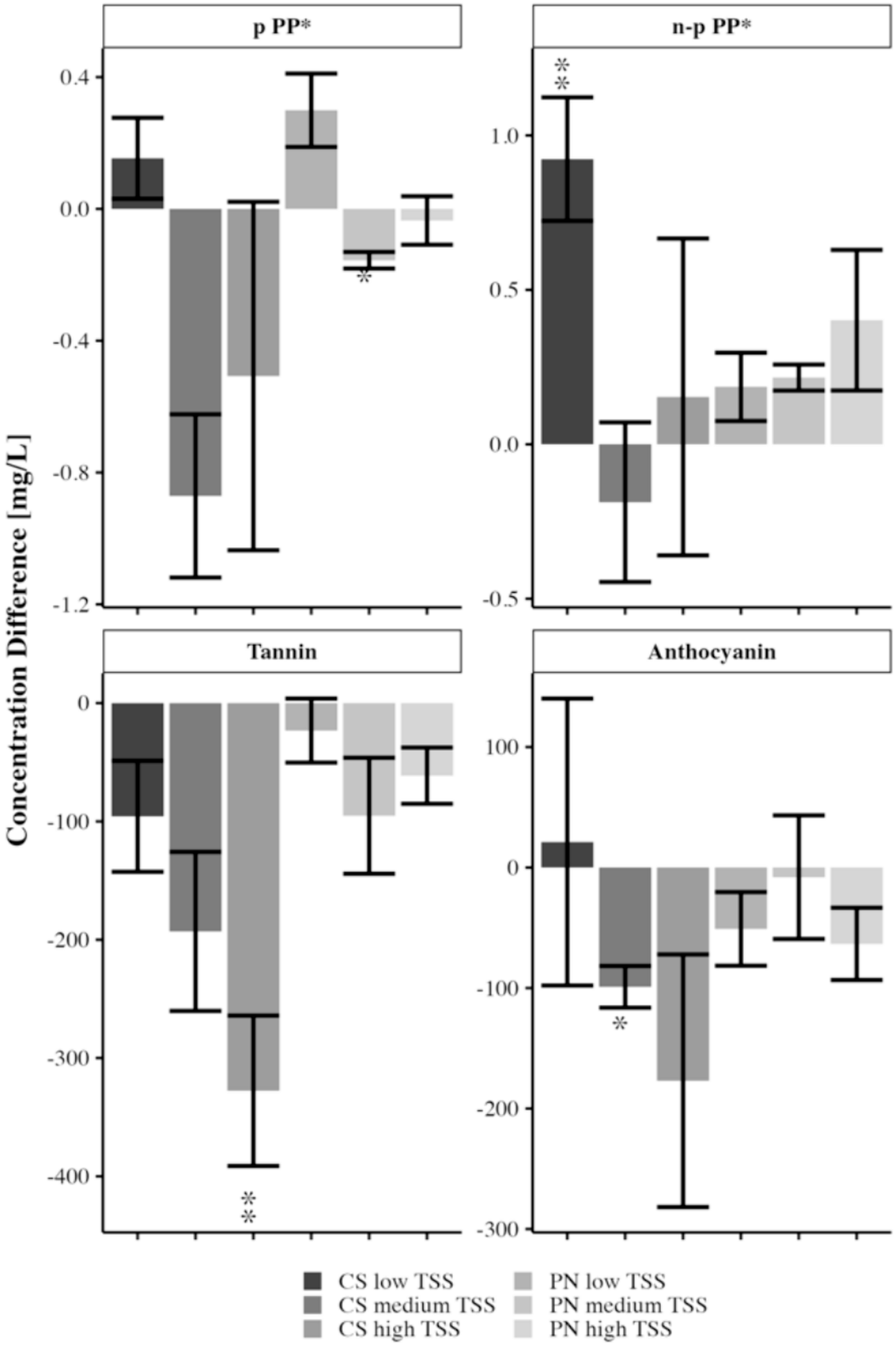

Fig. 4. Impact of pectin addition to fermenting musts on the concentration of polyphenols in wines after 12 months aging. A negative concentration difference shows a decrease in polyphenol concentrations compared to wines without additional pectin. Means presented with standard error of means (n = 3). * indicates significant concentration differences between control and pectin-enriched wines [(*) = $p < 0.05$, (**) = $p < 0.01$]. (*Precipitable polymeric pigments (p PP) and non-precipitable polymeric pigments (n-p PP) in absorption units).

interactions on polyphenol concentrations was remarkably similar to the effects observed in young wines before filling. Added pectin further reduced the concentrations of tannins and anthocyanins and increased non-precipitable polymeric pigment concentrations (Fig. 4). There was a high variability of their concentrations among samples that limits the determination of significant differences. The broad range of potential reactions during the aging process is one major source of this variation. Because the composition of the young wine was already altered by pectin interactions during fermentation, reactions during wine aging further increased the differences among samples.

Despite the high variability of samples, the findings outline a similar aging process of wines. Although the added pectin changed the wine composition during fermentation, pectin seems to impact wine composition during subsequent aging only indirectly. Given the lower tannin and anthocyanin concentrations of pectin-enriched wines, the initial composition after filling leaves fewer possibilities for further polymerization reactions that usually occur during wine aging. This seems to be the case in particular for precipitable polymeric pigments arising from the reaction of tannins and anthocyanins. In pectin-enriched wines, the concentrations of precipitable polymeric pigments were lower compared to control wines.

Anthocyanin concentrations were similarly affected in all wines by the added pectin during aging (Fig. 4 and Figure S3 in the supporting information). After one year of aging, anthocyanin concentrations in wines with added pectin were slightly lower compared to control wines, although most differences were not significant. Changes in the anthocyanin concentration caused by pectin interactions during fermentation are preserved throughout aging, but no further impact of pectin can be determined. It can be assumed that anthocyanin concentrations are reduced more by the various degradation and polymerization reactions than non-covalent interactions with pectin (Cheynier et al., 2006). The concentrations of non-precipitable pigments were also still slightly higher in pectin-enriched wines compared to the control. Given this persistent increase in non-precipitable polymeric pigment concentrations in pectin-enriched wines, it can be hypothesized that pectin interactions offer long-term stabilization for anthocyanins through this mechanism.

Pectin formed a significant amount of insoluble complexes with tannins during fermentation, leading to lower concentrations in the wine after fermentation. During aging, tannin concentrations decreased in all wines caused by oxidation or reactions with other polyphenols and wine compounds (Smith, Mcrae, & Bindon, 2015). The control wine of ‘Pinot noir’ showed a stronger reduction in tannin concentrations than the ‘Cabernet Sauvignon’, leading to similar tannin concentrations in control and pectin-enriched wines. This implies that the remaining tannins in ‘Pinot noir’ wines after fermentation are better protected in pectin-enriched wines. It has previously been reported that tannins can be stabilized by polysaccharide interactions (Bindon et al., 2016; Hensen et al., 2022; Osete-Alcaraz et al., 2019). ‘Pinot noir’ tannins seem to be particularly protected by polysaccharide interactions (Hensen et al., 2022). In ‘Cabernet Sauvignon’ wines, the added pectin further reduced tannin concentrations during aging. Tannins in high TSS ‘Cabernet Sauvignon’ wines still formed insoluble complexes with the additional pectin or were influenced by the changed wine composition after fermentation. Because of the complex nature of reactions and interactions during wine aging, there are plenty of possible pathways by which high pectin concentrations in ‘Cabernet Sauvignon’ must reduce tannin concentrations.

All results combined outline a supposedly positive impact of pectin on red wine composition and properties. With their capability to interact with anthocyanins, pectin increases the concentration of stable non-precipitable polymeric pigments with a potentially positive effect on color stability. Despite this potential, the measured color changes in the samples were found to be insignificant. In young wines the positive impact of an increased pigment formation is obscured by the abundance of anthocyanins, that mainly contribute to the color (Table S1). During aging, different samples developed extremely different. The added pectin had to some extent a stabilizing effect to the natural variability of changes during aging, but overall, this effect was still stronger than any impact by the formation of pigments from pectin.

Although only a small proportion of these non-precipitable polymeric pigments is sufficiently stable to withstand changes during fermentation, they still contribute significantly to the total pigments in wines. In contrast to these stabilizing interactions, pectin precipitated tannins and some anthocyanins, which limited the formation of precipitable polymeric pigments during wine aging. Noteworthy, most of the pectin interactions occurred during fermentation, and effects after filling were generally smaller. The reduced tannin concentration might result in a less astringent mouthfeel, which would need further confirmation. Moreover, polysaccharides play a crucial role in fostering the creation of ternary complexes involving polyphenols and proteins (Sommer, Weber, & Harbertson, 2019). It is possible that similar ternary complexes might be formed between polysaccharides, anthocyanins, and tannins, although this hypothesis necessitates validation in forthcoming experiments, which should include investigation of the role of ternary complexes in the wine’s sensory properties.

During winemaking, cell wall polysaccharides, in particular pectic polysaccharides, are extracted together with polyphenols from grapes. Only recently, Weilack et al. (2023) demonstrated that grape-derived pectic polysaccharides form pigments with anthocyanins. Together with the present results, it can be assumed that grape pectin forms non-precipitable polymeric pigments in red wines, however to a different extent depending on the grape variety. Especially in wines with high concentrations of polysaccharides, the formation of stable polymeric pigments from pectin and anthocyanins will improve color stability and pigment concentration.

4. Conclusions

The presented findings expand the established understanding of structural diversity of polymeric wine pigments. Precipitable and non-precipitable polymeric pigments formed through anthocyanin polymerization with flavanols or tannins must be complemented by pectin-anthocyanin complexes. Regardless of their origin, all compounds comprise similar physico-chemical characteristics that are used for quantification. The current limited diversification of analytes in the assay used for quantification might be overcome by changes in the sample handling. Besides this analytical challenge, the completely different structure of these polymeric pigments might evoke changes in sensory properties, which should be explored further.

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Jan-Peter Hensen: Writing – original draft, Investigation, Formal analysis. **Fiona Hoening:** Investigation. **Tamara Bogdanovic:** Investigation. **Andreas Schieber:** Writing – review & editing, Supervision, Funding acquisition. **Fabian Weber:** Writing – review & editing, Supervision, Funding acquisition, Conceptualization.

Declaration of competing interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: Hensen, Jan-Peter reports financial support was provided by German Ministry for Economic Affairs and Energy (via AiF) and the FEI (Forschungskreis der Ernährungsindustrie e.V., Bonn). If there are other authors, they 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.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.foodres.2024.114442.

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