

# **Influence of the ripening progress of grapes on the phenolic composition and sensory properties of red wine**

*Einfluss des Reifefortschritts von Weintrauben auf die phenolchemische Zusammensetzung und die sensorischen Eigenschaften von Rotwein*

Vom Fachbereich Chemie der Rheinland-Pfälzischen Technischen Universität Kaiserslautern-Landau zur Verleihung des akademischen Grades "Doktor der Naturwissenschaften" genehmigte Dissertation

DE - 386



vorgelegt von

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Kaiserslautern, 12.06.2025

Die dieser Arbeit zugrundeliegenden praktischen Arbeiten wurden im Zeitraum vom 01.02.2020 bis zum 31.08.2023 am Weincampus Neustadt/Dienstleistungszentrum Ländlicher Raum Rheinpfalz, Institut für Weinbau und Oenologie, im Arbeitskreis von Prof. Dr. Dominik Durner durchgeführt.

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Hiermit versichere ich, dass ich die vorliegende Arbeit eigenständig verfasst und keine anderen als die angegebenen Quellen und Hilfsmittel verwendet, sowie Literaturzitate kenntlich gemacht habe. Die Arbeit liegt weder in gleicher noch in ähnlicher Form in einem anderen Prüfungsverfahren vor.

Kaiserslautern, den \_\_\_\_\_

## Danksagung

Ich möchte mich ganz herzlich bei all den Leuten bedanken, die mich in den letzten Jahren und besonders im Zusammenhang dieser Dissertation unterstützt und beraten haben.

Zunächst gilt mein Dank Prof. Dr. Dominik Durner, der mich nach dem Abschluss meiner Masterarbeit für dieses spannende und vielseitige Forschungsprojekt gewinnen konnte. Ich bin sehr dankbar für den kreativen Freiraum, den ich bei der Bearbeitung des Themas hatte, den fachlichen Austausch und die zahlreichen Diskussionen, die mich zum Hinterfragen meiner Forschungsergebnisse angeregt haben.

Weiterhin möchte ich mich bei den Mitgliedern der Prüfungskommission bedanken, allen voran Prof. Dr. Elke Richling für die Übernahme der Betreuung sowie der Erstellung des Erstgutachten und die gute Zusammenarbeit bei der Veröffentlichung unserer Forschungsergebnisse. Vielen Dank auch an Prof. Dr. Wolfgang Kleist für die Übernahme der Aufgabe des dritten Prüfers und den fachlichen Austausch nach dem Promovierendenseminar des Fachbereichs Chemie. Prof. Dr. Werner Thiel möchte ich danken für die Übernahme des Vorsitzes der Prüfungskommission und die gute Zusammenarbeit während meiner Tätigkeit in den studentischen Gremien des Fachbereichs.

Ein großes Dankeschön gilt Dr. Pascal Wegmann-Herr, der mich bei allen Themen rund um Weinbau und Weinbereitung beraten und unterstützt hat. Bei der Arbeit im Technikum konnte ich viel lernen und die gemeinsamen Gespräche haben mir geholfen den Praxisbezug und die Relevanz meines Forschungsthemas besser zu verstehen.

Mein Dank gilt auch unseren Projektpartnern Dr. Ingrid Weilack und Dr. Jan-Peter Hensen unter der Leitung von Dr. Fabian Weber für die gute Zusammenarbeit und den fachlichen Austausch.

Ein besonderer Dank geht an Daniel Zimmermann und Dr. Patrick Nickolaus, die mich mit ihrem Wissen rund um das Thema Polyphenolanalytik in der Theorie und der praktischen Tätigkeit im Labor unterstützt haben. Ich möchte mich bedanken für die vielen Gespräche und das gemeinsame Lachen, das meine Zeit in Neustadt so besonders gemacht hat.

Sandra Klink, Martha Wicks-Müller, Xenia Manzel und Anette Schormann möchte ich herzlich für die Unterstützung bei der Planung und Durchführung der Sensorik Projekte danken.

Dr. Jonas Müller, Florian Schraut sowie den Kolleg\*innen vom Staatsweingut danke ich für die tatkräftige Unterstützung bei der Lese und der Weinbereitung.

An dieser Stelle möchte ich mich auch bei allen Promovierenden und Kolleg\*innen am Weincampus Neustadt und DLR Rheinpfalz bedanken. Es war sehr schön mit euch zu arbeiten und die gemeinsamen Aktionen nach Feierabend werden mir gut in Erinnerung bleiben. Besonders danken möchte ich dabei Dr. Hannah Renner, Dr. Svetlana Cvetkova und Dr. Thi Nguyen für die vielen Gespräche – fachlicher und privater Natur – im Büro und die gemeinsamen Trainingssessions im Fitnessstudio. Weiter möchte ich Michael Wacker, Dr. Marcel Hensel, Fabian Marnet, Gaby Goergens, Prof. Dr. Uli Fischer, Marc Weber, Dr. Jörg Gottmann, Dr. Chantal Wingerter, Lisa Käßler, Dr. Caterina Szmania, Louis Backmann, Caroline Dietzel, Thomas Roos und Julian Lueck danken.

Herzlich bedanken möchte ich mich auch bei den Absolventen, die ich mitbetreuen durfte, Erika Markusevics, Julian Hantke und Julian Hauser. Die Eigeninitiative und das Interesse an den Fragestellungen dieses Forschungsprojekts haben einen wichtigen Beitrag zum Gelingen dieser Arbeit geleistet.

Ein Dankeschön gilt auch meinem ehemaligen Nachbarn in Mußbach Bernd Bassler für die herzliche Aufnahme als „echte Mußbacherin“ und die gemeinsamen Gespräche.

Meinen neuen Kolleg\*innen am LKA Niedersachsen möchte ich für ihre Unterstützung und das Verständnis danken, das sie mir in den letzten Monaten während des Schreibens gezeigt haben.

Zum Schluss möchte noch ich meiner Familie, Raphael und meinen Freunden aus dem Studium Sophie, Daniel und Britta danken. Für Motivation, Unterstützung und gemeinsame Unternehmungen, um etwas Abwechslung zu bekommen. Ohne euch wäre ich nicht so weit gekommen. Danke!

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Feifel, S., Hantke, J., Wegmann-Herr, P., Richling, E., and Durner, D. "Impact of grape berry size on the phenolic composition of Pinot noir wine" (submitted to *OENO One*).

Feifel, S., Zimmermann, D., Schaub, M., Wegmann-Herr, P., Richling, E., and Durner, D. (2025), "Influence of grape maturity and maceration time on sensory characteristics and phenolics in Pinot noir and Cabernet-Sauvignon wines", *OENO One*, Vol. 59 No. 2. <https://doi.org/10.20870/oeno-one.2025.59.2.8446>.

### ***Publications in technical journals***

Feifel, S. (2022). Immer noch eine offene Frage: Phenolische Reife im Fokus. *DAS DEUTSCHE WEINMAGAZIN*, 16-17, 36 ff.

### ***Publications in conference proceedings***

Feifel, S., Hensen, J.-P., Weilack, I., Weber, F., Wegmann-Herr, P., and Durner, D. (2023), "Impact of climate change on grape cluster structure, grape constituents, and processability", *BIO Web of Conferences*, Vol. 56, p. 1016. <https://doi.org/10.1051/bioconf/20235601016>.

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Feifel, S., Wegmann-Herr, P., and Durner, D. (2021). Influence of berry maturity, maceration time and wine maturation on the polyphenols and sensory characteristics of Pinot noir and Cabernet Sauvignon. Macrowine 2021, Virtual conference, June 23<sup>rd</sup> – 30<sup>th</sup>, 2021. [Poster presentation]

Feifel, S., Wegmann-Herr, P., and Durner, D. (2022). Saignée - The Remedy for an Early Harvest? 2<sup>nd</sup> Wine Graduates' Forum/ DWV Congress, Virtual conference, April 11<sup>th</sup>, 2022. [Short oral presentation]

Feifel, S., Weilack, I., Wegmann-Herr, P., Weber, F., and Durner, D. (2022) Influence of Potential Alcohol and pH Adjustment on Polyphenols and Sensory Characteristics of Red Wines Produced at Different Harvest Time Points. In *Vino Analytica Scientia*, Neustadt an der Weinstraße, Germany, July 3<sup>rd</sup> – 7<sup>th</sup>, 2022. [Short oral presentation]

Feifel, S., Hensen, J.-P., Weilack, I., Weber, F., Wegmann-Herr, P., and Durner, D. (2022). Impact of climate change on grape cluster structure, grape constituents, and processability. 43<sup>rd</sup> World Congress of Vine and Wine, Ensenada, Mexico, October 30<sup>th</sup> – November 4<sup>th</sup>, 2022. [Oral presentation]

Weilack, I., Feifel, S., Wegmann-Herr, P., Durner, D., and Weber, F. (2022). Role of the tannin to anthocyanin ratio in the formation of polymeric pigments and its influence on red wine mouthfeel. 73<sup>rd</sup> ASEV National Conference, San Diego, California, USA, June 19 – 22, 2022, *Technical Abstracts*, p. 71. [Oral presentation]

Weber, F., Weilack, I., Hensen, J. P., Feifel, S., Wegmann-Herr, P., and Durner, D. (2022) Phenolische Traubenreife – Definition, Bestimmung und Bedeutung. Deutscher Lebensmittelchemikertag, Hamburg, Germany, September 19-21, 2022. *Lebensmittelchemie*, 76: S2-137-S2-137. <https://doi.org/10.1002/lemi.202259094> [Poster presentation]

## Seminars and workshops

Feifel, S., Müller, L., and Markusevics, E. (2020). Anpassung der Rotweintechnologie an die klimabedingte Varianz der phenolischen Traubenreife. Aus der Forschung für die Praxis, Neustadt an der Weinstraße, Germany, August 19<sup>th</sup>, 2020. [Oral presentation and wine tasting]

Feifel, S., Wegmann-Herr, P., and Durner, D. (2022). Climate Change: A New Pinot noir Wine Style? 5<sup>th</sup> Young Researchers Symposium, Kaiserslautern, Germany, July 22<sup>nd</sup>, 2022. [Oral presentation]

Feifel, S., Weilack, I., Wegmann-Herr, P., Weber, F., and Durner, D. (2022) Influence of Potential Alcohol and pH Adjustment on Polyphenols and Sensory Characteristics of Red Wines Produced at Different Harvest Time Points. Tag der Forschung an der Hochschule Kaiserslautern, Pirmasens, Germany, September 29<sup>th</sup>, 2022. [Oral presentation]

Feifel, S., Weilack, I., Hensen, J.-P., Wegmann-Herr, P., Weber, F., and Durner, D. (2023). Impact of climate change on the enology of Pinot noir wine. 62. Arbeitstagung des Forschungsrings Deutscher Weinbau, Veitshörsheim, Germany, May 2<sup>nd</sup>, 2023. [Oral presentation]

## Zusammenfassung

Ob der Anbau von Weinreben erfolgreich ist, hängt von verschiedenen Umweltfaktoren ab, da Weinreben sehr empfindlich auf Wetter- und Klimaveränderungen reagieren. Es hat sich gezeigt, dass die globale Erwärmung und der damit verbundene Anstieg der Wachstumsgradtage (GDD) eine fortgeschrittene und/oder beschleunigte Reifung von Weintrauben auslösen. Frühere Studien deuten darauf hin, dass sich mit fortschreitendem Klimawandel die Reifekinetik verschiedener Traubenbestandteile, wie z. B. die Akkumulation von Zuckern und Anthocyanen, entkoppeln könnte. Daher könnten herkömmliche Indikatoren für die Traubenreife wie der potenzielle Alkoholgehalt oder das Verhältnis von Zucker zu Säure möglicherweise keine konkreten Informationen mehr über die Reife anderer Traubenbestandteile liefern. Die Phenolische Reife ist ein bekanntes, aber nur vage definiertes Konzept für rote Rebsorten, um den optimalen Reifegrad zu ermitteln, wobei der Gehalt und die Extrahierbarkeit von Phenolen aus Trauben im Mittelpunkt stehen. In dieser Arbeit wurde der Einfluss des Reifegrads zweier roter Rebsorten von internationaler Bedeutung (Pinot noir und Cabernet Sauvignon) auf die phenolchemische Zusammensetzung und die sensorischen Eigenschaften von Wein in der Europäischen Weinbauzone A (cool climate) untersucht. Es wurde ferner gezeigt, wie sich die phenolische Reife im Wein widerspiegelt, und es wurden Indikatoren für die im Wein wahrgenommene phenolische Reife ermittelt. Anthocyane und nicht präzipitierbare polymere Pigmente korrelierten positiv mit der fortschreitenden Traubenreife und waren unabhängig von einer möglichen Alkoholanreicherung und den Weinbereitungsverfahren, was sie zu geeigneten Indikatoren für die phenolische Reife macht, wenn die Zuckerakkumulation von der Reifungskinetik anderer Traubenbestandteile entkoppelt wird. Es wurden Sortenunterschiede festgestellt, die darauf hindeuten, dass bei Sorten mit niedrigem Gesamtphenolgehalt wie Pinot noir die Anthocyane berücksichtigt werden sollten, während bei Sorten mit hohem Gesamtphenolgehalt wie Cabernet Sauvignon die nicht präzipitierbaren polymeren Pigmente im Vordergrund stehen sollten. Es wurde ein experimentelles Modell entwickelt, bei dem phenolchemische und sensorische Daten von Pinot noir und Cabernet Sauvignon aus zwei aufeinanderfolgenden Jahrgängen verwendet wurden, um festzustellen, inwieweit der potenzielle Alkohol als Lösungsmittel fungiert, das die Extraktion der phenolischen Verbindungen während der Weinbereitung moduliert. Es zeigte sich, dass die Wirkung des potenziellen Alkohols auf die einzelnen Polyphenole unterschiedlich ist. Die in dieser Arbeit vorgestellten Ergebnisse deuten jedoch darauf hin, dass weder eine Erhöhung des potenziellen Alkoholgehalts noch Weinbereitungsverfahren wie eine verlängerte Mazerationzeit die sensorischen Eigenschaften von Wein aus unreifen Trauben in Richtung der Eigenschaften von Weinen der Spätlese manipulieren können. Der Einfluss des potenziellen Alkohols oder der Mazerationzeit auf die phenolchemische Zusammensetzung des Rotweins unterschied sich vom Einfluss der Traubenreife. Es wurde nachgewiesen, dass weinbauliche Strategien wie Entlaubung und Erntezeitpunkt die phenolische Zusammensetzung von Pinot noir beeinflussen, jedoch hatten diese Strategien nur einen geringen Einfluss auf die Beerengrößenverteilung im Weinberg. Die Beerengröße hatte einen signifikanten Einfluss auf das Polyphenolprofil von Pinot noir Wein. Kleine Beeren erzeugten Weine mit dem höchsten Gesamtphenolgehalt. Diese Arbeit zeigt, dass der Reifefortschritt von Weintrauben die phenolchemische Zusammensetzung und Extraktion in Rotwein sowie die sensorischen Eigenschaften erheblich beeinflusst. Die Ergebnisse zeigen auch, dass die Traubenreife den Einfluss der Weinbereitungsmethoden überwiegt.

## Abstract

Successful cultivation of grapevine depends on various environmental factors, because grapevine are very sensitive to changes in weather and climate. Global warming and related increases in growing degree days (GDD) have been shown to trigger advanced and/or accelerated ripening of grapes. Previous studies suggest that with progressing climate change, the ripening kinetics of different grape constituents, such as sugar and anthocyanin accumulation might decouple. Therefore, traditional indicators for grape maturity such as potential alcohol or the ratio of total soluble solids to titratable acidity might no longer provide accurate information about the maturity of other grape constituents. Phenolic maturity is a well-known, but vaguely defined concept for red grape varieties to identify optimal maturity focusing on the content and extractability of phenolics from grapes. In this thesis, the influence of the ripening progress of two red varieties of international importance (Pinot noir and Cabernet Sauvignon) on the phenolic composition and sensory properties of wine was investigated in the European winegrowing zone A (cool climate). It was further shown how phenolic maturity is reflected in wine and indicators for phenolic maturity perceived in wine were identified. Anthocyanins and non-precipitable polymeric pigments were positively correlated with progressing grape maturity and were independent of potential alcohol enhancement and winemaking procedures, making them suitable indicators for phenolic maturity if sugar accumulation is decoupled from the ripening kinetics of other grape constituents. Varietal differences were observed, suggesting that varieties with low phenolic contents such as Pinot noir should focus on anthocyanins, whereas varieties with high phenolic contents such as Cabernet Sauvignon should focus on non-precipitable polymeric pigments. An experimental model using phenol and sensory data from Pinot noir and Cabernet Sauvignon of two consecutive vintages was developed to determine the extent to which the potential alcohol acts as a solvent modulating the extraction of phenolic compounds during winemaking. The effect of potential alcohol enhancement was shown to be different for individual polyphenols. However, the results presented in this thesis suggest that neither potential alcohol enhancement nor winemaking techniques such as extended maceration time can manipulate the sensory characteristics of wine produced from immature grapes towards the characteristics of late harvest wines. The influence of potential alcohol or maceration time on the phenolic composition of red wine was different from the influence of grape maturity. Viticultural strategies such as leaf removal and timing of harvest were shown to influence the phenolic composition of Pinot noir wine, however these strategies only had a marginal effect on the berry size distribution in the vineyard. Berry size significantly influenced the phenolic composition of Pinot noir wine. Small berries produced wines with the highest phenolic content. This thesis shows that grape ripening significantly influences the phenolic composition and extraction in red wine as well as the sensory properties. The results also show that grape maturity outweighs the influence of winemaking techniques.

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## Abbreviations

<b>ANOVA</b>	Analysis of variance
<b>CON</b>	Control
<b>CS</b>	Cabernet Sauvignon
<b>CVA</b>	Canonical variates analysis
<b>EH</b>	Early harvest
<b>ESI</b>	Electrospray ionization
<b>EXP</b>	Exposed
<b>GDD</b>	Growing degree days
<b>H1-3</b>	Harvest date
<b>ha</b>	Hectares
<b>HI</b>	Huglin Index
<b>HPLC</b>	High performance liquid chromatography
<b>L</b>	Large berry size
<b>LH</b>	Late harvest
<b>M</b>	Medium berry size
<b>MCP</b>	Methyl cellulose precipitation
<b>mDP</b>	Mean degree of polymerization
<b>MFA</b>	Multiple factor analysis
<b>mhL</b>	millions of hectoliters
<b>MLF</b>	Malolactic fermentation
<b>MS</b>	Mass spectrometry
<b>np-PP</b>	non-precipitable polymeric pigments
<b>OIV</b>	International Organization of Vine and Wine
<b>PC(A)</b>	Principal component (analysis)
<b>PLS</b>	Partial least squares regression
<b>PN</b>	Pinot noir
<b>p-PP</b>	precipitable polymeric pigments
<b>QToF</b>	Quadrupole time-of-flight
<b>S</b>	Small berry size
<b>SD</b>	Standard deviation
<b>TSS</b>	Total soluble solids

# 1 Introduction

Viticulture has a long history, dating back to the Neolithic period (ca. 8500-4000 BC) (McGovern *et al.*, 1996). Archeological finds such as decorated pottery jars from Georgia suggest that grapevine cultivation originates in the Caucasus between the Caspian and Black Seas, predating chemical evidence of resinated wine in a pottery jar found at the site of Hajji Firuz Tepe in Iran (McGovern *et al.*, 1997; McGovern, 1999). Grape cultivation and winemaking then spread southward and around the Mediterranean. Wine consumption had and still has socioreligious purposes. It was used as sacrificial offering in religious events and buried with kings and pharaohs for their afterlife (McGovern *et al.*, 1996; Soleas *et al.*, 1997). Wine has always been a precious good and is therefore important for international trade. The International Organization of Vine and Wine (OIV) estimated the world wine production in 2024 to be between 227 and 235 millions of hectoliters (mHL) (OIV, 2024) sourced from approximately 7.2 million hectares vineyard surface (Barker, 2024). In 2023 the world wine export reached 36.0 billion Euro which is the second highest ever recorded although the export volume decreased to 99 mHL which is the lowest volume since 2010. This trend is reflected in the average world export price of wine in 2023 at 3.62 Euro per liter which is the highest ever recorded (Barker, 2024). The OIV reports that the reasons for the rise in prices are higher production costs, transport and global inflationary pressures. However, the world's vineyard surface area is also continuously declining since 2000 (Barker, 2024) which is caused by extreme weather events such as flooding e.g. in the Ahr valley, Germany in July 2021 (Seidel, 2021) or Tuscany, Italy in 2024 (Dupuy, 2024), wildfires e.g. in Northern Portugal in 2017 and 2024 (Meininger's International, 2024), in California in 2017, 2018 and 2020 (Bassler, 2024), in Australia in 2019-2020 (Roberts, 2020), and in Chile in 2023 (Williams, 2023), just to name the most recent cases. But even in regions without weather events as extreme as described before, crop yield has been declining due to changing climatic conditions, atypical meteorological events such as early frosts, heavy rainfall and prolonged drought (OIV, 2024), but also changes in phytophagous insect activity (Reineke and Thiéry, 2016) as well as increased incidences of fungal and viral diseases influenced by climate change (Rienth *et al.*, 2021). Climate change, however, does not only affect crop yield but also grape and wine composition as well as wine quality (van Leeuwen *et al.*, 2024). Previous climatology studies have shown that with increasing temperatures the periods between phenological events such as budburst, floraison, véraison and harvest have become shorter and tend to occur earlier (Duchêne and Schneider, 2005; Jones and Davis, 2000). During grape ripening sugar as well as secondary plant metabolites such as polyphenols accumulate in the berries, whereas concentrations of certain organic acids and polyphenols decrease. Berry maturity and timing of harvest are therefore crucial for winemakers in order to produce high quality wine (Bindon

*et al.*, 2014a). Traditionally the sugar-to-acid concentration ratio is used by winemakers as a maturity indicator in addition to their personal preferences to determine the timing of harvest. However, Jones and Davis (2000) as well as Mira de Orduña (2010) have shown that higher sugar-to-acid ratios associated with warmer vintages may negatively affect wine quality. In addition to sugars and acids, polyphenols such as anthocyanins, tannins and flavan-3-ols significantly influence sensory characteristics of red wine as they provide the red color, taste and mouthfeel (Gil *et al.*, 2012; Noble, 1998). The concept of “phenolic maturity” was therefore introduced by Glories (1986) and later extended by Saint-Cricq de Gaulejac *et al.* (1998) to provide a new maturity indicator for red grape varieties. Grape berries reach optimal “phenolic maturity” when the anthocyanin concentration in the skins has reached a maximum while the concentration of seed tannins has decreased from véraison (Glories, 1986). In addition, the extractability of the different phenolic compounds as well as the sensory characteristics of tannins need to be considered (Saint-Cricq de Gaulejac *et al.*, 1998). In contrast to sugar-to-acid ratios as maturity indicators, optimal phenolic maturity is difficult to determine for winemakers. It is therefore necessary to assess and understand the influence of different levels of phenolic maturity on the wine composition and how it is reflected in the sensory characteristics of red wine.

## **2 Literature review**

### **2.1 Grape berries**

Grapes are berries which are organized in a cluster. A small pedicel attaches each berry to the rachis. The length of the pedicel determines whether the cluster structure is more compact or spread out (Ribéreau-Gayon *et al.*, 2005). The grape berry structure can generally be divided into three types of tissue - skin, pulp/flesh, and seeds – which differ in their chemical composition (Dalton, 2017). Depending on the grape variety, the skin makes up for 10 – 17 % of the berry weight (Ortega-Regules *et al.*, 2006), the seeds account for less than 6 % of the berry weight (Cadot *et al.*, 2006) and the pulp has the highest proportion of up to 77 % (Ortega-Regules *et al.*, 2006). The relationship between berry volume and seed number was found to be quadratic (May, 2000; Schumann, 1973).

#### **2.1.1 Grape composition**

The pulp represents the highest proportion of the berry weight (Ortega-Regules *et al.*, 2006). The cells of the pulp accumulate water, sugars and organic acids such as tartaric acid, malic acid, citric acid and succinic acid during ripening, which produce the grape juice (Dalton, 2017). The primary constituents after water are sugars with fructose being predominant, followed by

glucose (Ribéreau-Gayon *et al.*, 2005). For winemaking, the amount of sugar in the berry at harvest determines the potential alcohol that can be obtained during fermentation. Therefore, sugars are considered important indicators for grape maturity and wine quality (Dalton, 2017). Titratable acidity, another important maturity indicator, depends mostly on the content of tartaric, malic and citric acid. Depending on the variety, grape maturity and climate, titratable acidity can vary from 4.5 – 15 g/L tartaric acid equivalents (Ribéreau-Gayon *et al.*, 2005).

Grape juice is also a source for nitrogen-containing compounds, which are necessary for yeast metabolism. The nitrogen content is mainly composed of amino acids, peptides and proteins. The total nitrogen content in grape juice can vary from 60 – 2400 mg/L (Fleet, 2002). This corresponds to 20 – 25 % of the total nitrogen content of the berry (Ribéreau-Gayon *et al.*, 2005). The solid parts of the pulp (cytoplasm and pectocellulosic cell walls) constitute for less than 1 % of the pulp mass (Ribéreau-Gayon *et al.*, 2005).

Grape skins contain different phenolic compounds such as benzoic and cinnamic acid, flavonols, proanthocyanidins and anthocyanins as well as aromatic substances and aroma precursors (Obreque-Slér *et al.*, 2010; Ribéreau-Gayon *et al.*, 2005). The skins are also rich in organic acids like the pulp; however, citric acid is predominant, and the malic acid concentration is actively metabolized during berry ripening. Tartaric acid is mostly present as esters with phenolic acids such as caffeic and coumaric acid (Ribéreau-Gayon *et al.*, 2005). The skin cell walls are composed of polysaccharides, phenolic compounds and proteins, which are stabilized by ionic and covalent linkages. The structure of the cell walls is provided by hemicellulose, pectins, cellulose microfibrils and structural proteins, with cellulose and pectins accounting for 30 - 40 % of the total polysaccharide content (Huang and Huang, 2001; Nunan *et al.*, 1997).

Grape seeds differ in their composition from skins and pulp. They contain approximately 35 – 40 % fiber, 7 – 20 % oil, 11 % protein, 7 % phenolic compounds, 7 % water, 3 % minerals, as well as sugars and salts (Campos *et al.*, 2008; Cao and Ito, 2003; Lachman *et al.*, 2013; Mironeasa *et al.*, 2010; Murga *et al.*, 2000; Rogiers *et al.*, 2015; Shinagawa *et al.*, 2015). The main fatty acids found in seeds are oleic and linoleic acid (Ovcharova *et al.*, 2016; Ribéreau-Gayon *et al.*, 2005). Although seeds account for less than 6 % of the berry weight, they are an important source for phenolic compounds. Depending on the variety the seeds contain 20 – 50 % of the berry's total polyphenols (Ribéreau-Gayon *et al.*, 2005).

Polyphenols are phytochemicals that belong to the secondary plant metabolism. Phytochemicals are plant-derived chemical compounds which are important for the survival of the plants by protecting them against other organisms, controlling essential functions such as growth or reproductive systems and are necessary for proper functioning (Molyneux *et al.*, 2007). Polyphenols can be divided into two categories: non-flavonoids, which include phenolic acids and stilbenes, and flavonoids, which comprise anthocyanins, flavan-3-ols and flavonols.

In plants phenolic compounds can be found mainly in the seeds and skin and to a lesser extent in rachis and pulp (Obreque-Sl er *et al.*, 2010). Further information on polyphenols will be provided in chapter 2.2.1.2.

## 2.1.2 Grape maturity

Phenological processes (Chapter 2.1.3) such as berry setting and shattering modulate the number of berries that get fertilized and kept in the cluster to ripen. These mechanisms are important for the grape vine to adjust to weather conditions (temperature, humidity) and the related sugar availability in order to provide enough nutrients and energy for the berries to reach maturity (Rib ereau-Gayon *et al.*, 2005). Grapes follow the ripening stages that are common to all berries. Berry growth can be divided into three stages as shown in Figure 1, taking parameters such as berry diameter, weight and volume into consideration (Coombe and Hale, 1973; Kennedy *et al.*, 2000).

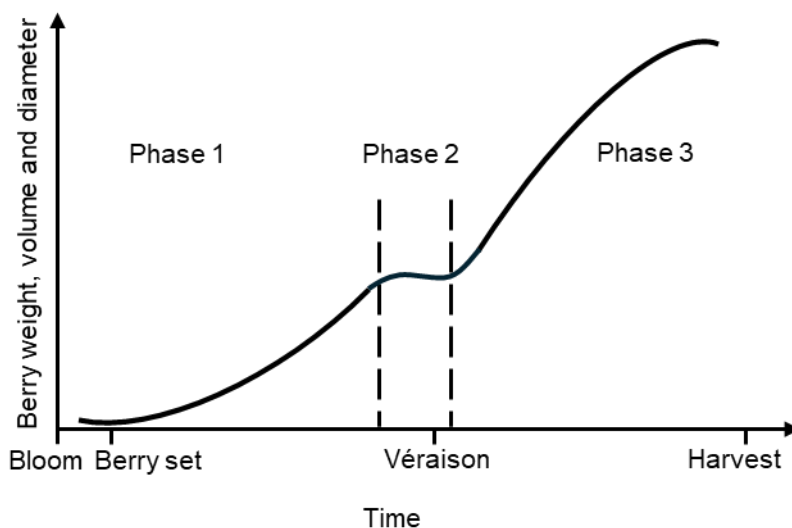


Figure 1 Developmental stages of the grape berry (Rib ereau-Gayon *et al.*, 2005).

An initial rapid growth period (first stage) is characterized by a rapid accumulation of acids into the berries due to their intense metabolic activity. The number of seeds per berry is directly related to the concentration of growth hormones (cytokinins and gibberellins) present in the berries (Coombe, 1960; Rib ereau-Gayon *et al.*, 2005). Seeded grape varieties may contain up to four fully functional seeds (Ristic and Iland, 2005). The second growth stage is marked by slower metabolic activity and the appearance of color in colored varieties, which is called v eraison. Growth substance synthesis is depleting and the concentration of abscisic acid increases (Coombe and Hale, 1973; Rib ereau-Gayon *et al.*, 2005). The third stage is a second growth phase leading to maturation. In this phase the grape accumulates sugars, cations such as potassium, amino acids and phenolic compounds. Simultaneously the concentrations of

malic acid and ammonium decrease. The size of grape berries at harvest depends on several factors such as the number of cells per berry, the aforementioned accumulation processes but there have also been reports of a close relationship between the dimension of the berry and the number of seeds it contains (Coombe and Hale, 1973; Ribéreau-Gayon *et al.*, 2005). Ristic and Iland (2005) also suggested that berry development is affected by seed development. The maximum seed weight is reached between one to two weeks prior to véraison, then it declines by 20-30 % until harvest (Downey *et al.*, 2003). At harvest, seeds still account for less than 6 % of the berry weight (Cadot *et al.*, 2006; Downey *et al.*, 2003; Ribéreau-Gayon *et al.*, 2005). The biosynthesis of tannins commences with flower development. Their accumulation occurs rapidly until reaching a maximum between berry-set and véraison (Downey *et al.*, 2003; Hanlin and Downey, 2009). With progressing maturation, the degree of tannin polymerization increases. Downey *et al.* (2003) observed that seed proanthocyanidins are shorter (mean degree of polymerization (mDP) ~ 5) than skin proanthocyanidins (mDP ~ 40) and differ in their composition. In addition to polymerization, tannin extractability decreases with progressing maturation due to increased interactions with other cellular components such as grape cell walls. Downey *et al.* (2003) suggested that the accumulation of tannins in the seeds is independent of that in the skins.

Monomeric flavan-3-ol content in berries decreases drastically during ripening (Kennedy *et al.*, 2000). Kennedy *et al.* (2000) observed that the flavan-3-ol content in berries decreased by 90% between véraison and harvest. The composition of monomers and terminal proanthocyanidin units changes during maturation, whereas extension unit composition remains the same. Gallic acid concentration was reported to remain low throughout the season, leading to the suggestion that epicatechin-3-gallate hydrolysis does not contribute to the epicatechin pool in the berry (Kennedy *et al.*, 2000; Singleton, 1966).

Calderon Orellana *et al.* (2014) reported that skin weight per berry was positively and linearly correlated with berry size. At maturity, red grape skins contain phenolic compounds like benzoic and cinnamic acids, flavonols, proanthocyanidins (tannins) and anthocyanins (Ribéreau-Gayon *et al.*, 2005). Kontoudakis *et al.* (2011) showed that the accumulation of tannins and anthocyanins in the skins occurs simultaneously to an increase in sugar concentration. Skin tannin concentration (mg/g berry fresh weight) decreases slightly (approx. 10%) with increasing berry size (g berry fresh weight) (Roby *et al.*, 2004). Anthocyanin content per berry was found to be positively correlated with berry size. Their concentration, however, decreases with berry size in a similar matter as skin tannins (Roby *et al.*, 2004).

The content of seed tannins per berry generally increases with berry size. Seed tannin content has a positive linear correlation with both seed number and seed mass per berry, but the correlation is slightly stronger with seed mass per berry than seed number (Roby *et al.*, 2004).

Kontoudakis *et al.* (2011) suggested that heterogeneity in grape phenolic maturity affects wine quality and ageing potential due to differences in proanthocyanidin contents. Therefore, a reduction in berry heterogeneity (size and maturity) might improve winemaking processes and wine quality. Barbagallo *et al.* (2011) suggested that reducing the grape size might be desirable due to higher anthocyanin concentrations and less diluted tannin contents. Viticultural strategies to modulate grape heterogeneity are discussed further in chapter 2.1.4.2.

### **2.1.3 Grape phenology**

Phenology is the study of periodically occurring biological phenomena that are correlated with climatic conditions. For *Vitis vinifera* grapevines the most important developmental stages are budburst, floraison (flowering), véraison (color change) and harvest (grape maturity) (Jones and Davis, 2000). The duration and timing of each stage is influenced by different factors such as grape variety, climate and geographic location (Jones, 1998). The timing of these stages is related to the ability of the vine to yield fruit. Weather events such as late frost during budburst or heavy rain during floraison can disrupt the stage and lead to a reduced yield. With progressing climate change, the timing of these phenological stages has been related to vintage quality (Jones, 1998; Jones and Davis, 2000).

A uniform scale to describe phenological stages (BBCH-Scale) was introduced by Hack *et al.* (1992). Lorenz *et al.* (1995) extended the BBCH-Scale descriptors for grapevine. The extended BBCH-Scale for grapevine divides the phenological development into macro- and micro-stages. The scale starts with the principal growth stage 0 “sprouting”, which ends in the micro-stage 09 “budburst”. Flowering is part of the principal growth stage 6 and is divided into micro-stages that describe the development from the beginning of flowering (BBCH 60) to full bloom (BBCH 65) until the end of flowering (BBCH 69). The development of fruits is described in principal growth stage 7, which is followed by the ripening of berries in stage 8. This stage includes both véraison (BBCH 83) and harvest (BBCH 89). The last principal growth stage takes place after harvest and describes the discoloration and falling of the leaves (Lorenz *et al.*, 1995). In viticulture, these stages represent important anchor points for plant protection treatments or treatments to improve the quality of the crop, such as leaf removal or cluster thinning.

### **2.1.4 Influence factors on grape maturity**

#### **2.1.4.1 Environmental**

Grape vine growth is affected by environmental conditions such as the soil in the vineyard or the climate (Jackson and Lombard, 1993). For ideal growing conditions the length of the

growing season needs to match the variety so that the late stages of berry ripening occur during the cooler part of the season but with temperatures still high enough to continue sugar accumulation and development of aroma and phenolic compounds (Jackson and Lombard, 1993). Due to climate change, extreme weather events tend to occur more often. Though less common, excessive cool temperatures during berry ripening can result in acidic wines with an unripe, vegetative aroma. High temperatures during the late stages of berry ripening on the other hand, can result in unbalanced wines with very high potential alcohol, low acidity, a cooked fruit aroma and an overall lack in complexity (Pons *et al.*, 2017).

Tools to visualize climate change are temperature indices such as the Huglin Index (HI) introduced in 1978 by Huglin or growing degree days (GDD) (McMaster and Wilhelm, 1997). Growing degree days are heat units that present the heat sum of days where the average temperature was higher than the base temperature (10 °C). It is assumed that the base temperature is necessary for vine growth. Growing degree days can either be expressed as GDD per year or as GDD per growing season which is different for the Northern (April-October) and Southern hemisphere (October-April) (McMaster and Wilhelm, 1997; Schultz and Jones, 2010). The index by Huglin (1978) also addresses the changes in temperatures from day- to nighttime, which is often perceived as more accurate.

Increased temperatures related to climate change lead to earlier phenological events and shorter intervals (Duchêne and Schneider, 2005; Feifel *et al.*, 2023; Jones and Davis, 2000; van Leeuwen and Darriet, 2016). Kliewer and Torres (1972) studied the effects of day and night temperatures on red wine color. Their study showed that cooler night temperatures of 15 – 20 °C compared to 25 – 30 °C promoted Pinot noir skin coloration. The effect of day temperatures was less pronounced, however, lower temperatures of 20 °C instead of 30 °C also enhanced the color of Pinot noir (Kliewer and Torres, 1972). These findings are in agreement with Pirie (1977) who suggested that the optimum temperatures for anthocyanin synthesis range from 17 – 26 °C. Temperatures too cold or hot can therefore be associated with poor color characteristics (Winkler *et al.*, 1974).

In a long-term climatology study from Bordeaux, high temperatures were related to increased sugar to total acid ratios in Cabernet Sauvignon and Merlot grapes (Jones and Davis, 2000). Higher temperatures in the growing season and as a result higher sugar levels in grape juice were associated with stress reactions in the yeast metabolism. The increased pH influences the microbial ecology and therefore increases the chances for unfavorable off-flavors (Mira de Orduña, 2010).

Related to temperature, solar radiation is another important factor for grapevine development. High solar radiation favors the synthesis and accumulation of sugars, phenolic compounds and aromatic compounds during berry ripening (Riou *et al.*, 1994). Increased photosynthesis due to high solar radiation also increases the demand for water. Precipitation is another

atmospheric variable which is influenced by climate change. The soil water balance determines the soil water availability for the vine and therefore its water status. Low availability of water results in water stress which is reflected in various effects, depending on the phenological stage (Austin and Bondari, 1988). During early stages, water stress increases the risk for poor flower-clustering development and berry set. Water stress before and after véraison lead to smaller berries and an increased risk of failure for the fruit to mature. Berries from vines with water stress also had a lower skin pigment content. Overall, water stress at each stage of ripening reduced the fresh fruit yield (Hardie and Considine, 1976).

Other studies have suggested that water deficits at specific stages of grape development might also be favorable for wine quality. A water deficit before véraison can stimulate anthocyanin accumulation and increase skin phenolic concentrations (Deluc *et al.*, 2007; Ojeda *et al.*, 2002).

#### **2.1.4.2 Viticultural**

In viticulture, different strategies can be applied to prevent or counteract the effects of environmental conditions. Canopy management like defoliation can modulate the sun exposure for grape bunches (Haselgrove *et al.*, 2000), berry temperature and enhance air circulation. In vintages with higher precipitation and humidity, defoliation can reduce the risk for *Botrytis cinerea* infection and other fungal diseases (English *et al.*, 1989; Jackson and Lombard, 1993; Smart *et al.*, 2017). Enhanced sun exposure due to defoliation was shown to significantly increase anthocyanin concentrations, while pH, sugar concentration and berry weight were not significantly different from the control (Lee and Skinkis, 2013; Wicks and Kliewer, 1983). Anthocyanins in the skins of berries act as a protection for photosynthetic tissues by reducing the light stress via light absorbance in the visible range (Clifford, 2000; Jimenez-Garcia *et al.*, 2013; Paredes-López *et al.*, 2010; Zhang *et al.*, 2014). Their protective character, however, is limited to light in the visible range as they cannot protect plant tissues from high UV light stress (Zhao *et al.*, 2022).

In hot climates with high sun exposure, bunch shading can be applied to prevent high berry temperatures which might inhibit anthocyanin metabolism (Haselgrove *et al.*, 2000), but also reduce the risk for sunburn which could lead to lower yields and increased chances for off-flavors in the wine (Gambetta *et al.*, 2020).

Within a vineyard, but also within grape clusters, berries vary in size and in ripeness. Kontoudakis *et al.* (2011) reported a Gaussian bell-shaped distribution for berry density at all four sampling points which could be replicated in a second vintage. These findings show that a heterogeneity of berry maturity is present from the beginning of berry ripening until the time of harvest (Kontoudakis *et al.*, 2011). A high uniformity of fruit ripeness is usually preferred in

winemaking to avoid over- or underripe characteristics (Calderon Orellana *et al.*, 2014; Carroll *et al.*, 1978). Wines produced from batches with a high proportion of underripe berries received higher ratings for tartness which were lacking balance and lower ratings for aroma, flavor and overall quality. Wines from overripe berries also received lower ratings for overall quality, however, the judges had a variety of reasons without a definite trend (Carroll *et al.*, 1978). Armstrong *et al.* (2021) investigated the effect of grape heterogeneity on the chemical composition and sensory characteristics of Cabernet Sauvignon wine by first sorting the grapes into different density categories and then producing wines of low, moderate and high heterogeneity in comparison to an unsorted control. Concentrations of anthocyanins and phenolics were significantly higher in low heterogeneity wines than moderate and high heterogeneity wines. Armstrong *et al.* (2021) suggested that higher proportions of less dense grapes in the moderate and high heterogeneity treatment resulted in a dilution of phenolics. However, the differences in chemical composition were not reflected in the color density of the wines, suggesting that grape heterogeneity increased the stabilization of pigments in wines with higher heterogeneity (Armstrong *et al.*, 2021).

Yield reducing practices such as cluster thinning or selective berry thinning can be applied to improve grape maturity (Sivilotti *et al.*, 2020), however, Calderon Orellana (2012) reported that cluster thinning resulted in no significant changes in fruit uniformity at harvest. Berry size influences the chemical composition of red wine, because the concentration of all soluble solids increases proportionally with increasing berry size (Roby *et al.*, 2004). The higher surface-to-volume ratio of small berries is often discussed in comparison to wines produced from large berries. In theory, wines produced from small berries should have higher concentrations of skin-associated phenolics such as anthocyanins and should therefore be darker in color (Matthews and Nuzzo, 2007). Previous studies by Gil Cortiella *et al.* (2020) and Barbagallo *et al.* (2011), however, have shown that the often-assumed dilution effects regarding larger berries do not take all physiological factors of berries such as berry skin thickness into consideration. Gil Cortiella *et al.* (2020) have shown that the extraction of phenolics from berry skins is stronger related to the weight proportion of the skin than the size of the berry.

#### **2.1.4.3 Grape variety**

Romero-Cascales *et al.* (2005) reported that anthocyanin content and extractability is strongly influenced by grape variety. Monastrell grapes were found to have the highest content of anthocyanins, however they are difficult to extract compared to varieties such as Cabernet Sauvignon or Syrah which have lower contents, but a higher extractability and even a higher wine color intensity as a result (Romero-Cascales *et al.*, 2005).

Cabernet Sauvignon is an important red grape variety on the international wine market, which is commonly cultivated in warmer climates. Worldwide, the variety is grown on 341,000 ha (OIV, 2017). Germany, however, contributes only 483 ha (Deutsches Weininstitut, 2024) because the growing conditions were traditionally categorized as “cool climate” in the European winegrowing zone A (European Union). Cabernet Sauvignon is characterized by its late ripening and small berries (8.76 mm ± 1.6 mm (Friend *et al.*, 2009)). Hence, sunshine and long warm days result in a better outcome (Dalton, 2017). With progression of global warming growing conditions in Germany might become more suitable for Cabernet Sauvignon, making it a promising alternative to traditionally cultivated varieties. Cabernet Sauvignon berries are rich in seeds, tannins and anthocyanins, producing a dark, red colored wine. Cabernet Sauvignon has a large variety of anthocyanins due to acylated and coumarylated forms (Dalton, 2017). Cabernet Sauvignon skins contain almost twice the amount of anthocyanins (1754 ± 16 mg/kg berries (Guerrero *et al.*, 2009)) compared to Pinot noir (794 ± 33 mg/kg berries (Mazza *et al.*, 1999)).

Pinot noir is an important red variety for the wine economy, but also for research. In Germany, Pinot noir has the largest acreage of red varieties. Internationally, Germany has the third largest acreage (11.519 ha in 2023) of Pinot noir after France and the USA (Statistisches Bundesamt, 2024). Pinot noir wine is a representative of the “cool climate varieties”, which have only five monoacylated anthocyanins compared to “warm climate varieties” (Dalton, 2017; Fong *et al.*, 1971). The grape clusters are “pine cone shaped” (Dalton, 2017) with berries ranging in diameter between 5-8 mm or larger than 10 mm (Friend *et al.*, 2009). Hensen *et al.* (2023) reported significantly higher berry weights for Pinot noir (131.68 ± 5.38 g/100 berries at 22.7 Brix) compared to Cabernet Sauvignon (105.73 ± 0.66 g/100 berries at 22.7 Brix). The seed proportion per berry on the other hand was significantly higher in Cabernet Sauvignon (5.12 ± 0.17 % at 22.7 Brix; Pinot noir: 3.61 ± 0.04 % at 22.7 Brix) (Hensen *et al.*, 2023). The differences in climatic growing conditions as well as varietal differences in berry size and composition allow for a representative comparison of grape varieties during winemaking, wine composition and sensory characteristics.

## **2.2 Wine**

### **2.2.1 Winemaking**

The choice of processes during winemaking influences the color, aroma, flavor, taste and mouthfeel of wine. After harvest and destemming of the grapes, the main difference between white and red winemaking is that white varieties are commonly pressed to ferment the juice whereas red varieties are macerated with the grape solids (Unterkofler *et al.*, 2020).

Compounds located in the grape pulp are readily extracted after crushing. Those found in the skins can require a few days, while the extraction from seeds can take up to weeks as the ethanol increases (Waterhouse *et al.*, 2016). For alcoholic fermentation to occur, yeast is required to transform grape juice into wine. Wild yeasts can be found on grape skins which would start a spontaneous fermentation if no single yeast strain is added by the winemaker. Single yeast strains of *Saccharomyces cerevisiae* are commonly used to guarantee a consistent wine quality and reduce the chances for off-flavors, because they are reliable in terms of fermentation and tolerate more stressors such as temperature, ethanol and sulfur dioxide (Boulton, 1999; Unterkofler *et al.*, 2020).

In the first stages of maceration anthocyanins and skin tannins are extracted. With increasing ethanol in the later stages of maceration seed tannins and flavan-3-ols get extracted (Boulton, 1999). Although seeds contain higher concentrations of polyphenols than skins, skin phenolics can be extracted more easily and represent therefore the majority of phenolic compounds in wine (Meyer and Hernandez, 1970). During red wine fermentation, floating solids such as skins or seeds rise to the top of the fermenter due to entrapped carbon dioxide from the yeast metabolism and create a cap (Jackson, 2008). In order to avoid mold formation on the top of the cap and to allow for a better extraction of the skins and seeds, the cap is punched down with a plunger multiple times per day. Mixing the cap with the underlying liquid also helps to regulate the temperature as the yeast activity underneath the cap produces heat (Schmid *et al.*, 2009). After completion of alcoholic fermentation, the wine can be macerated further, which is called extended maceration. The influence of maceration time on the extraction of phenolics will be highlighted in 2.2.2.2.

After alcoholic fermentation red wines commonly undergo malolactic fermentation (MLF). During MLF lactic acid bacteria are added to the wine in order to decrease the harsh acidity of malic acid by decarboxylating malic acid into lactic acid (Moreno-Arribas and Polo, 2009).

## **2.2.2 Factors influencing the extraction and composition of phenolics**

### **2.2.2.1 Grape maturity**

With progressing grape maturity, the concentration and composition of sensory active compounds in grape berries change (Chapter 2.1.2) as well as their extractability. Many studies have been conducted to assess the influence of grape maturity on the extractability and composition of phenolics in red varieties. Sherman *et al.* (2017) investigated the effect of grape maturity on the extractability of phenolics in Merlot wines by using three harvest time points [20 Brix (unripe), 24 Brix (ripe), and 28 Brix (overripe)] and then adjusting the preferment soluble solids to match the other two harvest time points. The full-factorial design was achieved using chaptalization (addition of sugar) for unripe and ripe berries or saignée (bleeding-off,

removal of juice) and watering back for ripe and overripe berries. Anthocyanin concentrations in Merlot wine were higher with increasing grape maturity. The effect of potential alcohol adjustment on anthocyanin concentration in wine was only significant at the second harvest time point. Enhancing the potential alcohol resulted in lower anthocyanin concentrations. Sherman *et al.* (2017) suggested the water-soluble characteristics of anthocyanins as a possible reason for higher anthocyanin concentrations in low-alcohol ferments. Polymeric pigments increased significantly with higher ethanol concentrations and tannin concentrations were highest in wines produced from overripe grape at high potential alcohol (Sherman *et al.*, 2017). These observations suggest that anthocyanin extractability is mainly dependent on phenolic maturity, whereas the extraction of tannins and formation of polymeric pigments are influenced by both phenolic maturity and potential alcohol. Sacchi *et al.* (2005) suggest that the limiting factor for anthocyanin extractability is the physical barrier due to their location in the vacuoles of the hypodermal cells. Progressing grape maturity increases the permeability of these skin cells and membranes due to changes in the polysaccharide and pectin composition in the skin cell walls (Bautista-Ortín *et al.*, 2016; Ortega-Regules *et al.*, 2006). Previous studies have shown that the extractability of seed-associated tannins are more linked to the composition of the wine matrix than the composition of the seeds (Rousserie *et al.*, 2020). Tannin extraction is therefore difficult to predict from berry ripeness alone, however, winemaking techniques such as extending maceration time (Chapter 2.2.2.2), chaptalization or increasing fermentation temperature have been shown to modulate the extraction (Sacchi *et al.*, 2005). In contrast to anthocyanins, tannin extractability seems to be hindered by limited solubility. Higher potential alcohol content either by later harvesting or chaptalization, but also extending the extraction time at high alcohol concentrations can therefore increase the amount of tannin soluble in wine (Oszmianski *et al.*, 1986; Singleton and Draper, 1964).

The extractability of monomeric and oligomeric flavanols from grape seeds is influenced by seed texture. Hensen *et al.* (2023) showed that Pinot noir seeds became harder and tougher with progressing grape maturity, suggesting a lower extractability of phenolics. However, with progressing maturity concentrations of monomeric and dimeric flavanols also decreased in seeds. Hence, lower concentrations of these smaller flavanols paired with lower extractabilities result in lower concentrations of them in the wine (Hensen *et al.*, 2023).

### **2.2.2.2 Maceration Time**

Maceration time has a crucial role in modulating the extraction of phenolics as it prolongs the contact with skins and seeds after completion of alcoholic fermentation. Extending the maceration time has been shown to increase the concentration of condensed tannins (proanthocyanidins) and polysaccharides in wine (Sacchi *et al.*, 2005; Waterhouse *et al.*,

2016). Bautista-Ortín *et al.* (2015), however, showed that grape cell walls have a high re-adsorption capacity for proanthocyanidins which could lead to a lower astringency perception in the wine. Casassa *et al.* (2013a) showed that anthocyanin concentration peaked between day 4 and 6 of maceration. A study by Frost *et al.* (2018) suggests that extended maceration reduces the concentration of anthocyanins due to the ionic adsorption of anthocyanins by yeast cell walls. Anthocyanins can also be adsorbed onto bitartrate crystals or other solids (Cheynier *et al.*, 2006).

Once the yeast activity after alcoholic fermentation slows down, the cap made up of skins, seeds and other grape solids starts to sink. The cap can therefore no longer provide a carbon dioxide barrier to prevent oxidation of the wine. As a result the formation of polymeric pigments from anthocyanins reacting with yeast metabolites, tannins and flavanols is favored (Chapter 2.2.1.2.6) (Adams *et al.*, 2004; Waterhouse *et al.*, 2016). Higher contents of polymeric pigments due to extended maceration are often related to increased color stability (Puertas *et al.*, 2008). As a less favorable effect, post-fermentation oxygen exposure can also increase the content of acetic acid due to the oxidative metabolism of *Acetobacter pasteurianus* and *Acetobacter aceti* (Casassa *et al.*, 2013a; Drysdale and Fleet, 1988).

Extended maceration is known to enhance seed extraction (Casassa *et al.*, 2013a; del Llaudy *et al.*, 2008; Gil *et al.*, 2012) due to the higher ethanol concentration in the must (Casassa and Harbertson, 2016). González-Manzano *et al.* (2004) showed in a model wine system that after three weeks of maceration seed tannins contributed 90 % of the total wine tannins. In contrast to seed-associated phenolics, monomeric and oligomeric tannins from skins get extracted early during maceration (Koyama *et al.*, 2007). Overall, extending maceration time by 20 days can enhance the tannin extraction by up to 80 % depending on the ethanol concentration (Casassa *et al.*, 2013a).

## **2.1.1 Chemical composition of red wine**

### **2.2.1.1 Wine constituents**

Besides water, ethanol is a major ingredient in wine. According to European law, wines are allowed to contain up to 15 % vol alcohol, with a few local exceptions, where up to 20 % vol alcohol is accepted (European Union). An alcohol content of 16 % vol is commonly considered the maximum that yeast can survive, however, there are specific strains on the market that claim to survive up to 25 % vol (Ribéreau-Gayon *et al.*, 2006; Tarziu, 2023). Organic acids represent another important part of the wine composition. They contribute to microbiological and physicochemical wine stability as well as organoleptic properties. Tartaric acid, malic acid and citric acid are the three major organic acids in grapes and therefore also in wine. During alcoholic and malolactic fermentation other organic acids such as pyruvic acid, lactic acid,

succinic acid, acetic acid, fumaric acid, oxaloacetic acid and citramalic acid are produced (Ribéreau-Gayon *et al.*, 2006). Acetic acid is the main contributor to volatile acidity, which is considered negative for wine quality if the concentration is too high.

During fermentation other volatile compounds are produced such as higher alcohols and their esters. They can reach concentrations in a range from 150 – 550 mg/L and express intense odors which contribute to the wine aroma. Traces of fatty acids can also be found among the volatile components in wine (Ribéreau-Gayon and Peynaud, 1982).

In dry red wines, there are only small amounts of residual sugars present in the wine. However, sugar derivatives such as glycosides can be found in the presence of anthocyanins or aroma precursors (Ribéreau-Gayon *et al.*, 2006). Pectic substances contribute to a smooth and soft mouthfeel and a full-bodied character in wine as they interact with tannins (Ribéreau-Gayon *et al.*, 2006). Glycerol is present in small quantities (0.5 – 1.5 % w/w) and has a slight contribution to the sweetness perception in wine (Waterhouse *et al.*, 2016). Minerals such as potassium, calcium, iron, copper, magnesium and manganese contribute to the salification of organic acids and therefore the acidobasic buffer capacity of wine (Ribéreau-Gayon *et al.*, 2006; Waterhouse *et al.*, 2016). Similar to grapes, amino acids are the most prevalent form of total nitrogen by weight in wine. After completion of alcoholic fermentation the relative proportion of proline in wine is higher than in grape juice, because yeasts cannot assimilate proline in the absence of oxygen (Ribéreau-Gayon *et al.*, 2006).

In red wine, polyphenols are major contributors to the wine composition and the organoleptic properties. They are individually presented in the following chapter.

### 2.2.1.2 Polyphenols

#### 2.2.1.2.1 Flavonoids

Phenol (C<sub>6</sub>-OH skeleton) is the basic building block of polyphenols and therefore the namesake. Polyphenolic structures are characterized by an aromatic nuclei and at least one hydroxyl group (Fulcrand *et al.*, 2006). The largest group of polyphenols with about 6500 known structures are flavonoids (C<sub>6</sub>-C<sub>3</sub>-C<sub>6</sub> skeleton). Their structure is characterized by two aromatic rings (A and B) which are connected by an oxygenated heterocycle (C) (Figure 2) (Fulcrand *et al.*, 2006; Manach *et al.*, 2004).

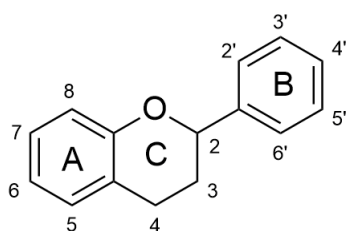


Figure 2 Generalized flavonoid structure (Manach *et al.*, 2004).

Depending on the oxidation state of the pyran ring (C), flavonoids can be classified into several subclasses: flavanols, proanthocyanidins, flavan-3,4-diols, anthocyanidins, flavonols, flavons, flavanonols and flavanons (Waterhouse *et al.*, 2016). Wine flavonoids are commonly present in their glycosylated form. The position of glycosylation is usually C-3 or the hydroxyl groups in position C-5 or C-7 (Manach *et al.*, 2004; Ribéreau-Gayon *et al.*, 2006; Watzl and Rechkemmer, 2001). Flavanols, proanthocyanidins and anthocyanidins will be explained in the following subchapters.

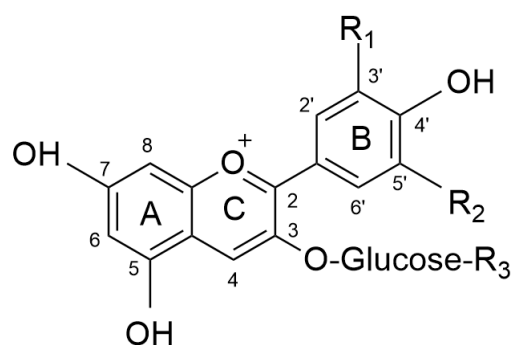
#### **2.2.1.2.2 Anthocyanins**

Anthocyanins are the most visible group amongst polyphenols in a variety of fruits and flowers. They provide colors in a range from red to purple and blue. These colors help the plants to attract pollinators or seed dispersers (Burns *et al.*, 2002). In fruit, the commonly water-soluble pigments can be found mainly in the external layer of the skin. Anthocyanins are located in anthocyanoplasts within the vacuoles of plant cells (Paredes-López *et al.*, 2010). Fruits accumulate anthocyanins in different amounts in their epicarp (skin) and mesocarp (flesh). There are three types of fruits: those that accumulate anthocyanins in their skin only, others contain them in skin and flesh, and some have anthocyanins only in their skin as a response to a light stimulus. Red grape varieties cover all three of these groups (Jaakola, 2013).

The fully unsaturated structure of anthocyanins allows aids to protect photosynthetic tissues by the reduction of light stress due to light absorbance in the visible range (Clifford, 2000; Jimenez-Garcia *et al.*, 2013; Paredes-López *et al.*, 2010; Zhang *et al.*, 2014). Anthocyanins also contribute to the protection of plants against pathogen attacks and cold temperatures. The genes of the anthocyanin biosynthesis pathway can be considered cold-regulation genes (Christie *et al.*, 1994). The accumulation of anthocyanins can be a response to water deficit stress or nitrogen deficiency. Due to their antioxidant capacity anthocyanins and other phenolic compounds are said to be beneficial to human health (Petroni and Tonelli, 2011; Soubeyrand *et al.*, 2014).

Anthocyanins comprise anthocyanidins (aglycones) and their glycosides. Figure 3 shows the structures of the six common anthocyanidins: delphinidin, cyanidin, malvidin, petunidin, peonidin, and pelargonidin. Cyanidin is the most common anthocyanidin in fruit (Jimenez-Garcia *et al.*, 2013; Wrolstad *et al.*, 2005). In red *Vitis vinifera* varieties, however, malvidin is the major anthocyanidin. Pelargonidin is usually not considered an anthocyanidin found in *Vitis vinifera*, but He *et al.* (2010) reported traces of pelargonidin in Cabernet Sauvignon and Pinot noir skin. Non-*vinifera* varieties can also contain 3,5-O-diglucosides of these anthocyanidins as well as pelargonidin (Wang *et al.*, 2003). Each grape variety has its own specific

anthocyanin fingerprint which can be used as a proof of authenticity for wine in terms of origin of the product or adulteration of the grape variety (Clifford, 2000).



Anthocyanidins	R <sub>1</sub>	R <sub>2</sub>
Pelargonidin	-H	-H
Cyanidin	-OH	-H
Peonidin	-OCH <sub>3</sub>	-H
Delphinidin	-OH	-OH
Petunidin	-OCH <sub>3</sub>	-OH
Malvidin	-OCH <sub>3</sub>	-OCH <sub>3</sub>

R<sub>3</sub> = -Acetyl, -*p*-Coumaroyl, -Caffeoyl

Figure 3 Structure of anthocyanidin-3-O-glucosides and the substitution patterns for the six anthocyanins found in nature (Adams, 2006; Wrolstad *et al.*, 2005).

The six anthocyanidins shown in Figure 3 are the basis component of more than 540 anthocyanin pigments which have been identified in nature. Most of their structural variation is a result from the type, number and position of the glycosidic substitution and the possible type and number of aliphatic or aromatic acids attached to the sugar residues (Jimenez-Garcia *et al.*, 2013; Wrolstad *et al.*, 2005). The most common glycosides are glucose, galactose, rhamnose, and arabinose which can be present as mono-, di- or triglycosides. Substitution usually takes place at C-3, but less frequently in the C-5 or C-7 position (Clifford, 2000; Jimenez-Garcia *et al.*, 2013). Glycosylation increases the stability of anthocyanins as their aglycones are very unstable (Jaakola, 2013; Zhang *et al.*, 2014). Anthocyanins can be acylated in position C-4 of the sugar (R<sub>3</sub>, Figure 3) on position C-3 of the anthocyanidin. Acetic acid and *p*-coumaric acid have been identified as the major acylating acids in anthocyanidin-3-monoglucosides of grapes (Anderson *et al.*, 1970; Markakis, 1982).

Anthocyanins are key compounds in red wine making, because they provide information about the degree of grape maturity, but the color intensity and hue are also considered quality indicators (Bindon *et al.*, 2014a). Phenolic compounds, including anthocyanins, are prone to oxidation which can be catalyzed by exposure to light, oxygen, metal ions, enzymes, sugars or changes in pH. In order to reduce unwanted oxidation during grape processing, winemaking and storage, these factors need to be taken into consideration (Patras *et al.*, 2010; Rhim, 2002).

### 2.2.1.2.3 Flavanols

High concentrations of monomeric flavan-3-ols can be found in grape seeds, but also to a lesser extent in skins, pedicels as well as traces in the mesocarp (Guerrero *et al.*, 2009). Flavan-3-ols and anthocyanidins differ from other flavonoids by the lack of an oxygen group in position C-4 of the heterocyclic C-ring. Two centers of asymmetry at C-2 and C-3 are created due to the lack of a double bond in the heterocycle and the presence of a hydroxyl group in position C-3. In nature, only flavanols with a 2R configuration have been reported. The second center of asymmetry determines whether the isomer is in *cis*- or *trans*-configuration. Isomers with a *cis*-configuration receive the prefix “epi-” (epimer; 3R) e.g. epicatechin. Free forms of flavan-3-ols or esterified with gallic acid (-3-O-gallate) occur naturally in fruit (Hollman and Arts, 2000; Ribéreau-Gayon *et al.*, 2006).

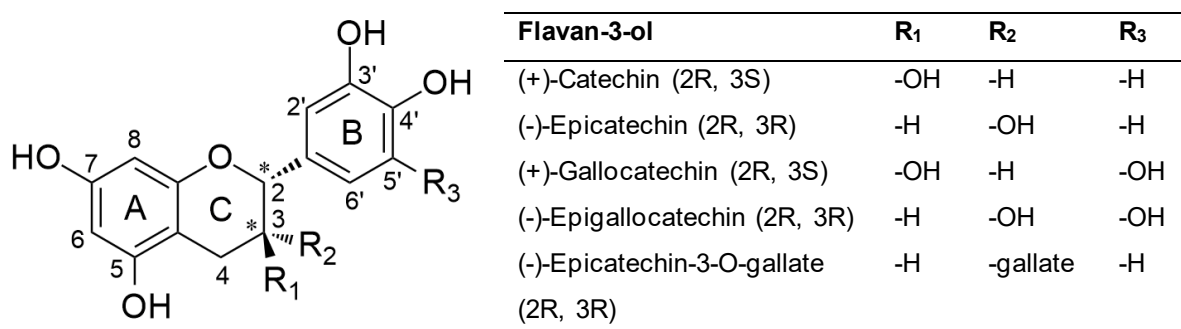


Figure 4 Generalized structure of flavan-3-ols and examples (Hollman and Arts, 2000).

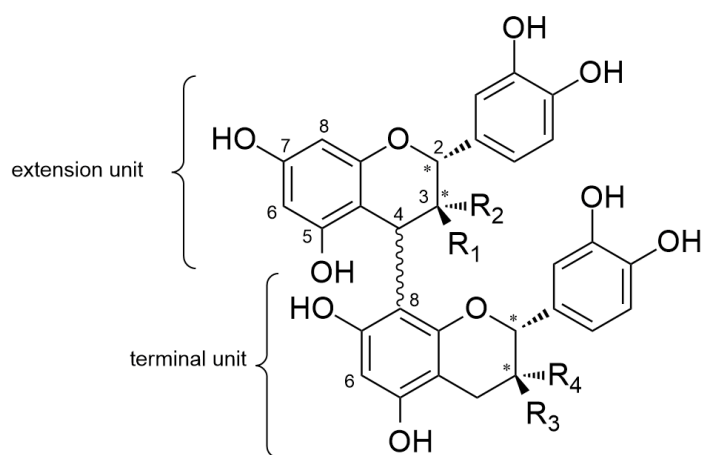
Figure 4 shows the most common flavan-3-ols in grapes: (+)-catechin, (-)-epicatechin, (+)-gallocatechin, (-)-epigallocatechin and (-)-epicatechin-3-O-gallate (Hanlin *et al.*, 2011).

### 2.2.1.2.4 Proanthocyanidins

The term condensed tannins or proanthocyanidins is used for oligomeric and polymeric flavonoids composed of subunits analogous to flavan-3-ols. The flavan-3-ol subunits are linked via interflavan bonds between C-4 and C-8 or less commonly between C-4 and C-6 (Hanlin *et al.*, 2011; Kennedy and Jones, 2001; Liang *et al.*, 2012). In contrast to condensed tannins hydrolysable tannins contain gallic acid (gallotannin) and ellagic acid (ellagitannin) subunits (Macáková *et al.*, 2014) and are easily hydrolysable either enzymatically or in acidic or basic conditions (Puech *et al.*, 1999). Hydrolysable tannins are introduced into the wine during barrel ageing, hence they are not derived from the grapes (Puech *et al.*, 1999). In wine research the terms (condensed) tannins and proanthocyanidins are often used synonymously.

The hydroxylation pattern of flavan-3-ols (Figure 4) creates two nucleophilic sites due to positions C-6 and C-8 at the A ring being partially negatively polarized. The formation of interflavan bonds linking C-4 and C-6 or C-8 of the flavonoids is possible. Dimeric

proanthocyanidins containing one of the described interflavan linkages are referred to as B-type proanthocyanidins (Cheynier *et al.*, 2006; Freitas and Mateus, 2001). Proanthocyanidins which consist of catechin and epicatechin subunits are called procyanidins. The most frequently reported B-type procyanidins are shown in Figure 5. With the number of monomeric subunits, the number of possible isomers increases exponentially. This means that for B-type procyanidin dimers there are eight different isomers which have been identified, while B-type procyanidin trimers already have 32 different isomers that are possible (Esatbeyoglu, 2011). Type-A procyanidins differentiate from type-B by an additional ether bond between the C5 or C-7 carbons of the terminal unit and the C-2 carbon of the upper unit (Ribéreau-Gayon *et al.*, 2006).



Procyanidin	R <sub>1</sub>	R <sub>2</sub>	R <sub>3</sub>	R <sub>4</sub>	
<b>B<sub>1</sub></b>	-H	-OH	-OH	-H	EC (4β→8)-Cat
<b>B<sub>2</sub></b>	-H	-OH	-OH	-H	EC (4β→8)-EC
<b>B<sub>3</sub></b>	-OH	-H	-OH	-H	Cat (4α→8)-Cat
<b>B<sub>4</sub></b>	-OH	-H	-H	-OH	Cat (4α→8)-EC
<b>B<sub>5</sub></b>	-H	-OH	-H	-OH	EC 4β→6)-EC
<b>B<sub>6</sub></b>	-OH	-H	-OH	-H	Cat (4α→6)-Cat
<b>B<sub>7</sub></b>	-H	-OH	-OH	-H	EC (4α→6)-Cat
<b>B<sub>8</sub></b>	-OH	-H	-H	-OH	Cat (4α→6)-EC

Figure 5 Generalized structure of type-B procyanidins with examples (Freitas and Mateus, 2001).

Trimeric procyanidins are divided into two categories: type-C, which are comprised of three subunits with two interflavan bonds corresponding to those of type-B dimers and type-D, which are trimers with two interflavan bonds of which one is type-A and one is type-B. The nomenclature of procyanidins can also be applied to prodelphinidins which are another group of proanthocyanidins containing additional gallo catechin and epigallocatechin subunits commonly as extension units (Ribéreau-Gayon *et al.*, 2006).

The composition of proanthocyanidins in grape skins and seeds differs in the mean degree of polymerization, degree of galloylation, and trihydroxylation at the B ring. Previous studies reported that the polymer length in skins is several-fold higher than in seeds (Downey *et al.*, 2003). The degree of galloylation provides information about the number of epicatechin-3-gallate subunits and studies have shown that seeds usually have a higher degree of galloylation than skins (Hixson *et al.*, 2015). In seeds the terminal as well as the extension subunits are catechin, epicatechin and epicatechin-3-gallate. In skins on the other hand catechin is the main terminal unit. In addition to epicatechin, catechin and epicatechin-3-gallate, epigallocatechin is another possible extension unit. Skin and seed proanthocyanidins can therefore be differentiated by the presence or absence of prodelfinidins. Due to the higher polarity of the prodelfinidins, skin tannins are expected to be more easily extracted during winemaking (Downey *et al.*, 2003; Souquet *et al.*, 1996).

#### 2.2.1.2.5 Phenolic acids

The second largest group of phenolic compounds are phenolic acids. Phenolic acids comprise the subclasses hydroxybenzoic acids and hydroxycinnamic acids. In nature, seven benzoic acids have been identified, of which five are common in wine. The generalized structure and the corresponding substituents of the five most common benzoic acids in wine are shown in Figure 6 (Rossouw and Marais, 2004).

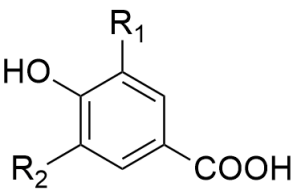
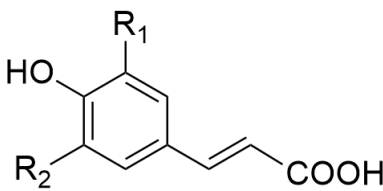
	<b>Hydroxybenzoic acid</b>	<b>R<sub>1</sub></b>	<b>R<sub>2</sub></b>
	<i>p</i> -Hydroxybenzoic acid	-H	-H
	Protocatechuic acid	-OH	-H
	Vanillic acid	-OCH <sub>3</sub>	-H
	Gallic acid	-OH	-OH
Syringic acid	-OCH <sub>3</sub>	-OCH <sub>3</sub>	
	<b>Hydroxycinnamic acid</b>	<b>R<sub>1</sub></b>	<b>R<sub>2</sub></b>
	<i>p</i> -Coumaric acid	-H	-H
	Caffeic acid	-OH	-H
	Ferulic acid	-OCH <sub>3</sub>	-H
	Sinapinic acid	-OCH <sub>3</sub>	-OCH <sub>3</sub>

Figure 6 Generalized structures of phenolic acids and examples (Rossouw and Marais, 2004).

Salicylic acid and gentisic acid are less common in wine and have only been detected in trace amounts. Phenolic acids are differentiated by the substitution of their benzene ring (Rossouw and Marais, 2004). Benzoic acids are mainly present in their glycosylated or esterified form,

but free forms can be released by acid hydrolysis or alkaline hydrolysis, respectively. Their free forms are more prevalent in red wine (Ribéreau-Gayon *et al.*, 2006).

Several cinnamic acids have been identified in grapes, however, caffeic acid and ferulic acid are the most common. The generalized structure of hydroxycinnamic acids is shown in Figure 6. In contrast to hydroxybenzoic acids, hydroxycinnamic acids are rarely found in their free form. Most hydroxycinnamic acids are esterified with tartaric acid, but they can also be simple glycosides of glucose. Phenolic acids are colorless in dilute alcohol solutions such as wine, however, when exposed to oxygen, they eventually turn yellow due to oxidation (Paredes-López *et al.*, 2010; Ribéreau-Gayon *et al.*, 2006).

#### **2.2.1.2.6 Polymeric Pigments**

In grape berries and young wine, monomeric anthocyanins (Chapter 2.2.1.2.2) are the main contributors to their visual appearance. Somers (1966) demonstrated that during fermentation and wine ageing the initial concentration of monomeric anthocyanins in wine decreases steadily without major changes in color characteristics, indicating the existence of stable polymeric pigments related to anthocyanins. Somers (1968) suggested that the profile of polymeric and monomeric pigments could be used to determine the “chemical age” of a wine and that the formation of polymeric pigments is similar to the formation of tannins during wine ageing. Their polymeric character which is due to reactions of anthocyanins with monomeric and polymeric flavanols is the reason why they are called polymeric pigments (Somers, 1971). Somers (1971) showed that in aged dry red wines polymeric pigments are responsible for the major portion of color, ranging from 50 % (one year old wine) to 85 % (10 years old wine). In young wines, color intensity and hue are largely due to the monomeric anthocyanins extracted from the grapes, whereas with progressing wine ageing, the influence of more stable polymeric pigments increases. Polymeric pigments are less affected by bisulfite bleaching and provide color stability at higher pH than monomeric anthocyanins (Somers, 1971).

In their hydrated form anthocyanins act as nucleophiles which can compete with flavanols in tannin polymerization reactions (Chapter 2.2.1.2.4). Like monomeric flavan-3-ols, anthocyanins can either react with other monomeric flavan-3-ols or with oligomeric flavanols, allowing mainly two possible types of adducts: flavanol-anthocyanin (F-A<sup>+</sup>) and anthocyanin-flavanol (A-F<sup>+</sup>) adducts (Fulcrand *et al.*, 2006; Salas *et al.*, 2003). The formation of F-A<sup>+</sup> requires acid-catalyzed cleavage of interflavan bonds in proanthocyanidins, releasing an intermediate carbocation F<sup>+</sup> which acts as an electrophile (Haslam, 1980). The hydrated hemiketal form of anthocyanins (AOH; carbinol pseudo base, Figure 7) acts as a nucleophile, thus yielding the colorless dimer F-AOH, which then dehydrates to its red flavylium form (F-A<sup>+</sup>) (Salas *et al.*, 2003). Since the cleavage of proanthocyanidin interflavan bonds requires low pH,

the formation of these adducts is less likely at red wine pH, however, they can still be observed in small concentrations (Salas *et al.*, 2003). In the formation of A-F<sup>+</sup>, the anthocyanin is in its red flavylum form (A<sup>+</sup>, Figure 7), acting as an electrophile which reacts with a nucleophilic flavanol through the partially negatively charged carbons C-8/C-6 of the A ring (Fulcrand *et al.*, 2006; Salas *et al.*, 2003). A colorless flavene intermediate (A-F) is formed, which can either be oxidized into the red flavylum form (A-F<sup>+</sup>) and then dehydrated to yield the xanthylium cation or proceed to a colorless cyclic condensation product with a bicyclic A-type bond (Fulcrand *et al.*, 2006; Jurd, 1969; Remy-Tanneau *et al.*, 2003; Salas *et al.*, 2003; Somers, 1971).

At red wine pH, the hemiketal (carbinol pseudo base) form as well as the flavylum cation form of anthocyanins are present, allowing them to react with each other, because they can both act as electrophile and nucleophile. These reactions yield oligomeric anthocyanin-anthocyanin (A<sup>+</sup>-AOH) adducts (Bindon *et al.*, 2014b; Salas *et al.*, 2003).

In wine, yeast metabolites such as acetaldehyde are released during fermentation. However, acetaldehyde formation from ethanol can also be a result of oxidation during fermentation and wine ageing (Waterhouse *et al.*, 2016). Acetaldehyde is known to accelerate the polymerization of anthocyanins and flavanols, thus influencing red wine pigment composition and color characteristics (Cheynier *et al.*, 2006; Waterhouse *et al.*, 2016). The formation of ethyl-bridged tannin-anthocyanin linkages induced by acetaldehyde shifts the wine color from purple-red to brick red (Atanasova *et al.*, 2002; Francia-Aricha *et al.*, 1997; Nave *et al.*, 2010; Sheridan and Elias, 2016). At red wine pH, acetaldehyde can also induce the formation of methyl methine-linked anthocyanin-flavanol as well as anthocyanin-anthocyanin pigments which can further polymerize with an unknown proportion of anthocyanin subunits. Methyl methine-linked polymeric pigments shift the wine color towards a deep purple hue (Atanasova *et al.*, 2002).

### 2.2.2 Polyphenol analysis in wine

The phenolic content and composition in wine can be studied using different spectrophotometric assays, such as the protein precipitation assay introduced by Harbertson *et al.* (2003) or the assay for total oxidizable phenolics using the Folin-Ciocalteu reagent “Folin-Ciocalteu assay” (Folin and Ciocalteu, 1927; Singleton, 1966; Singleton *et al.*, 1999). The protein precipitation assay allows the quantification of iron reactive phenolics, tannins, protein-precipitable polymeric pigments as well as non-protein-precipitable polymeric pigments (Harbertson *et al.*, 2002; Harbertson *et al.*, 2003; Harbertson *et al.*, 2015). The Folin-Ciocalteu assay estimates the content of oxidizable phenolics by reaction of the hydroxyl groups with a phosphomolybdate reagent (Folin and Ciocalteu, 1927; Singleton, 1966; Singleton *et al.*, 1999). A common alternative to the protein-precipitation assay for tannin analysis is the

methylcellulose-precipitable (MCP) tannin assay by Sarneckis *et al.* (2006), which uses the polysaccharide polymer methylcellulose to precipitate tannin instead of the protein bovine serum albumin V (Harbertson *et al.*, 2003).

Monomeric and dimeric phenolic compounds can be identified and quantified using reversed-phase high-performance liquid chromatography (RP-HPLC). The compounds are separated by polarity with gradients from an aqueous solvent to an organic solvent, generally at a low pH (Beer *et al.*, 2004). There are two types of column material that are used as stationary phases: reversed-phase silica (C<sub>18</sub>) (Lamuella-Raventós and Waterhouse, 1994) and polystyrene divinylbenzene (Peng *et al.*, 2002; Price *et al.*, 1995). Polymeric phenolics up to heptamers can be quantified using normal-phase HPLC (NP-HPLC) (Hammerstone *et al.*, 1999).

Oligomeric and polymeric proanthocyanidins can also be analyzed using a liquid chromatography mass spectrometry system equipped with an electrospray ionization source (ESI) operated in the negative-ion mode and a quadrupole mass analyzer (Cheynier *et al.*, 1997a; Fulcrand *et al.*, 1999).

The mean degree of polymerization can be determined by phloroglucinolysis or thioacidolysis. In addition, phloroglucinolysis allows to identify the percentage of galloylation and average molecular weight of proanthocyanidins. The method was established by Kennedy and Jones (2001), who showed that phloroglucinolysis is as reliable as thioacidolysis using benzyl mercaptan (Lapierre *et al.*, 1986). Additionally, phloroglucinolysis has a higher selectivity in the formation of 3,4-*trans* adducts from 2,3-*trans* flavan-3-ol extension units than thioacidolysis (Kennedy and Jones, 2001; Obreque-Sliver *et al.*, 2012).

## **2.3 Sensory**

### **2.3.1 Sensory characteristics of polyphenols**

Polyphenols contribute significantly to the sensory characteristics of red wine. Color intensity and hue are commonly the first visual attributes that are evaluated during a tasting. Anthocyanins and polymeric pigments are the main contributors to red wine color. In young red wines, the anthocyanin composition is responsible for the hue. The hydroxylation and methylation of the aglycones on the B-ring determine the color range. An increasing hydroxylation causes an increase in blue, whereas increasing methylation results in a red hue (He *et al.*, 2012; Jimenez-Garcia *et al.*, 2013; Wrolstad *et al.*, 2005). Table 1 shows the color ranges of the six anthocyanidins found in nature.



When sulfur dioxide is added to red wine for stabilization, it reacts with monomeric anthocyanins to form a colorless sulfonate adduct. However, it is an equilibrium reaction and therefore the color intensity depends on the relative concentrations of the reactants (Harrison, 2017).

Copigmentation describes the color-stabilizing interactions and reaction of anthocyanins with other usually non-colored wine phenolics (copigmentation cofactors, “copigments”) such as flavanols (Chapter 2.2.1.2.3), hydroxybenzoic and hydroxycinnamic acids (Chapter 2.2.1.2.5). Due to copigmentation anthocyanins can exhibit more intense color than what would be expected from their concentration. This phenomenon is known as hyperchromic shift (Boulton, 2001). Certain copigmentation cofactors can also lead to a bathochromic shift in the wavelength of maximum absorbance (e.g. 5-20 nm higher), hence shifting the hue from a red solution towards blue-purple (Boulton, 2001).

Association of anthocyanins and copigments occurs by vertical stacking of the aromatic rings due their planar shape, hydrophobicity and  $\pi$ - $\pi$  interactions of the delocalized electrons of the aromatic rings, which reduces the discoloration of anthocyanins by preventing the formation of the colorless carbinol pseudo base (Figure 7) (Fulcrand *et al.*, 2006). During fermentation and wine ageing anthocyanins can react with hydroxycinnamic acids and pyruvic acid, a product from yeast metabolism, to form a second pyran ring between C-4 and the C-5 hydroxyl group, thus creating pyranoanthocyanins. Similar to polymeric pigments (Chapter 2.2.1.2.6) they are more stable against changes in pH and bisulfite bleaching (Cheynier *et al.*, 2006; Fulcrand *et al.*, 2006).

The polymerization of anthocyanins during fermentation and wine ageing is very important for the color stability of red wines. However, previous studies suggest that polymeric pigments in red wine also contribute to a smoother mouthfeel. A possible explanation for this phenomenon could be that polymeric pigments consisting of anthocyanins and tannins are more polar than condensed proanthocyanidins (Gawel, 1998). A study by McRae and Kennedy (2011) suggests that more polar polymers exhibit a lower astringency than non-polar proanthocyanidins. Astringency describes the perception of dryness after red wine consumption which occurs due to precipitation of salivary proteins induced by polyphenols. The precipitation of salivary proteins reduces the natural lubrication properties in the mouth which can feel dry, but also smooth or rough depending on the phenolic composition (Noble, 1998). Noble (2002) reported that the astringency perception of flavanols increases with the degree of polymerization, whereas bitterness decreases with chain length. Monomeric flavan-3-ols such as catechin and epicatechin are therefore more bitter while condensed proanthocyanidins are more astringent (Noble, 2002; Peleg *et al.*, 1999). However, Vidal *et al.* (2003) showed that the degree of galloylation influences the astringency and bitterness perception of tannins. Skin-derived tannins with a lower degree of galloylation were perceived

less astringent than seed-derived tannins (Brossaud *et al.*, 2001; Vidal *et al.*, 2003). Galloylation can increase the interaction between tannins and salivary proteins, thus increasing astringency perception (Cheynier *et al.*, 1997b).

Bitterness perception in wine can primarily be attributed to monomeric flavan-3-ols and ethanol. Due to their chiral difference (-)-epicatechin is perceived to be significantly more bitter than (+)-catechin and the sensation lasted significantly longer. Ethanol content was shown to significantly enhance bitterness intensity and duration (Noble, 1994).

## **2.3.2 Factors influencing sensory characteristics**

### **2.3.2.1 Grape maturity**

Sensory characteristics of wine depend on several factors; however, grape maturity is usually considered the most important factor. The influence of grape maturity on grape and wine composition is discussed in chapters 2.1.2 and 2.2.2.1, respectively. Sensory active grape metabolites accumulate or decline during ripening (Sherman *et al.*, 2017). Previous studies reported significant differences in aroma, taste and tactile characteristics in wine due to differences in grape maturity. In Moristell wine fruity aromas such as red or black fruit increased with progressing grape maturity, whereas astringency tended to decrease (Ferrero-del-Teso *et al.*, 2020). Trujillo *et al.* (2022) reported a decrease in red and fresh fruit aroma as well as overall aroma in wine produced from overripe Merlot grapes compared to “normal” mature grapes. This trend was also observed for Tempranillo wine, however, the differences were not significant (Trujillo *et al.*, 2022). Casassa *et al.* (2013b) showed that late harvested Merlot grapes improved sensory characteristics by increasing sweetness perception, fruit-derived aromas as well as the viscosity of the wine. Wines from early harvested grapes on the other hand received higher ratings in fresh vegetal aroma, acidity, and had lower color intensity (Casassa *et al.*, 2013b). These observations are in agreement with the findings of Bindon *et al.* (2014a) for Cabernet Sauvignon wine. Wines produced from earlier harvests were rated higher in red color, fresh green as well as red fruit aroma and palate, but lower in overall fruit, dark fruit, fruit aftertaste, vanilla, purple color, viscosity and bitterness. With progressing grape maturity wines received higher ratings in dark fruit and overall fruit aroma and palate, hotness, pungent, opacity, bitterness and earthy attributes. This study shows that overripe berries increase sensory attributes associated with high alcohol such as bitterness, hotness or pungent (Bindon *et al.*, 2014a). Sensory characteristics such as dark fruit, overall fruit flavor, vanilla and viscosity, which increased with progressing grape maturity were positively related to overall liking in a consumer preference study. Wines with higher ratings of acidity, red fruit and fresh green characteristics due to earlier harvested grapes were not well accepted (Bindon *et al.*, 2014a).

### 2.3.2.2 Maceration Time

Winemakers use maceration time as a tool to modulate the extraction of phenolics (Chapter 2.2.2.2), which influences wine composition and style. Frost *et al.* (2018) reported higher astringency ratings with increasing maceration time in Merlot wines. Extending maceration time increased the perception of red fruit aroma, whereas Merlot wines without maceration received significantly higher ratings for pepper spice aroma (Frost *et al.*, 2018). Casassa *et al.* (2013a) observed similar trends for astringency in extended maceration (30-day skin contact) Merlot wine, which were rated approximately 30 % more astringent than the control (10-day skin contact). These observations were related to higher concentrations of seed-associated tannins. Extended maceration wines were also perceived to have a more pronounced brown hue, which was explained by oxidative polymerization of anthocyanins with other phenols, producing polymeric pigments. Oxidative processes in extended maceration wines were reflected in higher polymeric pigments as well as higher acetic acid concentrations (Casassa *et al.*, 2013a). A higher oxidized character was also observed in extended maceration Cabernet Sauvignon wine. Extending maceration time reduced purple hue, red fruit and dark fruit aroma, and increased ratings for bitterness, astringency and red-brown hue (Casassa *et al.*, 2013c). Extending maceration time increases the total polysaccharide concentration in wine (Gil *et al.*, 2015). Polysaccharides were shown to contribute to the sensation of “fullness” in wine, while decreasing astringency, dryness, and roughness (Vidal *et al.*, 2004). The extent to which astringency is perceived in wine appears to be influenced by various factors, making it difficult to predict.

## 2.4 Statistical evaluation

In wine research, scientists encounter a large number of chemical and sensory parameters to discuss their experimental hypothesis. The first step to evaluate whether there are any significant differences between the experimental treatment is often an analysis of variance (ANOVA). ANOVA is a widely used statistical method for hypothesis testing which has the flexibility to cover a large number of experimental designs (Stähle and Wold, 1989). When using ANOVA, the effect of various factors (e.g. harvest date, grape variety, vintage, maceration time, etc.) on some response (e.g. anthocyanin concentration, color intensity, potential alcohol, etc.) is assessed. The factors can be divided into two distinct types of effects: random effects and fixed effects (Stähle and Wold, 1989). In sensory studies, panelists are considered a random effect, because they represent a random sample of the population and can therefore show a high variability. A random effect is used to reduce the interindividual variability. For a trained sensory panel, the tasting repetitions of each panelist have a much smaller variance and can hence be considered a fixed effect. Other examples of fixed effects

in the following studies are harvest date, berry size, maceration time or potential alcohol. It can be assumed that the experimenter has complete control over the properties of the factors, meaning that e.g. extended maceration will always have the same effect on the extraction of phenolics and that this effect will always differ in the same way (when under the same conditions) from the effect of e.g. harvest date (Stähle and Wold, 1989).

When studying factor effects, they can be divided into nested and crossed designs. A nested design is used when one level of the experimental design is subordinate to the next level, e.g. analytical or experimental replicates. These replicates are subordinate to the experimental treatment. A crossed design is used when the combination of two (or more) factors also has a specific meaning. For example, three harvest dates (H1, H2 and H3) can be combined with maceration time (7d and 14d) resulting in the combinations H1\_7d, H1\_14d, H2\_7d, H2\_14d, H3\_7d and H3\_14d. All combinations are tested with maceration time being treated on the same level as harvest date, because each combination has a specific meaning or effect on the wine (Stähle and Wold, 1989).

After identifying significant differences and trends of the treatments or influencing factors, individual variables can be presented as bar charts for example. For large data sets individual presentation of all variables is not always helpful and it cannot visualize crossed effects. One option to explain and visualize the variation of a data set is using a principal component analysis (PCA). A PCA reduces the cases-by-variables data table to its essential features which are called principal components (PC) (Greenacre *et al.*, 2022). Principal components reduce the original variables to a few linear combinations of them. These new combinations allow to maximally explain the variance of all the variables (Greenacre *et al.*, 2022). It is then possible to identify the strongest cause for variance in the data set. Principal component 1 accounts for the highest variance, followed by PC2 and so on. A PCA can be presented as two separate plots, one for the variables, e.g. sensory descriptors (loading plot) and one for the observations, e.g. experimental wines (score plot) or as a biplot which combines variables and observations. However, when discussing a PCA, it is important to keep in mind that the plot only shows two dimensions of a multi-dimensional data set. Relations between variables or samples (e.g. clustering) might not be visible when plotting just the first two principal components if the influence causing the relation is weaker than the first two PCs. However, PCA can be used to detect outliers, identify trends or patterns, or generate new hypotheses and are therefore a great tool to discuss multivariate data sets (Bro and Smilde, 2014).

Data from a PCA can be further used for a multiple factor analysis (MFA). MFA is often used in sensory evaluation when several sets of variables have been measured on the same set of observations (Abdi *et al.*, 2013). While PCA uses a single data table, MFA works with multiple data tables that either measure sets of variables collected on the same observations or the same variables are measured on different sets of observations (Abdi *et al.*, 2013).

A different approach that is often used for multiple data sets is a partial least squares (PLS) regression. PLS regression is a method for relating two data matrices by a linear multivariate model in order to predict a set of dependent variables from a set of independent variables or predictors (Abdi, 2010; Wold *et al.*, 2001). A PCA creates new principal components which are best to explain data matrix X, whereas a PLS regression finds components from this matrix X that best predict a second matrix Y (Abdi, 2010). In wine research this method could be applied to relate and explain sensory data (Y) with chemical data (X).

When sensory profiling data is presented in a PCA, usually the mean scores of panelists and tasting repetitions are used for each wine. This method does not take the interindividual variability of the panelists into account. A canonical variate analysis (CVA) is an alternative to PCA that extends on the univariate approach by maximizing product discrimination (Peltier *et al.*, 2015).

### **3 Aims of the thesis**

The grape ripening of two red grape varieties – Pinot noir and Cabernet Sauvignon – of international importance is investigated in the European winegrowing zone A (cool climate). It is shown how progressing grape maturity, viticultural strategies and winemaking techniques influence the phenolic composition and sensory characteristics of these red wines.

The effects of global warming and changing climate on viticulture, in detail higher GDD and changing ripening kinetics, challenge winemakers more than ever to determine optimal grape maturity and plan harvest accordingly. Traditional grape maturity indicators such as TSS or the ratio of sugar-to-acid may no longer be appropriate due to the decoupling of ripening kinetics of grape constituents where sugar accumulation might occur ahead of other quality-associated compounds. For red grape varieties, phenolic maturity might provide more accurate information on the grape maturity perceived in wine because the content and extractability of phenolics from grapes are taken into consideration when determining the time of harvest. Polyphenols such as anthocyanins and tannins are the main contributors to the typical red wine quality characteristics including color intensity, color stability, and astringency. It is therefore necessary to improve the understanding of the concept of phenolic maturity as well as to identify indicators for phenolic maturity in wine. These indicators can then be used in addition to the traditional parameters to determine optimal grape maturity. Suitable indicators for phenolic maturity in wine should be independent from potential alcohol and winemaking techniques such as extending maceration time. Sugar accumulates naturally during grape ripening but can also be enhanced in grape must during winemaking to increase potential alcohol in wine. Winemakers use different strategies to enhance the sugar content before

fermentation such as letting ripe berries dry and shrivel with continuous air flow which is a common procedure for Amarone wine production. Reverse osmosis and cryoextraction are other possible techniques to enhance the sugar content in grape must by the removal of water, whereas chaptalization requires the addition of sugar or concentrated grape juice to the grape must. During winemaking, potential alcohol acts as a solvent modulating the extraction of phenolic compounds. Alcohol is also known to contribute significantly to the sensory characteristics of red wine and is associated with sweetness and a full body but at high concentrations it can be perceived as bitter or hot and pungent. The influence of potential alcohol in grapes on phenolic and sensory characteristics of red wine is investigated and discussed in chapter 4. This study raises the question if potential alcohol is a suitable indicator for the phenolic composition in grapes.

Within a vineyard and within individual grape clusters, there are berries of varying size and maturity. The results from previous studies, however, are inconclusive regarding the question whether small berries are more mature and/or higher in quality than large berries. It is also not entirely clear if heterogeneity in berry size improves or lowers wine quality. The effect of berry size on the phenolic composition of Pinot noir wine is investigated and presented in chapter 5. Viticultural strategies such as defoliation aim to reduce ripening and berry size heterogeneity. The study presented in chapter 5 examines whether leaf removal and timing of harvest are suitable strategies to modulate the berry size distribution in the vineyard and if these measures lead to a higher phenolic maturity reflected in the wine composition.

The phenolic composition of red wine is influenced by maceration time. By extending the maceration time the proportion of phenolics, that are more readily extractable in the presence of ethanol, increases. Continuing maceration after completion of alcoholic fermentation also increases the oxygen exposure of young wine, thus favoring the formation of polymeric pigments and potentially improving color stability. The study in chapter 0 determines the extent to which different maceration times can modulate the phenolic composition and sensory characteristics of red wine made from grapes of different maturities. It also assesses whether extending maceration time can improve the sensory characteristics of wine produced from immature grape material.

In summary, the specific aims of this thesis (Chapters 4, 5 and 0) are as follows:

- Investigation of grape ripening in the European winegrowing zone A (cool climate) and how progressing grape maturity influences the phenolic composition and the sensory characteristics of Pinot noir and Cabernet Sauvignon wines (Chapters 4 and 0)
- Improve the understanding of the concept of phenolic maturity (Chapters 4, 5 and 0)

- Identification of indicators for phenolic maturity in wine independent from potential alcohol (Chapters 4, 5 and 0)
- Determination of the extent to which the potential alcohol acts as a solvent modulating the extraction of phenolic compounds during winemaking or if it is an indicator for the phenolic composition of grapes (Chapter 4)
- Investigation of the effect of berry size on the phenolic composition of Pinot noir wine (Chapter 5)
- Determination whether leaf removal leads to a higher uniformity in phenolic maturity reflected in wine produced from grapes of different harvest dates (Chapter 5)
- Assessing whether defoliation and/or timing of harvest are suitable to modulate the berry size distribution in the vineyard and phenolic composition of Pinot noir wine (Chapter 5)
- Determination of the extent to which different maceration times can modulate the phenolic composition and sensory characteristics of red wine made from grapes of different maturities (Chapter 0)
- Determination whether extending maceration time can improve the sensory characteristics of wine produced from immature grape material and if sensory effects can be explained by chemical data of phenols (Chapter 0)

## 4 Influence of Potential Alcohol in Grapes on Phenolic and Sensory Characteristics of Red Wine

Potential alcohol contributes significantly to the extraction of phenolics during red winemaking and affects the sensory characteristics of wine. Potential alcohol is related to the sugar accumulation in grape berries during ripening or can be enhanced by winemakers with various techniques, including chaptalization. However, 'grape maturity' cannot be simply limited to the sugar content in berries. The commonly used 'potential alcohol' only partially explains 'grape maturity' by indicating the presence of grape constituents with similar ripening kinetics, by supporting the extraction of grape constituents during red winemaking and by directly contributing to the sensory characteristics of a wine. Another portion of 'grape maturity' cannot be explained by this analytical parameter. This study allows new insights into the contribution of potential alcohol as well as the contribution of other grape constituents to a universal grape maturity model.

Progressing grape maturity changes the sensory characteristics of red wine, especially in color and phenol-related attributes of Pinot noir and Cabernet Sauvignon wines. Experimentally simulated grape maturity, namely the adjustment of potential alcohol and pH to late harvest conditions, did not achieve the sensory characteristics of a late harvest wine. In a climate change scenario with an earlier and faster sugar accumulation, decoupled from phenolic maturity, here simulated by the enhanced potential alcohol, wines might become imbalanced with a harsh mouthfeel and with the astringency characteristics and low color intensity of wines from unripe grapes while obtaining the body and sweetness perception of a late harvest wine. The analytical data confirmed that progressing grape maturity led to significant changes in the phenol profile. The effect of enhanced potential alcohol is different from the influence of naturally obtained potential alcohol by progressing grape maturity. The phenolic composition of wine made from immature grapes cannot be driven towards a phenol composition associated with a higher grape maturity by increasing the potential alcohol. In both grape varieties, a higher phenolic grape maturity shifted the phenolic profile from seed-associated to skin-associated phenolics. The adjustment of potential alcohol had only minor effects on seed- and skin-associated phenolics but positively affected the gallic acid concentrations and lowered the B-prodelphinidin concentrations in both grape varieties.

An experimental model to distinguish the influence of natural and simulated potential alcohol on individual sensory attributes revealed that natural and simulated potential alcohol had a similar effect on the herbaceous aroma in Pinot noir. However, simulated potential alcohol had no influence on the bell pepper aroma in Cabernet Sauvignon suggesting that the IBMP extraction is independent from alcohol. Natural and simulated potential alcohol had a similar contribution to the body of the wines irrespective of the grape variety. In Pinot noir, green

astringency perception was partially dependent on potential alcohol, suggesting that potential alcohol contributes to grape maturity by masking the unripe sensation. The experimental model also suggested that the alcohol itself is the main driver of bitterness in the wines, however, specific phenols – monomeric and oligomeric flavanols – due to varietal differences might also contribute to bitterness in relation with the alcohol.

The experimental model was further used to identify indicators for phenolic maturity. In Pinot noir, anthocyanins correlated well with natural potential alcohol and were unaffected by potential alcohol adjustment. In Cabernet Sauvignon, polymeric pigments provided the most accurate information about grape maturity perception in wine.

## **Influence of Potential Alcohol in Grapes on Phenolic and Sensory Characteristics of Red Wine**

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Disclaimer:

The presented work was performed by Weincampus Neustadt/DLR Rheinpfalz (Sandra Feifel and master thesis Erika Markusevics) and University of Bonn (Ingrid Weilack). For more information, refer to the author contribution section.

Author Contributions:

**Sandra Feifel:** Conceptualization, Methodology, Investigation, Winemaking, Chemical analyses, Sensory analysis, Writing – Original draft, Review and Editing. **Ingrid Weilack:** Conceptualization, Methodology, Investigation, Chemical analyses. **Erika Markusevics:** Methodology, Investigation, Chemical analyses, Sensory analysis. **Daniel Zimmermann:** Winemaking, Chemical analyses. **Pascal Wegmann-Herr:** Conceptualization, Winemaking, Writing - Review and Editing. **Fabian Weber:** Conceptualization, Writing - Review and Editing, Funding acquisition. **Elke Richling:** Supervision, Writing - Review and Editing. **Dominik Durner:** Conceptualization, Supervision, Writing – Original draft, Review and Editing, Funding acquisition.

All authors have read and agreed to the published version of the manuscript.

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Feifel, S., Weilack, I., Markusevics, E., Zimmermann, D., Wegmann-Herr, P., Weber, F., Richling, E., and Durner, D. (2024), "Influence of Potential Alcohol in Grapes on Phenolic and Sensory Characteristics of Red Wine", *Journal of agricultural and food chemistry*, Vol. 72 No. 22, pp. 12725–12737. <https://doi.org/10.1021/acs.jafc.4c01035>.

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# Influence of Potential Alcohol in Grapes on Phenolic and Sensory Characteristics of Red Wine

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Cite This: *J. Agric. Food Chem.* 2024, 72, 12725–12737

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**ABSTRACT:** Potential alcohol, as obtained by grape maturity, affects the extraction of phenolics during winemaking. The extent to which potential alcohol is correlated to phenolic and sensory characteristics of red wine was investigated. Decoupling of the ripening kinetics of grape constituents due to climate change emphasizes this question. The impact of potential alcohol, as naturally obtained by grape maturity or adjusted by sugar addition, representing high sugar but low phenolic maturity, on wine characteristics was investigated for two varieties over two vintages. Enhancement of potential alcohol to late harvest conditions did not achieve the sensory characteristics of wine made from phenolic mature grapes. An experimental model was developed revealing the contribution of potential alcohol to phenolic and sensory characteristics. In Pinot noir, anthocyanins correlated well with natural potential alcohol but were not influenced by enhanced potential alcohol. In Cabernet Sauvignon, polymeric pigments provided the most accurate information about grape maturity perception in wine.

**KEYWORDS:** red wine, potential alcohol, anthocyanins, tannins, polymeric pigments, sensory

## 1. INTRODUCTION

Increases in temperature over the past few decades are consequences of climate change which affect grape ripening and grape composition.<sup>1</sup> Higher temperatures trigger advanced phenology, shifting the ripening phase to warmer periods in the summer with earlier and faster sugar accumulation, increased sugar content, and lower acidity in the grapes.<sup>1–3</sup> Kliewer and Torres<sup>4</sup> and Spayd et al.<sup>5</sup> reported that anthocyanin content in red varieties is negatively affected by high temperatures. Sadras and Moran postulated decoupling of ripening kinetics of different grape constituents, which could eventually mean that the sugar accumulation occurs ahead of other compounds.<sup>6</sup> Accordingly, other concepts, besides sugar maturity, exist to describe grape maturity: physiological maturity, technological maturity, aroma maturity, and phenolic maturity.<sup>7</sup> Some of these concepts are better defined than others. The original definition of phenolic maturity was given by Glories considering it is optimal when the anthocyanin concentration in the skins reaches a maximum and seed tannin concentration has decreased from veraison.<sup>8</sup> Saint Cricq de Gaulejac et al. provided an extended definition of phenolic maturity, taking also the extractability of phenolic compounds and the quality of tannins perceived by sensory assessment into account.<sup>9</sup>

Sugars, often expressed as total soluble solids (Brix), and titratable acidity are most commonly used as indicators for grape maturity.<sup>10</sup> Recent research suggests that phenolic composition may additionally be used to monitor grape maturity but also to predict wine composition and style.<sup>11</sup> A previous study by Bindon et al. stated that measures of phenolic maturity did not necessarily track with a targeted “optimal ripeness” for red wine quality as defined by consumer

preference.<sup>12</sup> Many studies have been conducted to assess the effect of grape maturity on the sensory profile of red wines. Casassa et al. harvested Merlot grapes at two different grape maturities ( $20.35 \pm 0.16$  Brix, early harvest and  $24.91 \pm 0.46$  Brix, late harvest) and found that a later harvest resulted in wine with higher ratings for sweetness, viscosity, and fruit-derived aromas, while early harvest wine was described as having a sour taste, low color intensity, and the aroma of fresh vegetables.<sup>13</sup> In late harvest Cabernet Sauvignon wine, Bindon et al. and Schelezki et al. observed that ratings were lower for red color, red fruit, and fresh green/vegetative aroma, and higher for dark fruit aroma, overall fruitiness and aroma intensity, hotness, pungency, opacity, earthiness, and bitterness.<sup>13,14</sup>

Pineau et al. investigated the extent to which fruit ripeness and juice chaptalization affect the typicality of Marlborough Sauvignon Blanc wine. Sauvignon Blanc grapes were harvested at three different grape maturities (19.7, 22.0, and 23.5 Brix) and fermented as is, and in addition, the juice of the first and second harvest time points was chaptalized to 23.5 Brix. They found that chaptalization of the juice had nearly no impact on the flavor properties of the wine, but it greatly affected the taste and mouthfeel characteristics.<sup>15</sup> These findings are in agreement with Frost et al., who used a full factorial design with Cabernet Sauvignon at three maturity targets [20 Brix

Received: February 1, 2024

Revised: April 30, 2024

Accepted: April 30, 2024

Published: May 22, 2024



(unripe), 24 Brix (ripe), and 28 Brix (overripe)]. At each maturity target, TSS was adjusted to match the other two maturity targets by chaptalization or saignée followed by water-back. They observed that grape maturity primarily impacted the aroma profile of Cabernet Sauvignon wine, whereas chaptalization increased the perception of bitterness, hotness, and viscosity of the wine.<sup>16</sup> Sherman et al., on the other hand, concluded that both fruit maturity and chaptalization had significant effects on the sensory and chemical profiles of Merlot wines. They suggested, however, that wine ethanol concentration due to chaptalization is more important for the sensory properties than fruit maturity at harvest.<sup>17</sup>

Wine ethanol concentration is considered an important contributor to the sensory properties of a wine as it influences the astringency and bitterness perception<sup>18,19</sup> as well as the aroma<sup>20,21</sup> and is the key factor for the body of a wine.<sup>22</sup> Throughout grape ripening, the concentration of sugar and therefore the potential alcohol increases.<sup>10</sup> A study on grape ripening by Fournand et al. showed that anthocyanins accumulated in grapes until 170 g/L sugar (18 Brix) and then slightly decreased.<sup>23</sup> More complexity is observed for skin and seed tannins. Many recent studies on this topic follow the opinion that total skin tannin content increases with the progression of ripening, whereas the extractability of skin tannins decreases in the late stages of grape ripening.<sup>24</sup> A study by Ristic et al. on wine quality showed that higher concentrations of anthocyanins and skin tannins in Shiraz grape berries coupled with a lower concentration of seed tannins were associated with higher wine quality.<sup>25</sup>

In this study, an experimental model was developed to determine the effect of potential alcohol, as naturally obtained by grape maturity and its contribution to the sensory characteristics and phenolic composition of red wine. Phenolic data and descriptive sensory data of wines produced at three harvest time points from Pinot noir and Cabernet Sauvignon grapes were plotted against the natural potential alcohol over two vintages. The three harvest time points represent low, medium, and high grape maturity. The enhancement of potential alcohol, as obtained by sugar addition to premature grapes, was used to simulate low, medium, and high phenolic maturity but yield the same alcohol content irrespective of the harvest time point. In other words, the study investigated the contribution of potential alcohol as a solvent modulating the extraction of phenolic compounds during winemaking and whether potential alcohol can represent the phenolic maturity of grapes. The sensory characteristics as well as the monomeric and oligomeric phenolic compounds and parameters summarizing phenolic compound classes of the wines were analyzed and discussed with regard to which extent they were influenced by the potential alcohol, as part of grape maturity and as a solvent influencing the extraction during winemaking, and whether they are suitable for the experimental model. The experimental model incorporates an important cool climate variety with a well-studied warm climate variety in European winegrowing zone A (cool climate). In a climate change scenario, in which sugar accumulation in grapes occurs ahead of the ripening of other compounds, it is important to utilize maturity parameters indicating phenolic maturity independent of sugar accumulation.

## 2. MATERIALS AND METHODS

**2.1. Vineyard Site.** The experiment was conducted during the 2019 and 2020 vintage at the Weincampus Neustadt experimental

vineyard located in the Pfalz region of Germany (lat. 49°24' N; long. 8°11' E). *Vitis vinifera* L. cv. Pinot noir (PN; clone Mariafeld on SO4 rootstock) was planted in 1987 with a vine by row spacing of 1.88 m × 1.20 m and *Vitis vinifera* L. cv. Cabernet Sauvignon (CS; clone 1Gm on Binova 1Opp rootstock) was planted in 2008 with a vine by row spacing of 2.00 × 1.20 m. The growing degree days (GDD) were calculated with data from an on-site weather station yielding 1673 (2019) and 1683 GDD (2020) according to Method 1 by McMaster and Wilhelm.<sup>26</sup> A Huglin Index (HI) of 2089 (2019) and 2178 (2020) was calculated according to Huglin.<sup>27</sup>

**2.2. Wine Production.** Healthy grape bunches of each grape variety were harvested manually at three different harvest time points (early harvest "H1", commercial harvest "H2" and late harvest "H3") in both vintages 2019 and 2020 with a TSS range that is realistic for winemaking in Germany from 17 to 24 Brix.<sup>28</sup> Harvest dates were 3 weeks apart in 2019 and 2 weeks apart in 2020. Basic chemical analyses of the grape musts are shown in Supplemental Tables 1, 2, 3, and 4.

After destemming using an A1–280 M (Anton Wottle Maschinen- und Weinpressenbau GmbH, Poysdorf, Austria), grapes were crushed manually and transferred into 100 L experimental stainless-steel fermenters (70 kg each). At each harvest time point, an aliquot of the grape must was used as a control and an aliquot of the must was adjusted to 24.5 Brix (14 vol % potential alcohol) and pH 3.3 (Figure 1), the latter simulating high sugar maturity at low, medium, and high

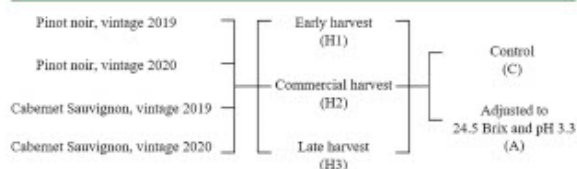


Figure 1. Experimental design.

phenolic maturity. TSS was adjusted by measuring the relative density in the juice using a hand-held density meter (DMA 35 Basic, Anton Paar, Graz, Austria), and then sucrose was added to each tank to make up the difference in TSS. A pH of 3.3 was chosen, as it corresponded to a late harvest in 2018 for both varieties. To adjust the pH, the juice was measured with a pH meter (Profiline pH 3110, WTW, Frankfurt, Germany) and then either potassium hydrogen carbonate (Kalinat, Erbslöh, Geisenheim, Germany) for deacidification or l(+)-tartaric acid (Roth, Karlsruhe, Germany) for acidification was added until a pH of 3.3 was reached. No SO<sub>2</sub> was added to the must to avoid any influence of SO<sub>2</sub> on the extraction.

Pinot noir and Cabernet Sauvignon musts were inoculated with a starter of 200 mg/L ZYMAFLORE RB2 yeast (LAFPORT, Bordeaux, France) and 300 mg/L LALVIN GO-FERM (LALLEMAND, Wien, Austria). Once fermentation commenced, fermenting wines were maintained at 24 °C and manually punched down three times per day. Fermentations were monitored with a hand-held density meter (DMA 35 Basic, Anton Paar, Graz, Austria). Wines were kept on skins and seeds until 2 days after reaching <1 g/L residual sugar. Solids were pressed off (1.6 bar) with a tank press (T1, Scharfenberger, Bad Dürkheim, Germany). The wines were transferred into 50 L carboys and inoculated with 10 mg/L *Oenococcus oeni* VP41 (ZEFÜG GmbH & Co. KG, Bingen, Germany). Two weeks after completion of malolactic fermentation, which was defined when <0.3 g/L malic acid was reached, 60 mg/L SO<sub>2</sub> (IOC Sulfivin K150, ZEFÜG GmbH & Co. KG, Bingen, Germany) was added. Wines were kept for 2 months at 15 °C until filtration and bottling. Filtration was done using a 0.45 µm membrane cartridge filter (Eaton Technologies, Langenlonsheim, Germany). Before bottling, the concentration of SO<sub>2</sub> was adjusted to obtain 35 mg/L free SO<sub>2</sub> (Tables 1, 2, 3, and 4). Wines were filled into 0.75 L Alsace-style wine bottles, closed with screw caps, and stored for 5 months at 16 °C until chemical analyses and sensory evaluation. All experimental treatments were carried out in triplicate (2019) and duplicate (2020). Wines of the 2019 vintage were used

Table 1. Basic Chemical Analyses of 2019 Pinot Noir Wine ( $n = 3$  Experimental Replicates)

		alcohol (g/L)	residual sugar (g/L)	pH	titratable acidity (g/L)
maturity	adjustment				
early harvest	no	70.4 ± 0.4 d <sup>a</sup>	0.0 ± 0.0 c	3.12 ± 0.04 d	8.1 ± 0.1 a
	yes	107.7 ± 1.5 ab	0.1 ± 0.1 bc	3.56 ± 0.06 bc	5.7 ± 0.5 b
commercial harvest	no	96.2 ± 0.9 c	0.2 ± 0.1 b	3.49 ± 0.02 c	5.8 ± 0.1 b
	yes	111.5 ± 5.9 a	0.1 ± 0.1 bc	3.54 ± 0.07 bc	5.7 ± 0.6 b
late harvest	no	102.9 ± 0.3 b	0.3 ± 0.1 a	3.64 ± 0.01 a	4.9 ± 0.1 c
	yes	111.0 ± 3.5 a	0.2 ± 0.1 ab	3.61 ± 0.02 ab	5.2 ± 0.1 bc
<i>p</i>		<0.0001	0.010	<0.0001	<0.0001

<sup>a</sup>Within a column, values followed by the same letter are not significantly different according to Fisher's LSD test at  $p \leq 0.05$ . Significant  $p$  values ( $p \leq 0.05$ ) are shown in italic letters.

Table 2. Basic Chemical Analyses of 2019 Cabernet Sauvignon Wine ( $n = 3$  Experimental Replicates)

		alcohol (g/L)	residual sugar (g/L)	pH	titratable acidity (g/L)
maturity	adjustment				
early harvest	no	73.6 ± 0.6 f <sup>a</sup>	0.0 ± 0.0	3.55 ± 0.02 d	5.7 ± 0.2 a
	yes	109.7 ± 0.4 c	0.0 ± 0.0	4.09 ± 0.03 a	4.0 ± 0.1 e
commercial harvest	no	94.0 ± 0.4 e	0.0 ± 0.0	3.71 ± 0.01 c	5.0 ± 0.0 b
	yes	111.8 ± 0.4 b	0.0 ± 0.0	3.99 ± 0.01 b	4.2 ± 0.1 de
late harvest	no	103.1 ± 0.3 d	0.0 ± 0.0	3.98 ± 0.07 b	4.5 ± 0.2 c
	yes	117.4 ± 1.4 a	0.0 ± 0.0	4.06 ± 0.08 ab	4.4 ± 0.2 bc
<i>p</i>		<0.0001		<0.0001	<0.0001

<sup>a</sup>Within a column, values followed by the same letter are not significantly different according to Fisher's LSD test at  $p \leq 0.05$ . Significant  $p$  values ( $p \leq 0.05$ ) are shown in italic letters.

Table 3. Basic Chemical Analyses of 2020 Pinot Noir Wine ( $n = 2$  Experimental Replicates)

		alcohol (g/L)	residual sugar (g/L)	pH	titratable acidity (g/L)
maturity	adjustment				
early harvest	no	69.9 ± 0.1 d <sup>a</sup>	0.6 ± 0.0 b	3.12 ± 0.03 d	7.2 ± 0.1 a
	yes	104.4 ± 1.5 b	0.5 ± 0.1 b	3.63 ± 0.02 b	4.8 ± 0.1 de
commercial harvest	no	89.8 ± 0.5 c	0.7 ± 0.1 b	3.44 ± 0.02 c	5.3 ± 0.3 bc
	yes	109.7 ± 0.2 a	0.4 ± 0.0 b	3.76 ± 0.07 a	4.6 ± 0.2 e
late harvest	no	105.9 ± 1.3 b	1.4 ± 0.3 a	3.45 ± 0.09 c	5.4 ± 0.4 b
	yes	111.0 ± 1.0 a	1.5 ± 0.1 a	3.54 ± 0.00 b	5.0 ± 0.0 cd
<i>p</i>		<0.0001	0.000	<0.0001	<0.0001

<sup>a</sup>Within a column, values followed by the same letter are not significantly different according to Fisher's LSD test at  $p \leq 0.05$ . Significant  $p$  values ( $p \leq 0.05$ ) are shown in italic letters.

Table 4. Basic Chemical Analyses of 2020 Cabernet Sauvignon Wine ( $n = 2$  Experimental Replicates)

		alcohol (g/L)	residual sugar (g/L)	pH	titratable acidity (g/L)
maturity	adjustment				
early harvest	no	75.1 ± 0.2 d <sup>a</sup>	0.1 ± 0.1 c	3.42 ± 0.06 d	5.8 ± 0.1 a
	yes	101.4 ± 1.5 b	0.0 ± 0.0 c	3.76 ± 0.01 a	4.9 ± 0.0 d
commercial harvest	no	91.9 ± 0.1 c	0.8 ± 0.1 ab	3.55 ± 0.01 c	5.4 ± 0.0 bc
	yes	106.0 ± 0.1 a	0.6 ± 0.0 b	3.49 ± 0.01 cd	5.7 ± 0.1 ab
late harvest	no	103.2 ± 1.4 ab	0.9 ± 0.1 a	3.71 ± 0.01 ab	5.1 ± 0.1 cd
	yes	100.8 ± 1.7 b	0.8 ± 0.1 ab	3.64 ± 0.07 b	5.5 ± 0.3 ab
<i>p</i>		<0.0001	0.001	0.001	0.010

<sup>a</sup>Within a column, values followed by the same letter are not significantly different according to Fisher's LSD test at  $p \leq 0.05$ . Significant  $p$  values ( $p \leq 0.05$ ) are shown in italic letters.

describe the effect of progressing grape maturity and adjusted potential alcohol on the sensory profiles and phenolic composition of Pinot noir and Cabernet Sauvignon. Wines of the 2019 and 2020 vintages were used to develop the maturity model.

**2.3. Chemicals.** Hydrochloric acid, propan-2-ol, anhydrous caffeine, Folin-Ciocalteu reagent, and sodium chloride were purchased from AppliChem (Darmstadt, Germany). Ethanol, orthophosphoric acid, acetic acid, and potassium metabisulfite were purchased from

ORG Laborchemie (Bunde, Germany). Procyanidin B2 and procyanidin C1 were purchased from PhytoLab (Vestenbergsgreuth, Germany). Caftaric acid, cyanidin-3-O-rutinoside, (–)-epicatechin-3-O-gallate, (–)-epigallocatechin, (+)-gallocatechin, and oenin chloride were purchased from PhytoPlan (Heidelberg, Germany). Urea, bovine serum albumin fraction V, l(+)-tartaric acid, and sodium carbonate were purchased from Roth (Karlsruhe, Germany). Caffeic acid, (+)-catechin hydrate, (–)-epicatechin, gallic acid monohydrate,

Table S. Descriptors, Definitions, Reference Standards, and Anchor Terms for Scales Used in the Sensory Descriptive Analysis

descriptor	definition	reference standard <sup>a</sup>
<b>Appearance attributes</b>		
color	how much the wine blocks the passage of light	
intensity	wine color from brown to red to purple	
color hue <sup>b</sup>		
<b>Aroma attributes for Pinot noir</b>		
green bell pepper	aroma of green bell pepper, unripe	4 cm <sup>3</sup> fresh green bell pepper to 100 mL base wine stirred for 10 min, then strained
red fruit	aroma of fresh red fruits such as sour cherry and red currant	35 mL sour cherry juice (REWE Beste Wahl) and 35 g crushed fresh red currant to 100 mL base wine stirred for 10 min, then strained
dark fruit	aroma of ripe dark fruits such as blackberry and blackcurrant	20 mL black currant juice (REWE Beste Wahl), 10 mL "superfruit blackberry juice" (Rauch, Happy Day) and 5 crushed fresh blackberries to 100 mL base wine, left overnight, then strained
jammy	aroma of sweet, cooked fruits like plum puree or strawberry jam	4 mL of strawberry syrup (Mönnin), 4 mL of prune juice (REWE Beste Wahl), and 1 tsp plum puree (jal) to 100 mL of base wine, left overnight, then strained
herbaceous	fresh, eucalyptus aroma with Mediterranean herbs	0.1 g of dried oregano (Ostmann), 1 juniper berry (0.15–0.2 g) (Ostmann), 0.35 g/L fresh rosemary, 1.5 g of green tea (Westminster), 1.5 g of black tea (Lipton), 3 pinches of black pepper (Ostmann), a pinch of cloves (Ostmann), and 1/3 cough drop eucalyptus (Wick) to 100 mL base wine, stirred for 10 min, then strained
<b>Aroma attributes for Cabernet Sauvignon</b>		
bell pepper	aroma of green and red bell pepper	4 cm <sup>3</sup> fresh green bell pepper and 4 cm <sup>3</sup> fresh red bell pepper to 100 mL base wine stirred for 10 min, then strained
sour cherry	aroma of sour cherry	50:50 sour cherry juice (REWE Beste Wahl) to base wine
dark fruit	aroma of ripe dark fruits such as blackberry and blackcurrant	20 mL of black currant juice (REWE Beste Wahl), 10 mL of "superfruit blackberry juice" (Rauch, Happy Day), and 5 crushed fresh blackberries to 100 mL base wine, left overnight, then strained
cooked plum	aroma of sweet, cooked fruits like plum puree	25 mL of prune juice (REWE Beste Wahl), 2 tsp plum puree (jal), and 1 diced prune (Seebecker) to 100 mL of base wine, left overnight, then strained
eucalyptus	fresh, eucalyptus aroma and herbs	1/2 cough drop eucalyptus (Wick) finely crushed, and 2 mL ethanol to 100 mL base wine
spices	spicy or savory aroma	in 100 mL of base wine: 3 pinches of black pepper (Ostmann), 1 soft licorice (Kafjes), a pinch of cloves (Ostmann), 1 soft licorice (Kafjes), stirred for 10 min, then strained
<b>Taste and tactile attributes</b>		
bitter	the intensity of bitter taste perceived in the back part of the palate	1.0 g/L caffeine (AppliChem (Darmstadt, Germany))
sour	the intensity of sour taste/acidity perceived in the mouth	2.0 g/L tartaric acid (Roth (Karlsruhe, Germany))
sweet	the intensity of sweetness perceived in the mouth	0.8 g/L tannic acid (Sigma-Aldrich (Steinheim, Germany))
dry	reduction of the natural lubrication in the mouth, giving a drying feeling	1.0 g/L aluminum sulfate (Sigma-Aldrich (Steinheim, Germany))
astringency	mouth feeling from rough to smooth	1.2 g/L catechin hydrate (Sigma-Aldrich (Steinheim, Germany)) and 100 crushed unripe grape seeds <sup>c</sup> , left overnight, then strained
green	palate drying effect with an unripe astringency	
full body <sup>d</sup>	perception of the body, weight, or thickness of the wine from light to full	
harsh	mouthfeel from harmonious to harsh	
mouthfeel <sup>e</sup>		

<sup>a</sup>Standards were produced with a red base wine, grape variety: Pinot noir, 2019 vintage. A volume of 1 L was used unless otherwise stated. <sup>b</sup>Scale was anchored on the left with brown hue, in the middle with red hue, and on the right with purple hue. <sup>c</sup>Scale was anchored on the left with rough and on the right with smooth. <sup>d</sup>Scale was anchored on the left with light and on the right with full. <sup>e</sup>Scale was anchored on the left with harmonious and on the right with harsh

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<https://doi.org/10.1021/acs.jafc.4c01035>  
J. Agric. Food Chem. 2024, 72, 12725–12737

sodium hydroxide, maleic acid, tannic acid, aluminum sulfate, triethanolamine, and iron(III) chloride were purchased from Sigma-Aldrich (Steinheim, Germany). Acetonitrile and formic acid were purchased from Bernd Kraft (Duisburg, Germany) and Honeywell (Morris Plains, New Jersey), respectively. Potassium dihydrogen phosphate was purchased from VWR International (Darmstadt, Germany). Ultrapure water was obtained from a PURELAB flex (ELGA LabWater, Celle, Germany) water purification system. All chromatographic solvents were HPLC grade.

**2.4. Basic Analytical Parameters.** Grape must parameters (relative density, pH, and titratable acidity) were measured by Fourier transform infrared spectroscopy (FT-IR) (WineScan FT120 Basic, FOSS GmbH, Hamburg, Germany) using a calibration method provided by the manufacturer. Wine parameters (alcohol, residual sugars, pH, titratable acidity, malic acid, and lactic acid) were measured by FT-IR (WineScan Auto, FOSS GmbH, Hamburg, Germany) using a calibration method provided by the manufacturer. Samples were centrifuged for 5 min at 4500 rpm and degassed prior to FT-IR analysis.

**2.5. Spectrophotometric Analysis.** The photometric analyses were scheduled to coincide with sensory evaluation. Iron reactive phenolics, tannins, protein-precipitable polymeric pigments (p-PP), and nonprecipitable polymeric pigments (np-PP) were measured by the assay described by Harbertson et al. with modified resuspension buffer.<sup>29–31</sup> The terms “large and small polymeric pigments” are no longer used since the size distinction between them has not been defined and a distinction according to their precipitability is therefore more appropriate.<sup>32</sup> A Jasco V-730 double-beam UV–visible spectrophotometer (Jasco GmbH, Pfungstadt, Germany) was used for all of the measurements.

**2.6. HPLC-DAD/FD Analysis.** The analysis was performed using an HPLC-DAD/FD (Jasco, Pfungstadt, Germany) comprising a PU-4180 pump, an AS-4150 autosampler, an MD-4010 photometric diode array detector, and an FP-4025 fluorescence detector.

Separation of phenolics was achieved with a reversed-phase Gemini NX-C18 (110 Å, 150 × 4.6 mm, 3 μm; Phenomenex, Torrance, USA) column, protected by a guard column containing the same material (Security Guard Cartridge System C18; Phenomenex, Torrance, USA) at a temperature of 25 °C (CO-4060 column oven; Jasco, Pfungstadt, Germany). A gradient consisting of eluent A {phosphate buffer [1.36 g/L KH<sub>2</sub>PO<sub>4</sub>, pH = 1.5 (85% H<sub>3</sub>PO<sub>4</sub>)]/ acetonitrile 95/5 (v/v)} and eluent B {phosphate buffer [1.36 g/L KH<sub>2</sub>PO<sub>4</sub>, pH = 1.5 (85% H<sub>3</sub>PO<sub>4</sub>)]/acetonitrile 50/50 (v/v)} was applied at a flow rate of 1.0 mL/min as follows: 0 min, 10% B; to 7 min, 34% B; to 12 min, 51% B; to 17 min, 68% B; to 22 min, 100% B; to 27 min, 100% B; to 28 min, 10% B; to 32 min, 10% B. The phenolic compounds were identified according to their order of elution and the retention times of reference compounds. The reference compounds were: malvidin-3-glucoside (mv-3-gl), caffeic acid, caftaric acid, gallic acid, (+)-catechin, and (–)-epicatechin. The analysis was performed at the characteristic wavelength of each compound: 520 nm for anthocyanins, 320 nm for hydroxycinnamic acids, and 280 nm for gallic acid. Flavan-3-ols were quantified by fluorescence with excitation and emission wavelengths of 280 and 310 nm, respectively, in order to avoid interferences with the other compounds. External standards were used for the quantification purposes. A calibration range of 0.5–125 mg/L was chosen for (–)-epicatechin and caftaric acid, 1.0–250 mg/L for gallic acid and (+)-catechin, and 1–450 mg/L for anthocyanins. Anthocyanins were quantified as mv-3-gl equivalents. An injection volume of 10 μL was used for all samples.

Samples were filtered through 0.45 μm cellulose acetate membranes (Graphic Controls, Totnes, UK) and then analyzed directly. ChromNAV (version 2.0, Jasco, Pfungstadt, Germany) was used for the HPLC data analysis. All quantitative analyses of phenolic composition were performed in duplicates.

**2.7. LC-QToF-MS Analysis.** The analysis was performed using an LC-QToF-MS (Agilent Technologies, Waldbronn, Germany) comprising an Agilent 1260 Infinity Binary Pump, an Agilent 1260 Infinity HiP Degasser, an Agilent 1260 Infinity HiP ALS Autosampler, an

Agilent Jet Stream Technology Ion Source (AJS) G-1958-65138, an Agilent 1260 DAD, and a 6530 Accurate-Mass Q-ToF LC/MS.

Separation of phenolics was achieved with a reversed-phase Kinetex C18 (100 Å, 150 × 2.10 mm, 2.6 μm; Phenomenex, Torrance, USA) column at a temperature of 40 °C (Agilent 1260 TCC; Agilent Technologies, Waldbronn, Germany). A gradient consisting of eluent A [H<sub>2</sub>O ultrapure/acetonitrile/formic acid 93/5/2 (v/v/v)] and eluent B [H<sub>2</sub>O ultrapure/acetonitrile/formic acid 5/93/2 (v/v/v)] was applied at a flow rate of 0.4 mL/min as follows: 0 min, 0% B; to 2 min, 0% B; to 18 min, 30% B; to 22 min, 100% B; to 23 min, 100% B; to 24 min, 0% B.

MS analysis was performed in positive ionization mode. The MS parameters were as follows: nebulizer pressure 35 psig; fragmentation at 170 V; skimming at 65 V; VCap 3000 V; gas flow 8 L/min; gas temperature 320 °C; sheath gas temperature 380 °C; rate 5 spectra/sec; scan at *m/z* 100–1700.

B-prodelphinidins and B-procyanidin gallates were identified according to their *m/z*. Gallocatechin, epigallocatechin, B-procyanidins, and C-procyanidins were identified according to the retention times of reference compounds. Cyanidin-3-rutinoside was used as internal standard, and external standards were used for quantification purposes. The peak areas of the external standards have been put in relation to the area of the internal standard. The quotient was graphically plotted against the concentration of external standards, and linear regression was used for quantitative evaluation. B-procyanidins, B-prodelphinidins, and B-procyanidin gallates (dimers) were quantified as procyanidin B2 equiv. C-procyanidins (trimers) were quantified as procyanidin C1 equiv. A calibration range of 0.1–15 mg/L was used for all reference compounds. An injection volume of 5 μL was used for all samples.

Samples were filtered through 0.45 μm cellulose acetate membranes (Graphic Controls, Totnes, U.K.) and then analyzed directly. MassHunter Workstation Software (Quantitative Analysis, version B.05.00, Build 5.0.291; Agilent Technologies, Waldbronn, Germany) was used for the MS data analysis. All the quantitative analyses of phenolic composition were performed in duplicates.

**2.8. Sensory Evaluation.** Before sensory evaluation, a group of five wine experts pre-evaluated all experimental wines, discussed them, and decided and agreed on a list of descriptors that discriminate between the wines. In a blind bench-tasting, the group of five wine experts could not find sensory differences among the experimental replicates. The experimental replicates were pooled for the sensory evaluation sessions.

Descriptive analysis (DA) was performed 5 months after bottling (11 months after alcoholic fermentation) by a trained sensory panel. The panel for the 2019 vintage consisted of 17 judges for Pinot noir wines (11 female and 6 male) and 19 judges for Cabernet Sauvignon wines (13 female and 6 male) with a range between 22 to 64 years of age. For the 2020 vintage, the panel consisted of 19 judges for Pinot noir wines (7 female and 12 male) and 18 judges for Cabernet Sauvignon wines (9 female and 9 male). Panelists attended three training sessions (90 min each) before evaluating Pinot noir wines and two training sessions (90 min each) before evaluating Cabernet Sauvignon wines. During the first training session for each grape variety, wines from this study were randomly presented to panelists, knowing the grape variety for which they signed up. Panelists were given a list of aroma descriptors that differentiate between grape maturity as well as taste and tactile descriptors (Table 5). Training for blind recognition of aroma, taste, and tactile attributes was carried out to create a common sense for these attributes. Other training sessions were carried out presenting the base wine and the reference standards (Table 5), undiluted and 1:2 diluted with wine, to the judges to familiarize them with the use of unstructured line scales. For training sessions and evaluation sessions, the unstructured line scales were anchored at the left with low intensity and at the right end with high intensity, unless stated otherwise in Table 5. Also part of the training was a trial-run DA, which entailed the evaluation of five random wines including one repetition from this study (separate for each grape variety) for their appearance, aroma, taste, and mouthfeel on the unstructured line scales, in order to familiarize panelists with the

wines and the evaluation process under the same conditions as those for the formal sessions.

Formal evaluation sessions were held repeatedly for Pinot noir ( $n = 2$ ) and for Cabernet Sauvignon ( $n = 2$ ) on separate days. Each session consisted of six samples. For aroma, taste, and tactile attributes, samples were presented to panelists (30 mL per sample at 16 °C) in black Sensus glasses (DIN 10960 wine-tasting glasses; Schott Zwiesel, Germany) covered with plastic lids. For appearance attributes, the same samples were presented in clear glasses (DIN 10960 wine-tasting glasses; Schott Zwiesel, Germany) covered with plastic lids. All samples were coded with a three-digit number. The wines were presented in a randomized order. Formal evaluations were held in an air-conditioned sensory laboratory with individual booths at 20 °C. Data were acquired using FIZZ software (Version 2.51 c 02, Biosystèmes, Couternon, France). All samples were expectorated. To avoid sensory fatigue, panelists were required to rest for 90 s while restoring their saliva with commercially available still water after each sample.

**2.9. Experimental Maturity Model.** In order to include both vintages in the model, interannual variations were normalized by dividing each individual data point by the arithmetic mean of all data points of the respective vintage. Afterward, the normalized data point was multiplied with the arithmetic mean of all data points for each parameter of both vintages combined. The data were plotted against the natural potential alcohol. A linear regression was performed for the data of the control wines showing the natural maturity progress. The comparison of the adjusted wines with the natural maturity progress at 14 vol % allowed for the assessment of the impact of the potential alcohol on analytical and sensory parameters. A heteroskedastic  $t$  test was performed to evaluate the effect of the simulated maturity. Figure 2 shows the hypothetical scenarios that are possible:

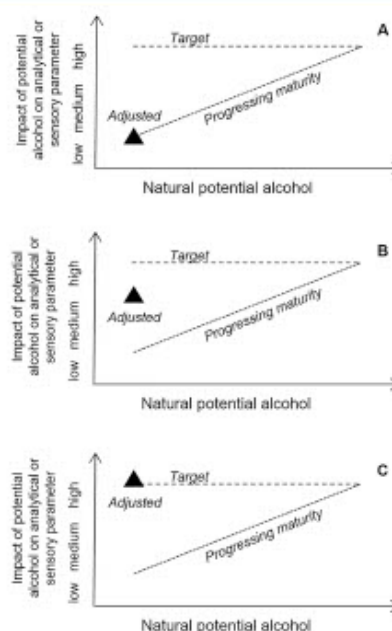
**2.10. Statistical Analysis.** The sensory data were processed by a three-way mixed model analysis of variance (ANOVA) considering a panelist as a random effect and wine and tasting replication as fixed effects. For the chemical data, the analytical repetitions and the experimental replicates were additionally regarded as separate factors. Fisher's least significant difference (LSD) posthoc test ( $p \leq 0.05$ ) was carried out for all data. Principal component analysis (PCA) was performed for chemical data using Pearson correlation ( $n - 1$ ).

All statistical analyses were performed using XLSTAT SENSORY (Version 2022.3.1.1329 (32 bit), Addinsoft SARL, Paris, France).

### 3. RESULTS AND DISCUSSION

#### 3.1. Influence of Natural Grape Maturity and Enhancement of Potential Alcohol on Sensory Profiles.

Figures 3 and 4 show the spider web graphs of the sensory attributes for 2019 Pinot noir (Figure 3) and 2019 Cabernet Sauvignon (Figure 4) wines produced from grapes of different maturity levels without (A) and with enhanced potential alcohol (B). The descriptive sensory analysis of the control wines of both grape varieties revealed significant differences in aroma, taste, mouthfeel, and color perception with progressing grape maturity. For both grape varieties, increasing grape maturity resulted in higher ratings in *color intensity*, *purple hue*, *dark fruit* aroma, *sweetness*, and a *full body*, which is in agreement with the findings of Cadot et al.<sup>33</sup> The observation of higher ratings in *acidity/sourness* and *green bell pepper/vegetal* aroma in early harvest Cabernet Sauvignon wines is in agreement with the results of Gil et al., Cadot et al., and Casassa et al.<sup>13,33,34</sup> In Pinot noir (Figure 3A), *harsh mouthfeel*, *dry astringency*, *rough astringency*, and *green astringency* decreased significantly with progressing grape maturity, whereas in Cabernet Sauvignon (Figure 4A), only *green astringency* decreased significantly. *Dry astringency* perception in Cabernet Sauvignon increased with progressing grape maturity, which is in agreement with the findings of Cadot et al.<sup>33</sup>

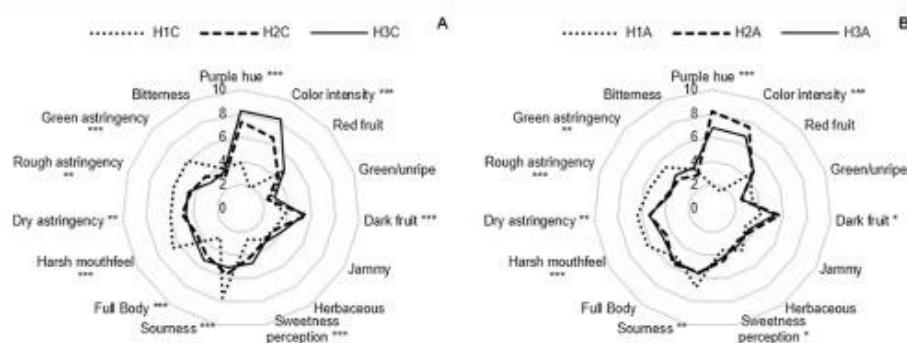


**Figure 2.** Hypothetical scenarios for the maturity model. Effects of progressing grape maturity and potential alcohol adjustment at early harvest (triangle) are plotted against those of natural potential alcohol. Scenario A: The parameter is not dependent on the potential alcohol; the content of substance(s) in the grape responsible for the parameter's intensity is the limiting factor. Scenario B: The parameter is partially dependent on potential alcohol; the extractability and/or the content of substance(s) in the grape responsible for the parameter's intensity is the limiting factor. Scenario C: The parameter is dependent on potential alcohol; the extractability of substance(s) from the grape responsible for the parameter's intensity is the limiting factor.

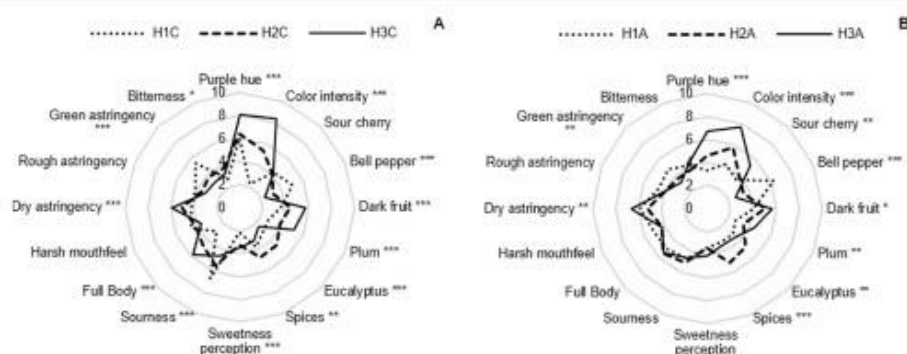
Figures 3B and 4B show the sensory profiles of wines produced from grapes of different maturity levels with an enhanced potential alcohol. Comparing the sensory profiles of wines with and without enhanced potential alcohol reveals that the effect of potential alcohol adjustment was very little, non-existent, or even enhancing unripe impressions. Most significant differences due to grape maturity were still present after alcohol adjustment, hence, the alcohol adjustment did not reach the sensory characteristics of a late harvest wine. In terms of sensory, if grape maturity would be solely dependent on potential alcohol, there should be no significant differences in the wines produced from grapes of different maturity levels with adjusted potential alcohol.

In Pinot noir (Figure 3B), early harvest wines were still rated significantly higher in *sourness* than commercial and late harvest wines even though the musts of all harvest time points were adjusted to the same pH. This observation can be explained by the significantly higher ratings of *green astringency*, *rough astringency*, *dry astringency*, and *harsh mouthfeel*, suggesting a halo-dumping effect. The minor effect of adjusted potential alcohol on astringency-related attributes suggests that more alcohol either did not affect the extraction of tannins or seed-associated phenolics or masked the changes in the phenolic composition.

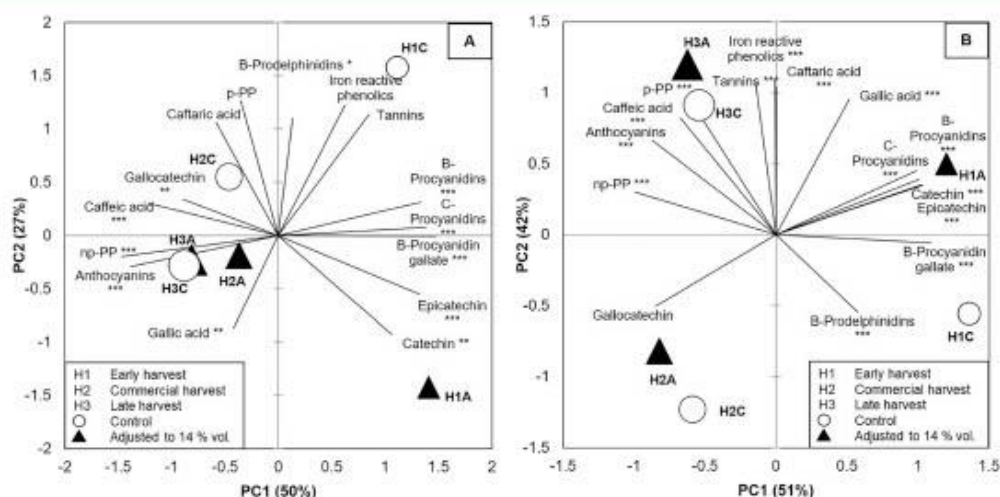
#### 3.2. Influence of Natural Grape Maturity and Enhancement of Potential Alcohol on the Phenolic



**Figure 3.** Sensory profiles for 2019 Pinot noir control (A) and adjusted (B) wines produced from grapes of different maturity levels H1–H3 (data expressed as mean values of 17 panelists and 2 panel replications). \*Significant at  $p < 0.05$ , \*\*significant at  $p < 0.01$ , \*\*\*significant at  $p < 0.001$ .



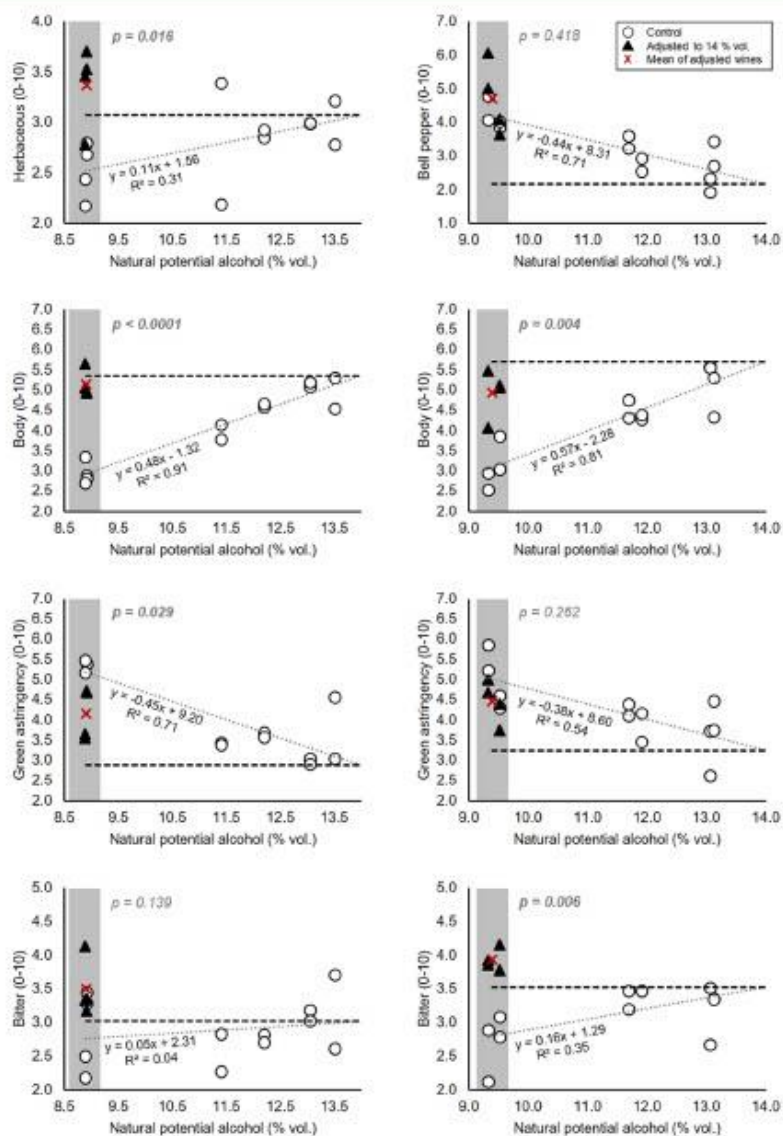
**Figure 4.** Sensory profiles for 2019 Cabernet Sauvignon control (A) and adjusted (B) wines produced from grapes of different maturity levels H1–H3 (data expressed as mean values of 19 panelists and 2 panel replications). \*Significant at  $p < 0.05$ , \*\*significant at  $p < 0.01$ , \*\*\*significant at  $p < 0.001$ .



**Figure 5.** Principal component analysis for 2019 Pinot noir (A) and Cabernet Sauvignon (B) wines produced from grapes of different maturity levels without (open symbols) and with potential alcohol adjustment (filled symbols) (data expressed as mean values of  $n = 3$  experimental replicates). PCA space was calculated for the 15 displayed chemical parameters. \*Significant at  $p < 0.05$ , \*\*significant at  $p < 0.01$ , \*\*\*significant at  $p < 0.001$ .

**Composition.** Figure 5 shows the principal component analysis (PCA) for Pinot noir (Figure 5A) and Cabernet Sauvignon (Figure 5B) wines produced from grapes of different maturity levels without and with potential alcohol

enhancement. For both grape varieties, PCA space was calculated for the same 15 chemical parameters. For Pinot noir, the first two PCs with eigenvalues  $> 1$  explained 77% of the cumulative variability, with PC1 accounting for 50% of the



**Figure 6.** Experimental model for the sensory data of 2019 and 2020 Pinot noir (left) and Cabernet Sauvignon (right) wines produced from grapes of different maturity levels without (circles) and with potential alcohol adjustment (triangles).

total variance (Figure 5A). For Cabernet Sauvignon, the first two PCs with eigenvalues > 1 explained 93% of the cumulative variability, with PC1 accounting for 51% of the total variance (Figure 5B). For both grape varieties, the natural grape maturity was separated on PC1 and the enhancement of the potential alcohol was separated on PC2.

For both grape varieties (Figure 5A,B), early harvest wines were positively loaded on PC1, which is explained by seed-associated phenolics such as catechin, epicatechin, B- and C-procyanidins, and B-procyanidin gallates. Late harvest wines were negatively loaded on PC1, which is explained by anthocyanins, caffeic acid, and np-PP. Pinot noir wines (Figure 5A) with enhanced potential alcohol were negatively loaded on PC2, which is explained by higher contents of gallic acid and lower contents of B-prodelphinidins. This suggests that seed-associated phenolics are better extracted at 14 vol % than skin-

associated phenolics. Cabernet Sauvignon wines (Figure 5B) were positively shifted on PC2 upon potential alcohol enhancement, which is explained by higher tannin and iron reactive phenolics concentrations. This observation is in agreement with the findings of Sherman et al., who showed that adjusted high-alcohol Merlot wines had significantly higher tannin concentrations in all maturity levels.<sup>17</sup> The shift of adjusted wines from control wines on PC2 became smaller with progressing grape maturity in both grape varieties (Figure 5A,B), which can be explained by the decreasing extent of potential alcohol adjustment. However, the results show that the effect of the adjusted potential alcohol is different from the effect of grape maturity. Accordingly, the phenolic composition of wines made from immature grapes cannot be driven toward a composition associated with a higher grape maturity by increasing the potential alcohol. These results also indicate that

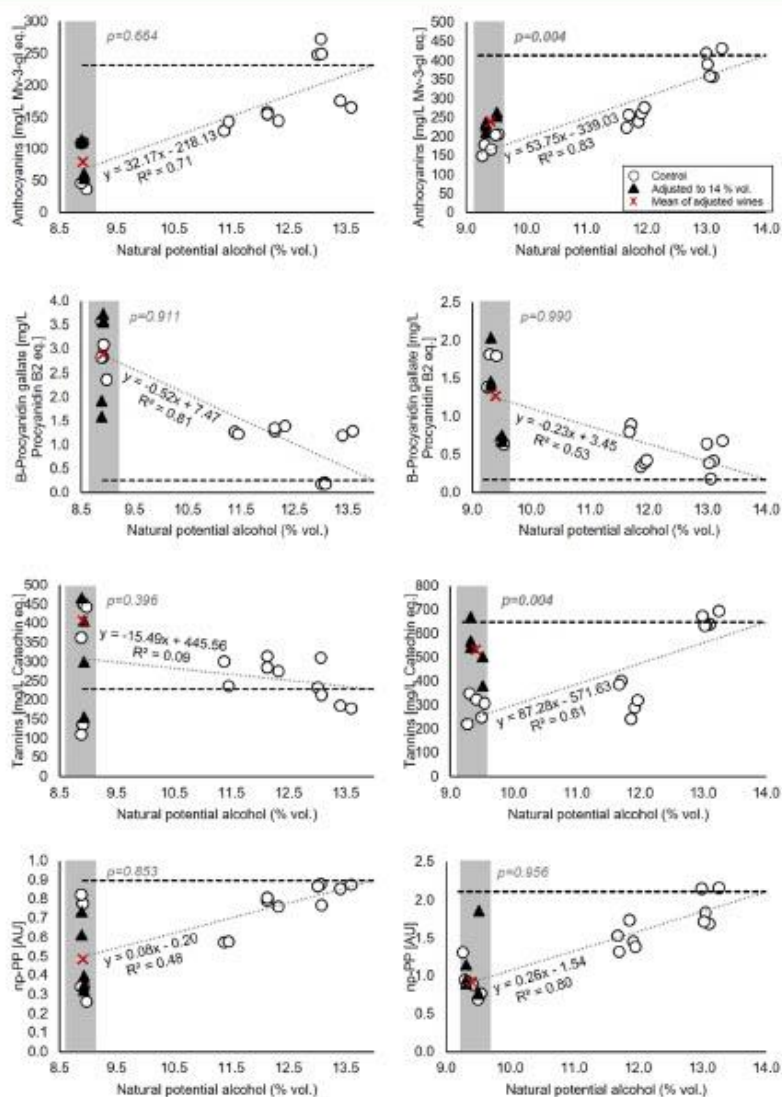


Figure 7. Experimental model for the chemical data of 2019 and 2020 Pinot noir (left) and Cabernet Sauvignon (right) wines produced from grapes of different maturity levels without (circles) and with potential alcohol adjustment (triangles).

grape maturity is not solely expressed by the sugar content in the berries or the potential alcohol, respectively. An earlier and faster sugar accumulation, decoupled from phenolic maturity due to climate change, here simulated by the enhanced potential alcohol, might result in wines with pronounced differences in the phenolic profile.

Pinot noir and Cabernet Sauvignon were used in this study because of their differences in phenolic profiles and concentrations. Other factors, such as different plantation years of the experimental vineyards and further viticulture factors, might contribute to the observed varietal differences. In both grape varieties, potential alcohol enhancement increased gallic acid concentrations and lowered B-prodelphinidin concentrations (Figure 5A,B); however, in Cabernet Sauvignon, both gallic acid and prodelphinidins were also related to early harvest wines (Figure 5B). Compared to Cabernet Sauvignon, Pinot noir had low tannin and iron

reactive phenolic concentrations that were not significantly affected by progressing grape maturity or enhanced potential alcohol concentration (Figure 5A). In Pinot noir, tannin and iron reactive phenolics concentrations were therefore not suited to differentiate between control and adjusted wines.

In conclusion, data displayed in Figures 3–5 showed that grape maturity and adjusted potential alcohol significantly influenced the sensory characteristics and phenolic composition of Pinot noir and Cabernet Sauvignon wines. The similarities and differences in the effects of grape maturity and adjusted potential alcohol were visualized and comprehensively discussed by utilizing 15 or 16 different sensory attributes and 15 different phenolic parameters. The data also revealed that individual parameters are influenced by grape maturity and the enhanced potential alcohol in different ways. In order to assess whether wines were made from grapes with low or high

phenolic maturity, it is necessary to examine individual sensory and phenolic parameters.

**3.3. Experimental Model Assessing the Contribution of Potential Alcohol on Wine Sensory.** The experimental model, which is hypothetically illustrated and explained in Figure 2, is applied for the measured sensory ratings of the wines, which are correlated to their natural potential alcohol (Figure 6). In Pinot noir, the rating for the aroma descriptor *herbaceous* increased with the natural potential alcohol. The adjusted alcohol significantly increased the *herbaceous* intensity and it became similar to a natural alcohol of 14% vol. Potential alcohol had therefore a contribution to an *herbaceous* aroma. The *herbaceous* aroma was defined as “cooling” or “refreshing” like eucalyptus (Table 5), which might be similar to an alcoholic aroma for the judges. In Cabernet Sauvignon, the rating of *bell pepper* aroma got lower with progressing grape maturity, which is in agreement with the findings of Bindon et al. and can be explained by decreasing concentrations of IBMP.<sup>12,35</sup> The ratings for *bell pepper* aroma at early harvest were not significantly affected by potential alcohol adjustment, suggesting that *bell pepper* aroma is independent of alcohol-supported extraction.

Ratings for *body* increased in both grape varieties with increasing natural potential alcohol. The parameter was strongly dependent on potential alcohol, which can be observed in the significantly higher ratings in the adjusted wines, which were close to the extrapolated value at 14 vol % vol. alcohol. Previous studies have shown that higher alcohol content in wine resulted in a higher viscosity perception and density perception and therefore a fuller body.<sup>22,36</sup> It is also possible that higher potential alcohol supported the extraction of macromolecules from the berries into the wine or increased the secretion of yeast-derived mannoproteins.<sup>35</sup> Okuda et al. showed that higher concentrations of macromolecules such as polysaccharides, proteins, and polymerized phenols increased the body of a wine<sup>36</sup> and Bindon et al. suggested that an additive effect of alcohol, glycerol, and mannoproteins contributed to the viscosity/body of a wine.<sup>12,37</sup>

For both grape varieties, the ratings for *green astringency* decreased as grape maturity progressed. In Pinot noir, *green astringency* is partially dependent on potential alcohol, which can be observed in the significantly lower rating of the adjusted wines. However, the effect was only half of the extrapolated value at 14% vol. alcohol, which suggests that the potential alcohol also partially contributes to maturity perception by masking the unripe sensation. In Cabernet Sauvignon, the effect of a higher ethanol concentration is less distinct than in Pinot noir. This might be due to Cabernet Sauvignon being a later ripening grape variety than Pinot noir. It is commonly grown in warmer climate regions; therefore, the seeds might be less lignified and hence have a stronger unripe impression.

In both grape varieties, *bitterness* ratings increased with higher potential alcohol irrespective of the alcohol being naturally present or adjusted. The model suggests that the alcohol itself is the main driver of bitterness in the wines and the influence of alcohol on the sensory perception of *bitterness* in wine has been discussed previously.<sup>18,19</sup> However, the increase in *bitterness* was significant only in Cabernet Sauvignon and not pronounced in Pinot noir. This indicates that specific phenols—monomeric and oligomeric—due to varietal differences also contributed to bitterness in relation to the alcohol.

**3.4. Experimental Model Assessing the Contribution of Potential Alcohol on Phenolic Parameters.** An increase in anthocyanin concentration with increasing natural potential alcohol was observed in both varietal wines (Figure 7), which is in agreement with previous studies.<sup>13</sup> In Pinot noir, the enhanced potential alcohol did not increase the anthocyanin content in wine, suggesting that it was limited by the concentration in the grapes due to immaturity. In Cabernet Sauvignon, on the other hand, anthocyanin concentration showed a significant increase by adjusting the potential alcohol, which is also reflected in the higher ratings in color intensity (Figure 4). The potential anthocyanin concentration could not be fully reached with the low natural potential alcohol present in the early harvested Cabernet Sauvignon grapes. This observation is in contrast to the literature stating that anthocyanins are extracted in the aqueous phase prior to fermentation, reach their maximum concentration in the first days of alcoholic fermentation, and decrease during the post fermentation maceration period.<sup>38,39</sup> Previous studies have shown that the grape skin cell wall composition, which is different for different grape varieties, is related to the extractability of anthocyanins into the wine.<sup>40</sup> The extractability index of Monastrell grapes was found to be higher than the extractability index of Cabernet Sauvignon, Syrah, and Merlot grapes. It was also dependent on the maturity of the grapes.<sup>40</sup> In the present study, the restricted anthocyanin extractability in early harvested Cabernet Sauvignon grapes was increased by adjusting the potential alcohol, eventually causing improved anthocyanin extraction, as described by Medina-Plaza et al., and higher concentrations.<sup>41</sup>

In both varieties, the B-procyanidin gallate concentration decreased with increasing natural potential alcohol (Figure 7), which is in agreement with the findings of Jordão et al. for Castelao Francês grapes and Touriga Francesa grapes and Obreque-Slier et al. for Cabernet Sauvignon grapes.<sup>42–44</sup> The decrease in B-procyanidin gallate is likely due to polymerization reactions with progressing maturity and/or lower extractability caused by lignification of the seeds.<sup>45</sup> However, the extraction of B-procyanidin gallate was not affected by the enhanced potential alcohol. This indicates that the B-procyanidin gallate concentration in the wine was limited to the B-procyanidin gallate concentration in the grapes, and it was already fully exhausted when being extracted with the low potential alcohol present. In contrast to these findings, Canals et al. have shown that alcohol significantly increased the extraction of Tempranillo seed phenolics.<sup>46</sup>

In Pinot noir, the tannin concentration merely decreased with increasing natural potential alcohol and did not change with adjusted potential alcohol (Figure 7). Overall, tannin concentrations in Pinot noir were at the lower end of the calibration used in the protein precipitation assay, which explains the poor reproducibility of the experimental replicates. Cabernet Sauvignon showed an increase in tannins with progressing grape maturity as well as a significant increase due to potential alcohol. Potential alcohol had a significant contribution to the tannin concentration in Cabernet Sauvignon but did not explain the phenolic maturity completely. Hensen et al. suggested that differences in the polysaccharide structure have a greater impact on the extractability of tannins than the changes in the tannin composition during ripening. They also suggested a strong impact of a varietal-specific polysaccharide composition on the phenolic composition of wines.<sup>47</sup> Other studies have shown

that tannin concentrations in wine were also dependent on the selective adsorption of tannins with high proportions of gallates into grape skins or flesh as well as the selective precipitation with skin-soluble polymers.<sup>48</sup>

Similar to anthocyanins, np-PP increased in both varieties with an increasing natural potential alcohol (Figure 7). The reproducibility of np-PP was low in wines from early harvest grapes. The adjustment of potential alcohol did not significantly affect np-PP, neither in Pinot noir nor in Cabernet Sauvignon. In Cabernet Sauvignon, both tannins and anthocyanins, being parts of polymeric pigments (np-PP and p-PP), were increased due to the enhancement of potential alcohol. Therefore, it was expected that polymeric pigment formation is affected by the adjustment of potential alcohol. In fact, Merrell et al. showed that higher initial anthocyanin and tannin concentrations favored polymeric pigment formation. Moreover, Merrell et al. described the anthocyanin-to-tannin ratio as a determinant factor for the formation of either np-PP and p-PP: more np-PP are formed with an excess of anthocyanins, whereas more p-PP are formed with an excess of tannins.<sup>11</sup> In the present study, tannin extraction in Cabernet Sauvignon was found to be more affected than anthocyanin extraction upon adjustment of potential alcohol; therefore, the anthocyanin-to-tannin ratio decreased. Indeed, p-PP significantly increased due to alcohol adjustment in Cabernet Sauvignon (Supplemental Figure 1), while np-PP were not affected. Since the wines in the present study were analyzed 11 months after alcoholic fermentation, it is assumed that the effect on p-PP is becoming more pronounced as more p-PP are being formed with ongoing wine aging.

Figure 7 reveals varietal differences with progressing grape maturity and enhanced potential alcohol. In Pinot noir wine, anthocyanin content increased significantly with increasing natural potential alcohol but was not affected by potential alcohol adjustment. Therefore, a high anthocyanin content in Pinot noir wine can reflect that it was produced from grapes with high phenolic maturity. For Cabernet Sauvignon wine, on the other hand, anthocyanins increased with increasing natural potential alcohol but also due to potential alcohol adjustment. Hence, the parameter anthocyanins is not suitable for Cabernet Sauvignon to draw conclusions about the phenolic maturity of grapes used for wine production. However, np-PP, which is related to anthocyanins, fit the criteria since they were independent of potential alcohol adjustment.

#### 4. CONCLUSIONS

Grape maturity cannot be limited to the sugar content in berries; the commonly used parameter "potential alcohol" indicates only the development of some grape constituents. However, alcohol supports the extraction of some grape constituents during red winemaking and directly contributes to the sensory characteristics of a wine. This study gave new insights into the contribution of potential alcohols to the phenolic and sensory characteristics of red wine. Natural and adjusted potential alcohols had a similar effect on the herbaceous aroma in Pinot noir. However, enhanced potential alcohol had no influence on the bell pepper aroma in Cabernet Sauvignon, suggesting that the IBMP extraction is independent of alcohol. Natural and adjusted potential alcohols had a similar effect on the body of the wines. Green astringency perception was partially influenced by potential alcohol, suggesting that alcohol contributes to grape maturity perception in Pinot noir wine by masking the unripe sensation.

Alcohol itself was found to be the main driver of bitterness in the wines; however, specific phenols also contribute to bitterness in relation to the alcohol.

An experimental model was developed revealing the relation between potential alcohol and phenolic parameters and sensory characteristics, respectively. Anthocyanins were found to provide the most accurate information about the grape maturity perception in Pinot noir. The anthocyanin content increased significantly with progressing grape maturity but was not affected by potential alcohol adjustment. Therefore, anthocyanins are suitable to reflect the maturity of grapes in Pinot noir wine. Nonprecipitable polymeric pigments provided the most accurate information about grape maturity perception in Cabernet Sauvignon wine. It can be assumed that varieties with a low phenolic content show a trend similar to that of Pinot noir, and varieties with a high phenolic content show a trend similar to that of Cabernet Sauvignon. In a climate change scenario, in which the sugar accumulation occurs ahead of other compounds, the sole use of potential alcohol as a grape maturity indicator could be misleading for the intended sensory characteristics of red wine.

#### ■ ASSOCIATED CONTENT

##### Supporting Information

The Supporting Information is available free of charge at <https://pubs.acs.org/doi/10.1021/acs.jafc.4c01035>.

Basic chemical analyses of 2019 Pinot noir must ( $n = 3$  experimental replicates), basic chemical analyses of 2019 Cabernet Sauvignon must ( $n = 3$  experimental replicates), basic chemical analyses of 2020 Pinot noir must ( $n = 2$  experimental replicates), basic chemical analyses of 2020 Cabernet Sauvignon must ( $n = 2$  experimental replicates), and maturity model for the chemical data of 2019 and 2020 Cabernet Sauvignon wines produced from grapes of different maturity levels without (circles) and with potential alcohol adjustment (triangles) (PDF)

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### Funding

This project was administered by the Research Association of the German Food Industry (FEI) and supported via the German Federation of Industrial Research Associations (AiF) within the program for promoting the Industrial Collective Research (IGF) of the Federal Ministry of Economic Affairs and Climate Action (BMWK), based on a resolution of the German Parliament; grant number AiF 20024N. Additional funding was provided by the University of Applied Sciences Kaiserslautern.

### Notes

The authors declare no competing financial interest.

### ACKNOWLEDGMENTS

The authors thank Daniel Munder and Luise Müller for their support. Participation in the sensory panel is gratefully acknowledged.

### ABBREVIATIONS

p-PPTabprecipitable polymeric pigments  
np-PPTabnonprecipitable polymeric pigments  
H1tabearly harvest  
H2tabcommercial harvest  
H3tablate harvest  
Ctabcontrol (natural potential alcohol)  
Atabadjusted potential alcohol

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## Supplemental Data

Supplemental Table 1. Basic chemical analyses of 2019 Pinot noir must (n = 3 experimental replicates)

	Relative density	pH	Titrateable acidity [g/L]
<b>Maturity</b>			
Early harvest	1.075 ± 0.000 c <sup>a</sup>	2.90 ± 0.00 c	16.2 ± 0.0 a
Commercial harvest	1.092 ± 0.000 b	3.12 ± 0.01 b	9.7 ± 0.2 b
Late harvest	1.096 ± 0.000 a	3.17 ± 0.01 a	6.9 ± 0.0 c
<i>p</i>	<i>&lt;0.0001</i>	<i>0.005</i>	<i>0.000</i>

<sup>a</sup> Within a column, values followed by the same letter are not significantly different according to Fisher's LSD test at  $p \leq 0.05$ . Significant  $p$  values ( $p \leq 0.05$ ) are shown in italic letters.

Supplemental Table 2. Basic chemical analyses of 2019 Cabernet Sauvignon must (n = 3 experimental replicates)

	Relative density	pH	Titrateable acidity [g/L]
<b>Maturity</b>			
Early harvest	1.075 ± 0.000 c <sup>a</sup>	3.32 ± 0.01 b	11.3 ± 0.2 a
Commercial harvest	1.090 ± 0.000 b	3.39 ± 0.04 a	7.2 ± 0.2 b
Late harvest	1.097 ± 0.001 a	3.43 ± 0.04 a	7.2 ± 0.3 b
<i>p</i>	<i>&lt;0.0001</i>	<i>0.007</i>	<i>&lt;0.0001</i>

<sup>a</sup> Within a column, values followed by the same letter are not significantly different according to Fisher's LSD test at  $p \leq 0.05$ . Significant  $p$  values ( $p \leq 0.05$ ) are shown in italic letters.

Supplemental Table 3. Basic chemical analyses of 2020 Pinot noir must (n = 2 experimental replicates)

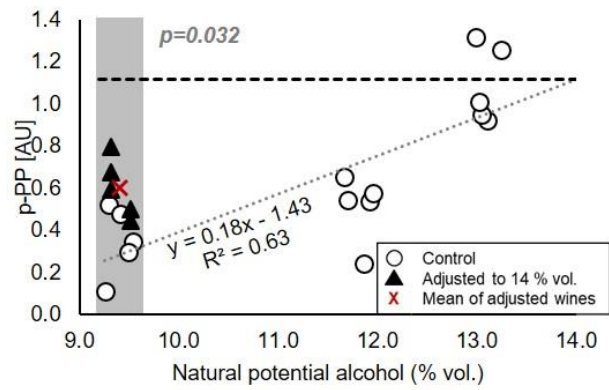
	Relative density	pH	Titratable acidity [g/L]
<b>Maturity</b>			
Early harvest	1.071 ± 0.000 c <sup>a</sup>	3.01 ± 0.01 c	13.3 ± 0.0 a
Commercial harvest	1.086 ± 0.001 b	3.25 ± 0.03 b	8.4 ± 0.3 b
Late harvest	1.102 ± 0.001 a	3.44 ± 0.08 a	7.3 ± 0.1 c
<i>p</i>	<i>&lt;0.0001</i>	<i>0.004</i>	<i>0.002</i>

<sup>a</sup> Within a column, values followed by the same letter are not significantly different according to Fisher's LSD test at  $p \leq 0.05$ . Significant  $p$  values ( $p \leq 0.05$ ) are shown in italic letters.

Supplemental Table 4. Basic chemical analyses of 2020 Cabernet Sauvignon must (n = 2 experimental replicates)

	Relative density	pH	Titratable acidity [g/L]
<b>Maturity</b>			
Early harvest	1.074 ± 0.000 c <sup>a</sup>	3.34 ± 0.06 b	10.5 ± 0.6 a
Commercial harvest	1.089 ± 0.000 b	3.64 ± 0.01 a	7.3 ± 0.1 b
Late harvest	1.099 ± 0.001 a	3.71 ± 0.11 a	6.6 ± 0.6 b
<i>p</i>	<i>&lt;0.0001</i>	<i>0.029</i>	<i>0.007</i>

<sup>a</sup> Within a column, values followed by the same letter are not significantly different according to Fisher's LSD test at  $p \leq 0.05$ . Significant  $p$  values ( $p \leq 0.05$ ) are shown in italic letters.



Supplemental Figure 1. Maturity model for the chemical data of 2019 and 2020 Cabernet Sauvignon wines produced from grapes of different maturity levels without (circles) and with potential alcohol adjustment (triangles).

## **5 Impact of Grape Berry Size on the Phenolic Composition of Pinot noir Wine**

The size of grape berries changes during ripening, however, there are also other factors that influence berry size. Grape varieties and clones are often discussed factors in this context. Previous studies have also shown that berry size can be manipulated by viticultural measures such as cluster exposure by leaf removal. Grape berry size influences the phenolic composition of red wine potentially by differences in phenolic maturity and different surface-to-volume ratios. Therefore, this study aimed to determine the impact of berry size on the phenolic composition of Pinot noir wine as well as to show whether more or less cluster exposure can lead to a higher uniformity in phenolic maturity reflected in wine produced from grapes of different harvest dates. Pinot noir vines were either defoliated when the berries reached pea size (BBCH stage 75) or left untreated as a control. Grapes were harvested manually at two different harvest dates and carefully sorted into three size categories of healthy and fully intact berries. The influence of cluster exposure and timing of harvest on the berry size distribution was marginal, which is different than what we expected. Microvinifications were performed on a 900 g berries scale. Chemical analysis showed that cluster exposure and harvest date as well as berry size affected the phenolic composition of Pinot noir wine. Cluster exposure enhanced the content of seed-associated phenolics whereas late harvesting of grapes increased the content of skin-associated phenolics. Small berries produced more concentrated wines than large berries. This was especially reflected in polymeric pigments and anthocyanins, which are often considered indicators for red wine quality.

## Impact of grape berry size on the phenolic composition of Pinot noir wine

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Disclaimer:

The presented work was performed exclusively by Weincampus Neustadt/DLR Rheinpfalz (Sandra Feifel and bachelor thesis Julian Hantke). For more information, refer to the author contribution section.

Author Contributions:

**Sandra Feifel:** Conceptualization, Methodology, Investigation, Winemaking, Chemical analyses, Sensory analysis, Writing – Original draft, Review and Editing. **Julian Hantke:** Conceptualization, Methodology, Investigation, Chemical analyses. **Pascal Wegmann-Herr:** Conceptualization, Writing - Review and Editing. **Elke Richling:** Supervision, Writing - Review and Editing. **Dominik Durner:** Conceptualization, Supervision, Writing – original draft, Review and Editing, Funding acquisition.

All authors have read and agreed to the submitted version of the manuscript.

Citation:

Feifel, S., Hantke, J., Wegmann-Herr, P., Richling, E., and Durner, D. "Impact of grape berry size on the phenolic composition of Pinot noir wine" (submitted to *OENO One*).

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# 1 Impact of grape berry size on the phenolic composition 2 of Pinot noir wine

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16

17 **Abstract**

18 Grape berry size is often discussed as a factor influencing the phenolic profile of red wine. The  
19 objective of this study was to investigate the effect of berry size on the phenolic composition of Pinot  
20 noir wine. Since grape berry sizes can change with progressing grape maturity, the study regarded  
21 early harvest timing at 20 Brix and late harvest timing at 24 Brix. Moreover, grape maturity may be  
22 influenced by sun exposure of grape clusters which was achieved by leaf removal in the grape zone  
23 of the canopy. Accordingly, the study investigated whether more or less cluster exposure has an  
24 impact on grape maturity, and therefore on grape berry sizes, eventually leading to higher uniformity  
25 in phenolic composition and concentration of phenolic compounds in Pinot noir wine. Different to  
26 what was expected, the effect of sun exposure of grape clusters and the impact of harvest timing on  
27 the berry size distribution was marginal. While late harvest of grapes increased the sugar content and  
28 the content of certain skin-associated phenolics, more sun exposure of grape clusters did not increase  
29 the sugar content but the content of seed-associated phenolics. This indicates lower phenolic maturity  
30 of grapes after leaf removal which might have been a result of foliar stress initiated by this treatment.  
31 Wines produced from small berries were richer in phenolics, especially in polymeric pigments,  
32 anthocyanins and tannins, than wines produced from large berries. This was true for early and late  
33 harvested grapes as well as more or less sun exposed grape clusters. The combined effect of exposed  
34 grape clusters and small berries produced wines with the highest content of anthocyanins and  
35 polymeric pigments. This suggests that those wines might have the highest ageing potential in terms  
36 of colour stability.

37

38 **Keywords:** phenolic composition, leaf removal, berry size, anthocyanins, tannins, polymeric  
39 pigments, Pinot noir

40

## 41 Introduction

42 The phenolic composition, concentration and extractability in grape berries change during the  
43 ripening process. Anthocyanins accumulate in the grape skins until they reach a maximum (Glories,  
44 1986; Sternad Lemut *et al.*, 2011), whereas the monomeric flavan-3-ol content gets lower due to  
45 polymerisation reactions (Freitas, 1995; Kennedy *et al.*, 2000a; Ribéreau-Gayon *et al.*, 2006).  
46 Monomeric and oligomeric flavanols are more easily extracted during winemaking while the grapes  
47 are unripe and the seed coat has not fully hardened, yet (Downey *et al.*, 2003; Hensen *et al.*, 2023;  
48 Kennedy *et al.*, 2000a; Kennedy *et al.*, 2000b).

49 While the sizes of grape berries increase with progressing grape maturity, studies have shown that  
50 intrinsic factors such as the number and weight of seeds in grape berries pre-determine the size the  
51 berries are going to reach (Coombe, 1960; Dai *et al.*, 2011; Walker *et al.*, 2005). Ristic and Iland  
52 (2005) reported that the berry development is affected by seed development. Other studies  
53 investigated the influence of different rootstock on the berry size (Walker *et al.*, 2005). Winemakers  
54 aim to achieve a homogenous grape maturity in vineyards to avoid unripe or overripe aromas and  
55 taste or a dilution of desirable characteristics (Calderon Orellana *et al.*, 2014). Calderon Orellana *et al.*  
56 (2014) have shown a positive linear correlation of the berry skin weight with the size of a berry.  
57 Roby *et al.* (2004) showed that the concentration of all soluble substances increase proportionally  
58 with an increase in berry size. Anthocyanins were shown to be the exception as their concentration  
59 decrease with increasing berry size while skin proanthocyanidins remain at a similar level (Lafontaine  
60 *et al.*, 2013; Roby *et al.*, 2004). Lafontaine *et al.* (2013) observed that the tannin concentration in  
61 wine increases with increasing berry size but is lower in high TSS (total soluble solids) wine made  
62 from berries with the same size category. In the same berry size category, the extraction of  
63 anthocyanins into the wine was found to be enhanced at higher TSS (Lafontaine *et al.*, 2013). Large  
64 berries have a lower surface-to-volume ratio than small berries, accordingly concentrations of skin  
65 phenolics per berry are lower (Matthews and Nuzzo, 2007). Gil Cortiella *et al.* (2020), however, have  
66 shown that large Carménère berries have thicker berry skins and therefore a greater proportion of  
67 skins (% m/m) (Gil Cortiella *et al.*, 2020). Wines produced from these Carménère grapes with a higher  
68 weight proportion of skins had higher concentrations of phenolics, terpenes and volatile acids, acetate  
69 esters and polysaccharides (Gil Cortiella *et al.*, 2020). Therefore, the concentration of anthocyanins  
70 also increased with the weight proportion of the skins (Barbagallo *et al.*, 2011; Gil Cortiella *et al.*,  
71 2020). Gil Cortiella *et al.* (2020) observed a positive correlation of a higher extraction of anthocyanins  
72 with a higher skin to juice ratio but not with the relative surface of the berries on their own. Therefore,  
73 the extraction of phenolics from the skins is stronger related to the proportion of the skin than the size

74 of the berry (Gil Cortiella *et al.*, 2020). The assumption that large berries are lower in quality or less  
75 desirable is often based on the conjecture of water diluting solutes which are important for wine  
76 quality. This theory does not take all physiological factors of berry growth into consideration. The  
77 increase in berry size during ripening is a complex process following a double sigmoid growth  
78 pattern, in which the dry weight as well as the fresh weight increases. In order to dilute the desired  
79 solute, the water intake would have to exceed the accumulation of solutes (Matthews and Nuzzo,  
80 2007). Roby and Matthews (2004) also reported that the proportions of skin, seeds and flesh that  
81 make up the fresh weight of Cabernet Sauvignon berries were not affected by a variation of berry  
82 size, indicating that berry skins are not being stretched like an inflating balloon. These findings as  
83 well as observations by Gil Cortiella *et al.* (2020) who presented more concentrated and deeper  
84 coloured wines produced from large berries raise the question whether berry size on its own is a  
85 suitable parameter to predict wine composition or colour intensity. Berry size as well as berry  
86 composition can be modulated by extrinsic factors such as environmental and viticultural factors,  
87 which has been demonstrated in previous studies (Dai *et al.*, 2011). The vine's physiological status  
88 and therefore the synthesis of secondary metabolites are altered by application of pruning treatments  
89 (Holt *et al.*, 2008), water deficits (Roby *et al.*, 2004; Roby and Matthews, 2004) and defoliation  
90 (Hunter *et al.*, 1991; Sternad Lemut *et al.*, 2011). At harvest, it is therefore difficult to determine  
91 whether a small berry is riper than a large berry.

92 During grape ripening, anthocyanins protect photosynthetic tissues by reducing light stress due to  
93 light absorbance in the visible range (Clifford, 2000; Jimenez-Garcia *et al.*, 2013; Paredes-López  
94 *et al.*, 2010; Y. Zhang *et al.*, 2014). However, anthocyanins cannot protect these tissues from high UV  
95 light stress (Zhao *et al.*, 2022). The accumulation of anthocyanins can also be a response to drought  
96 stress or nitrogen deficiency (Castellarin *et al.*, 2007; Petroni and Tonelli, 2011; Soubeyrand *et al.*,  
97 2014). Leaf removal is a common viticultural treatment to alter the grape cluster microclimate by  
98 enhancing the air circulation, sunlight exposure and berry temperature, depending on the time of  
99 application. It can reduce the risk of *Botrytis cinerea* infection in vintages with high humidity (English  
100 *et al.*, 1989; Smart *et al.*, 2017). Lee and Skinkis (2013) investigated the effect of leaf removal on  
101 berry maturity by reaching better sun exposure of grape clusters. Traditional berry maturity indicators  
102 such as pH, berry weight and TSS were not significantly affected by leaf removal. Anthocyanins,  
103 however, increased significantly due to the enhanced sun exposure (Lee and Skinkis, 2013; Wicks  
104 and Kliewer, 1983). Sternad Lemut *et al.* (2011) observed that three groups of phenolics were  
105 influenced by leaf removal. Hydroxycinnamic acids were only affected at early ripening stage,  
106 whereas anthocyanins and flavanols were significantly enhanced at the time of harvest. An increase

107 in berry weight after leaf removal was observed (Sternad Lemut *et al.*, 2011). This is in contrast to  
108 the findings of Ollat and Gaudillere (1998) who showed a decrease in berry weight after leaf removal  
109 and Chorti *et al.* (2010) or Tardáguila *et al.* (2008) who reported no significant differences in berry  
110 size.

111 Previous studies reported contrasting findings on whether small or large berries produce wines with  
112 higher colour intensity or other quality traits. Viticultural practices such as berry cluster exposure by  
113 leaf removal are extrinsic factors that influence grape berry size and grape composition, however,  
114 previous observations differed strongly on the impact of more or less berry cluster exposure on berry  
115 size. The aim of this study was to determine whether more or less berry cluster exposure leads to a  
116 higher uniformity in composition and concentration of phenolic compounds in wine produced from  
117 grapes of different harvest dates. Rather than using different grape vine clones in order to modulate  
118 the mean berry size and berry size heterogeneity, an approach of more or less berry cluster exposure  
119 and different harvest dates was chosen as viticultural measures for winemakers to potentially  
120 modulate the berry size distribution in their vineyard and phenolic composition of their wine.

121

## 122 **Materials and methods**

### 123 **1. Vineyard site**

124 The study was conducted during the 2020 vintage at the Weincampus Neustadt experimental vineyard  
125 located in the Pfalz region of Germany (lat. 49°24' N; long. 8°11' E). *Vitis vinifera* L. cv. Pinot noir  
126 (PN; clone Mariafeld on SO4 rootstock) was planted in 1987 with a vine by row spacing of 1.88 m ×  
127 1.20 m. The trellis system was a single guyot with vertical shoot positioning and five shoots per vine.  
128 The number of observed clusters were two clusters per shoot on average. The vineyard row  
129 orientation is North to South. No irrigation system was used. The growing degree days (GDD) were  
130 calculated with data from an on-site weather station yielding 1683 GDD according to “Method 1” by  
131 McMaster and Wilhelm (1997). A Huglin Index (HI) of 2178 was calculated according to Huglin  
132 (1978). Temperature and precipitation data for the vegetation period of the 2020 vintage is provided  
133 in Supplemental Figure 1.

### 134 **2. Leaf removal treatments**

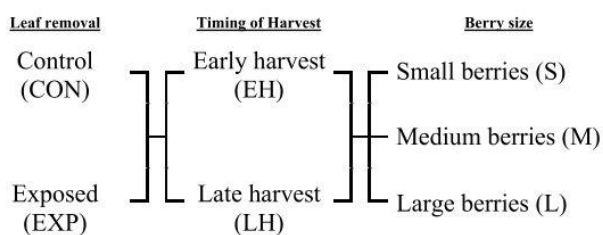
135 The experimental vineyard covered 12 rows with a total acreage of 0.28 ha. All rows received two-  
136 sided mechanical leaf removal around the bunches by ERO pulsed air defoliator VITIPulse (ERO,  
137 Simmern, Germany) at BBCH stage 69 (end of flowering). The reason for this treatment was to reduce

138 cluster compactness and enhance air ventilation in order to reduce the risk of fungal infection such as  
 139 *Botrytis cinerea* (English *et al.*, 1989; Sivilotti *et al.*, 2016; Smart *et al.*, 2017). Six rows were chosen  
 140 at random for an additional one-sided leaf removal treatment at BBCH stage 75 (pea-sized berries)  
 141 using the same device as mentioned above. This procedure was chosen for increased sun exposure of  
 142 the bunches. The percentage of leaf area reduced was less than 10 %. Hypothetically, better sun  
 143 exposure might have an influence on the ripening progress and berry size heterogeneity (Lee and  
 144 Skinkis, 2013; Sternad Lemut *et al.*, 2011; Wicks and Kliewer, 1983). The rows which did not receive  
 145 leaf removal at BBCH stage 75 were considered as control (CON) and the rows with leaf removal at  
 146 BBCH stage 75 were considered as exposed (EXP).

### 147 3. Wine production

148 Healthy grape bunches of both treatments, CON and EXP, were harvested manually into separate  
 149 containers. Harvests were conducted for both treatments at two different time points which were two  
 150 weeks apart from each other (early harvest: EH; late harvest: LH). At EH the CON treatment yielded  
 151  $13.0 \pm 1.4$  t/ha and the EXP treatment yielded  $9.2 \pm 1.4$  t/ha. At LH the CON treatment yielded  
 152  $13.2 \pm 2.2$  t/ha and the EXP treatment yielded  $11.1 \pm 1.1$  t/ha. Random berries of the grape bunches  
 153 were cut from the rachis using scissors to yield fully intact berries with <1 mm of pedicel still attached  
 154 to them. The berries were then sorted into three size categories using trays with holes (Supplemental  
 155 Figure 2) in different diameters (small berries <13 mm; medium berries 13-15 mm; large berries  
 156 >15 mm) as shown in Figure 1.

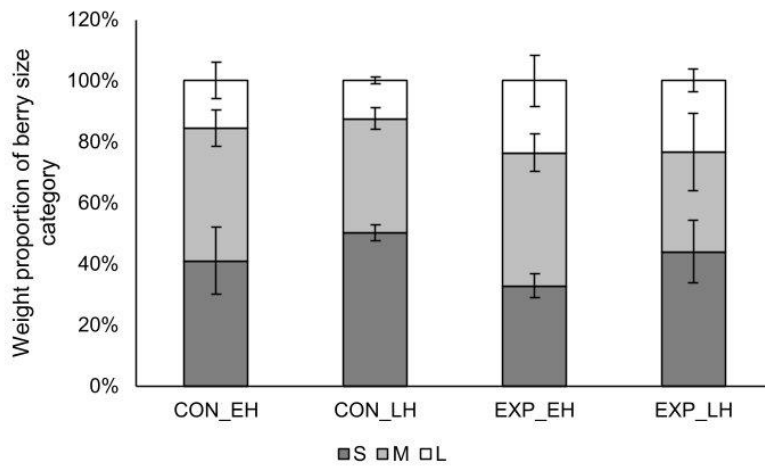
157



158 **Figure 1. Schematic representation of the experimental design**

159 The berry size distribution by weight proportions is shown in Figure 2 for all four treatments. On  
 160 average, the berries had one seed per berry, which did not differ significantly across treatments.

161



162

163 **Figure 2. Berry size distribution by weight proportions of a 66-berry sample for two harvest**  
 164 **dates (early (EH) and late harvest (LH)) and two leaf removal treatments (exposed (EXP) and**  
 165 **control (CON)) (data expressed as mean values  $\pm$  standard deviation of n = 3 replicates).**

166 Table 1 shows the weight proportions of Pinot noir skins, seeds and pulp of different berry sizes. With  
 167 increasing berry size, the proportion of skins gets lower whereas the proportion of pulp increases.  
 168 The proportion of seeds remains the same for all berry sizes.

169 **Table 1. Weight proportions of Pinot noir skins, seeds and pulp of different berry sizes (n =**  
 170 **1000 berries per size category).**

Berry size	Skins	Seeds	Pulp
S	26.9%	4.8%	68.3%
M	25.1%	4.8%	70.0%
L	20.6%	4.8%	74.6%

171

172 Table 2 shows the surface to volume ratios of Pinot noir berries of different sizes at early and late  
 173 harvest of the control and the exposed treatment. With an increase in berry size, the surface to volume  
 174 ratio decreased which is in agreement with the literature (Matthews and Nuzzo, 2007). The surface  
 175 to volume ratios of medium sized and large berries were the same across the harvest dates and leaf  
 176 removal treatments. The surface to volume ratios for the small berries of the treatment with high berry  
 177 cluster exposure were slightly higher than those of the control treatment.

178 **Table 2. Surface to volume ratio of Pinot noir berries of different sizes at early (EH) and late**  
 179 **harvest (LH) of the control (CON) and the exposed (EXP) treatment (n = 200 berries per size**  
 180 **category)**

Berry size	CON EH	CON LH	EXP EH	EXP LH
S	0.59	0.56	0.63	0.65
M	0.43	0.43	0.43	0.43
L	0.38	0.38	0.38	0.38

181

182 Three lots of 900 g berries for each size category (S, M, and L), different cluster exposure (CON and  
 183 EXP) and time of harvest (EH and LH) were manually crushed in plastic bags and the resulting musts  
 184 transferred into 1 L UPPHETTA coffee makers (IKEA, Delft, The Netherlands). Potential alcohol  
 185 adjustment was not performed, because previous studies have shown that the enhanced alcohol has  
 186 an influence on the extraction of phenolics (Feifel *et al.*, 2024). The TSS of each lot are reported in  
 187 Supplemental Table 1. The musts were inoculated with 200 mg/L ZYMAFLORE® RB2 yeast  
 188 (LAFFORT®, Bordeaux, France) and 300 mg/L LALVIN® GO-FERM (LALLEMAND, Wien,  
 189 Austria). Parafilm® (Bemis Company, Inc., Neenah, USA) was used around the lid of the glass vessel  
 190 to reduce the risk of contamination. Once fermentation commenced, wines were manually punched  
 191 down three times per day using the built-in plunger. This procedure took approximately 15 seconds  
 192 per fermenter. After the grape solids were compressed at the bottom of the glass vessel, the plunger  
 193 was pulled up again. Fermentations were monitored with a handheld density meter (DMA 35 Basic,  
 194 Anton Paar, Graz, Austria). On day 5, fermenting wines were co-inoculated with 10 mg/L  
 195 *Oenococcus oeni* VP41 (ZEFÜG GmbH & Co. KG, Bingen, Germany) and 1 mg/L *Lactobacillus*  
 196 *plantarum* ML Prime™ (LALLEMAND Inc., Aurillac, France), because previous studies have  
 197 reported improved malolactic fermentation (MLF) for co-cultures compared to single MLF cultures  
 198 (Devi and Anu-Appaiah, 2021; B. Zhang *et al.*, 2024). Wines were kept on skins and seeds for a total  
 199 maceration time of 7 days. After maceration, all wines were < 1 g/L residual sugar. Wines were  
 200 manually pressed off using the plunger of the coffee maker and transferred to 0.5 L Bordeaux style  
 201 wine bottles with lose screw caps to complete malolactic fermentation. Two weeks after completion  
 202 of malolactic fermentation which was defined when < 0.3 g/L malic acid was reached, 60 mg/L SO<sub>2</sub>  
 203 (IOC Sulfivin K150, ZEFÜG GmbH & Co. KG, Bingen, Germany) was added. Each wine was racked  
 204 off the solids, filled into 0.25 L Bordeaux style wine bottles, and stored at 16°C for 3 months. All  
 205 experimental treatments were carried out in triplicate.

#### 206 4. Chemicals

207 Hydrochloric acid, propan-2-ol, and sodium chloride were purchased from AppliChem (Darmstadt,  
208 Germany). Ethanol, ortho-phosphoric acid, acetic acid, and potassium metabisulfite were purchased  
209 from ORG Laborchemie (Bunde, Germany). Procyanidin B2 and procyanidin C1 were purchased  
210 from PhytoLab (Vestenbergsgreuth, Germany). Caffeic acid, cyanidin-3-*O*-rutinoside, (-)-  
211 epicatechin-3-*O*-gallate, (-)-epigallocatechin, (+)-gallocatechin, and oenin chloride were purchased  
212 from PhytoPlan (Heidelberg, Germany). Bovine serum albumin fraction V, and L-(+)-tartaric acid  
213 were purchased from Roth (Karlsruhe, Germany). Caffeic acid, (+)-catechin hydrate, (-)-epicatechin,  
214 gallic acid monohydrate, sodium hydroxide, sodium dodecyl sulfate, maleic acid, triethanolamine and  
215 iron(III) chloride were purchased from Sigma Aldrich (Steinheim, Germany). Acetonitrile and formic  
216 acid were purchased from Bernd Kraft (Duisburg, Germany) and Honeywell (Morris Plains, New  
217 Jersey), respectively. Potassium dihydrogen phosphate was purchased from VWR International  
218 (Darmstadt, Germany). Ultrapure water was obtained from a PURELAB flex (ELGA LabWater,  
219 Celle, Germany) water purification system. All chromatographic solvents were HPLC grade.

#### 220 5. Basic analytical parameters

221 Wine parameters (alcohol, residual sugars, pH, titratable acidity) were measured by FT-IR  
222 (WineScan™ Auto, FOSS GmbH, Hamburg, Germany) using a calibration method provided by the  
223 manufacturer. Samples were centrifuged for 5 min at 4500 rpm and degassed prior to FT-IR analysis.

#### 224 6. Spectrophotometric analysis

225 Iron reactive phenolics, tannins, protein-precipitable polymeric pigments (p-PP), and nonprecipitable  
226 polymeric pigments (np-PP) were measured by the assay described by Harbertson *et al.* (Harbertson  
227 *et al.*, 2002; Harbertson *et al.*, 2003) The terms “large and small polymeric pigments” are no longer  
228 used since the size distinction between them has not been defined and a distinction according to their  
229 precipitability is therefore more appropriate (Harbertson *et al.*, 2013). A Jasco V-730 double-beam  
230 UV–visible spectrophotometer (Jasco GmbH, Pfungstadt, Germany) was used for all the  
231 measurements.

#### 232 7. HPLC-DAD/FD analysis

233 The analysis was performed using an HPLC-DAD/FD (Jasco, Pfungstadt, Germany) comprising a  
234 PU-4180 pump, an AS-4150 autosampler, an MD-4010 photometric diode array detector, and an FP-

235 4025 fluorescence detector. Separation and quantification of phenolics was achieved with the method  
236 described by Feifel *et al.* (2024).

237 Samples were filtered through 0.45 µm cellulose acetate membranes (Graphic Controls, Totnes, UK)  
238 and then analysed directly. ChromNAV (version 2.0, Jasco, Pfungstadt, Germany) was used for the  
239 HPLC data analysis. All quantitative analyses of phenolic composition were performed in duplicates.

#### 240 **8. LC-QToF-MS analysis.**

241 The analysis was performed using an LC-QToF-MS (Agilent Technologies, Waldbronn, Germany)  
242 comprising an Agilent 1260 Infinity Binary Pump, an Agilent 1260 Infinity HiP Degasser, an Agilent  
243 1260 Infinity HiP ALS Autosampler, an Agilent Jet Stream Technology Ion Source (AJS) G-1958-  
244 65138, an Agilent 1260 DAD, and a 6530 Accurate-Mass Q-TOF LC/MS. Separation and  
245 quantification of phenolics was achieved with the method described by Feifel *et al.* (2024).

246 Samples were filtered through 0.45 µm cellulose acetate membranes (Graphic Controls, Totnes, U.K.)  
247 and then analysed directly. MassHunter Workstation Software (Quantitative Analysis, version  
248 B.05.00, Build 5.0.291; Agilent Technologies, Waldbronn, Germany) was used for the MS data  
249 analysis. All the quantitative analyses of phenolic composition were performed in duplicates.

#### 250 **9. Statistical analysis.**

251 The data was processed by a three-way mixed model analysis of variance (ANOVA). Analytical  
252 repetitions and experimental replicates were regarded as separate factors. Fisher's least significant  
253 difference (LSD) post-hoc test ( $p \leq 0.05$ ) was carried out for all data. Principal component analysis  
254 (PCA) was performed for chemical data using Pearson correlation ( $n-1$ ). All statistical analyses were  
255 performed using XLSTAT SENSORY (Version 2023.3.0 (32 bit), Addinsoft SARL, Paris, France).

256

## 257 **Results and Discussion**

### 258 **1. Basic chemical parameters of Pinot noir wine**

259 Table 3 shows the basic chemical parameters of Pinot noir wine from the control and the treatment  
260 with high berry cluster exposure at early and late harvest produced from different berry sizes. Late  
261 harvest significantly increased the alcohol content and decreased titratable acidity which is in  
262 agreement with our previous findings (Feifel *et al.*, 2024). The treatment with high berry cluster  
263 exposure produced wines with significantly higher titratable acidity and lower pH which is in

264 agreement with the findings of Main and Morris (2004) for Cynthiana wine in hot vintages. Hunter  
 265 *et al.* (1991) also observed slightly higher titratable acidity as well as total soluble solids in berries  
 266 from partially defoliated vines compared to non-defoliated vines, however, the differences were not  
 267 significant. They suggest that partial leaf removal improves canopy microclimate and photosynthetic  
 268 activity (Hunter *et al.*, 1991). Wines produced from small berries had significantly higher alcohol and  
 269 titratable acidity concentrations and a lower pH indicating that they are more concentrated than large  
 270 berries.

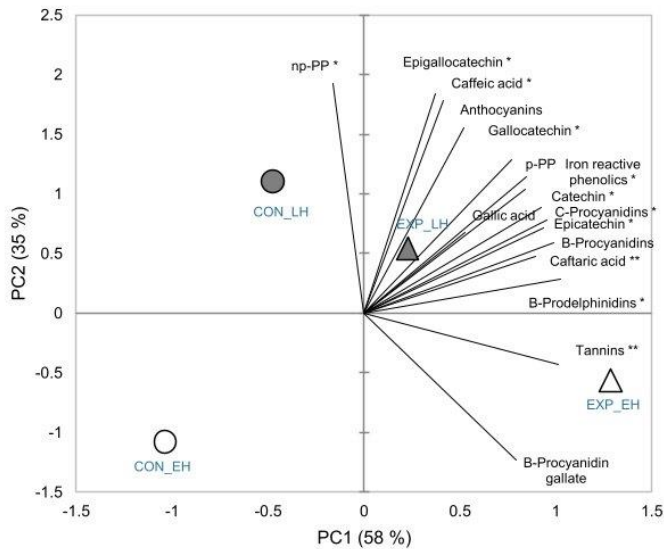
271 **Table 3. Basic chemical analyses of Pinot noir wine (n = 3 experimental replicates ± SD)**

			Alcohol [%v/v]	Residual sugar [g/L]	pH	Titratable acidity [TA g/L]	
<b>Harvest date</b>	<b>Cluster exposure</b>	<b>Berry size</b>					
Early	Control	small	11.8 ± 0.3 cd <sup>a</sup>	1.0 ± 0.1 abc	3.50 ± 0.02 bc	6.2 ± 0.0 bc	
		medium	10.7 ± 0.1 ab	0.8 ± 0.1 a	3.62 ± 0.03 de	5.7 ± 0.0 ab	
		large	10.3 ± 0.3 a	0.7 ± 0.3 a	3.62 ± 0.05 de	5.8 ± 0.1 ab	
	Exposed	small	12.0 ± 0.4 de	0.9 ± 0.1 ab	3.31 ± 0.14 a	6.9 ± 0.7 d	
		medium	11.1 ± 0.6 bc	1.1 ± 0.2 abc	3.58 ± 0.07 bcde	5.9 ± 0.3 ab	
		large	10.8 ± 0.3 ab	0.9 ± 0.1 ab	3.61 ± 0.04 cde	5.9 ± 0.2 ab	
	Late	Control	small	14.4 ± 0.7 h	1.2 ± 0.3 bcd	3.49 ± 0.07 b	6.0 ± 0.3 abc
			medium	13.5 ± 0.6 g	1.4 ± 0.2 d	3.63 ± 0.03 de	5.7 ± 0.1 a
			large	12.6 ± 0.7 ef	1.2 ± 0.2 bcd	3.67 ± 0.07 e	5.6 ± 0.1 a
Exposed		small	14.4 ± 0.3 h	1.2 ± 0.3 bcd	3.36 ± 0.08 a	6.5 ± 0.5 cd	
		medium	13.4 ± 0.2 g	1.2 ± 0.1 bcd	3.55 ± 0.05 bcd	5.8 ± 0.1 ab	
		large	12.8 ± 0.2 fg	1.3 ± 0.2 cd	3.66 ± 0.05 de	5.6 ± 0.1 a	
<i>p</i>		<0.0001	0.004	<0.0001	0.001		
<b>Harvest date</b>							
Early		11.1 ± 0.7 a	0.9 ± 0.2 a	3.54 ± 0.13 a	6.1 ± 0.5 b		
Late		13.5 ± 0.8 b	1.3 ± 0.2 b	3.56 ± 0.12 a	5.8 ± 0.4 a		
<i>p</i>		<0.0001	<0.0001	0.358	0.036		
<b>Cluster exposure</b>							
	Control	12.2 ± 1.6 a	1.1 ± 0.3 a	3.59 ± 0.08 b	5.8 ± 0.2 a		
	Exposed	12.4 ± 1.3 a	1.1 ± 0.2 a	3.51 ± 0.15 a	6.1 ± 0.6 b		
<i>p</i>		0.154	0.506	0.003	0.014		
<b>Berry size</b>							
	small	13.2 ± 1.4 c	1.1 ± 0.2 a	3.42 ± 0.11 a	6.4 ± 0.5 b		
	medium	12.2 ± 1.4 b	1.1 ± 0.3 a	3.60 ± 0.05 b	5.8 ± 0.2 a		
	large	11.6 ± 1.2 a	1.1 ± 0.3 a	3.64 ± 0.05 b	5.7 ± 0.2 a		
<i>p</i>		<0.0001	0.694	<0.0001	<0.0001		

272 <sup>a</sup> Within a column, values followed by the same letter are not significantly different according to Fisher's LSD test at  
 273  $p \leq 0.05$ . Significant  $p$  values ( $p \leq 0.05$ ) are shown in italic letters.  
 274

275 **2. Influence of cluster exposure and harvest timing on the phenolic composition of Pinot**  
276 **noir wine.**

277 In order to systematically manipulate the size of grape berries, cluster exposure and harvest timing  
278 were investigated as factors according to previous studies (Abi-Habib *et al.*, 2021; Hunter *et al.*, 1991;  
279 Mirás-Avalos *et al.*, 2019; Sternad Lemut *et al.*, 2011). Initially, it was important to determine how  
280 cluster exposure and timing of harvest influence the phenolic composition of Pinot noir wine  
281 irrespective of berry sizes. Therefore, PCA space was calculated for the wines produced from  
282 medium-sized berries of control and exposed treatment for early and late harvest grapes on 16  
283 phenolic parameters (Figure 3).



284  
285 **Figure 3. Principal component analysis for Pinot noir wines of the exposed treatment (EXP,**  
286 **triangle) and control (CON, circle) produced from medium-sized berries for early harvest (EH)**  
287 **and late harvest (LH) grapes (data expressed as mean values of n = 3 experimental replicates).**  
288 **PCA space was calculated on 16 displayed phenolic parameters (\* significant at  $p < 0.05$ ,**  
289 **\*\* significant at  $p < 0.01$ , \*\*\* significant at  $p < 0.001$ ).**

290 In Figure 3, the first two PCs with eigenvalues  $> 1$  explained 93% of the cumulative variability, with  
291 PC1 accounting for 58% of the total variance. Cluster exposure was separated on PC1, harvest timing  
292 was separated on PC2. Wines of the exposed treatment were positively loaded on PC1, which is  
293 explained by higher concentrations of tannins, B-prodelphinidins, caftaric acid, B-procyanidins, C-  
294 procyanidins, epicatechin, catechin, gallic acid, p-PP, and iron reactive phenolics. Late harvest wines

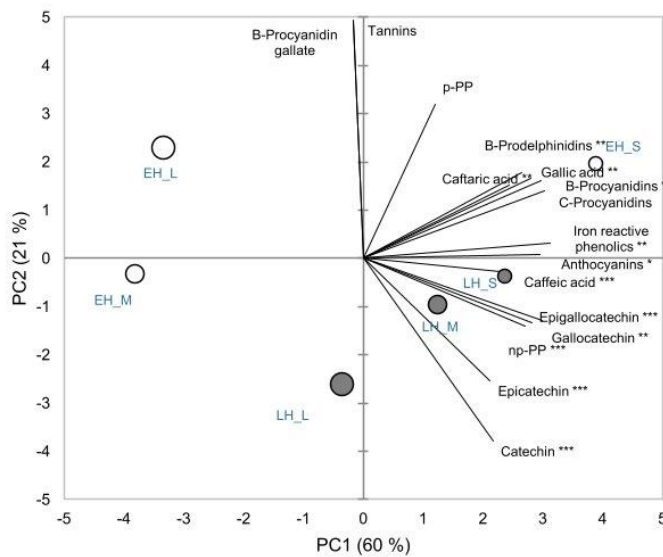
295 were positively loaded on PC2, which is explained by higher concentrations of epigallocatechin,  
296 caffeic acid, anthocyanins, np-PP, and lower B-procyanidin gallate concentrations. Figure 3  
297 demonstrates that the influence of cluster exposure was different from the influence of harvest timing.  
298 The exposed treatment enhanced the content of seed-associated phenolics which is in agreement with  
299 the findings of Baiano *et al.* (2015) and Sivilotti *et al.* (2016), whereas late harvest increased the  
300 content of skin-associated phenolics (Sivilotti *et al.*, 2016). The impact of cluster exposure on the  
301 phenolic composition was more pronounced at early harvest than at late harvest suggesting that vines  
302 adjust their carbon and nitrogen distribution after leaf removal. The temporary foliar stress after leaf  
303 removal can reduce cell division rates pre-véraison and hence postpone grape ripening (Palliotti *et*  
304 *al.*, 2013). The higher seed phenolic concentrations in the wine could have been either induced by  
305 higher phenolic concentrations in the seeds or a higher extractability of seed-associated phenolics.  
306 With progressing ripening, the differences due to cluster exposure were less pronounced because the  
307 vines had more time to adjust their carbon and nitrogen distribution after leaf removal.

### 308 **3. Influence of different berry sizes on the phenolic composition of Pinot noir wine.**

#### 309 **3.1 Harvest timing**

310 For control and exposed treatment, PCA space was calculated on the same 16 phenolic parameters.  
311 Figures 4 and 5 show the PCA for Pinot noir wines produced from grapes of different berry sizes of  
312 early and late harvest timing. For control (Figure 4), the first two PCs with eigenvalues > 1 explained  
313 81% of the cumulative variability, with PC1 accounting for 60% of the total variance. For the exposed  
314 treatment (Figure 5), the first two PCs with eigenvalues > 1 explained 91% of the cumulative  
315 variability, with PC1 accounting for 68% of the total variance. In both PCA, berry sizes were  
316 separated on PC1, and harvest timing was separated on PC2. Small berries were positively loaded on  
317 PC1, which is explained by higher concentrations of caftaric acid, gallic acid, B-prodelphinidins, B-  
318 and C-procyanidins, iron reactive phenolics, anthocyanins, caffeic acid, epigallocatechin,  
319 galocatechin, and np-PP for the control (Figure 4) and by higher concentrations of galocatechin, B-  
320 procyanidin gallate, gallic acid, B- and C-procyanidins, p-PP, iron reactive phenolics, caffeic acid,  
321 epigallocatechin, and anthocyanins for the exposed treatment (Figure 5). Large berries were  
322 negatively loaded on PC1, which is explained by lower contents of the aforementioned phenolics for  
323 both treatments. Early harvest wines were positively shifted on PC2 which is explained by a higher  
324 tannin content for both treatments. Late harvest wines were negatively loaded on PC2. At early  
325 harvest, the shift on PC1 from small to medium-sized berries was more pronounced than at late  
326 harvest, which could be observed for the control and the exposed treatment (Figures 4 and 5). This

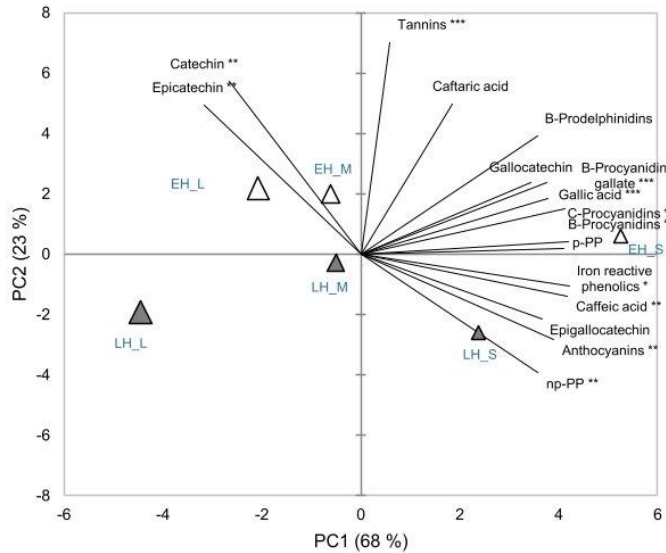
327 suggests that the phenolic composition of wines produced from small and medium-sized berries was  
 328 less distinguishable with progressing grape maturity. The difference between small and medium-sized  
 329 berries was also more pronounced than the difference between medium-sized and large berries. The  
 330 phenolic composition was more heterogeneous in small berries than in large berries. For the control,  
 331 the overall differences between the berry sizes at late harvest were smaller than at early harvest.  
 332 Previous studies suggest that the different expression of certain genes in the berries are responsible  
 333 for the synchronization of their transcriptional states toward maturity. Berries that are lagging in  
 334 maturity have been shown to complete their transcriptional program in a shorter period through  
 335 altered gene expressions and ripening-related hormone dynamics (Gouthu *et al.*, 2014). For the  
 336 exposed treatment, the overall difference of size categories remained similar for both harvest dates.  
 337 This suggests that enhanced leaf removal leads to more homogeneous grape ripening at earlier harvest  
 338 dates.



339

340 **Figure 4. Principal component analysis for Pinot noir wines of the control produced from**  
 341 **grapes of different maturity levels (early harvest (EH), open symbols; late harvest (LH), filled**  
 342 **symbols) and with different berry sizes (data expressed as mean values of n = 3 experimental**  
 343 **replicates). PCA space was calculated on the 16 displayed phenolic parameters (\* significant at**  
 344 **p < 0.05, \*\* significant at p < 0.01, \*\*\* significant at p < 0.001).**

345



346

347 **Figure 5. Principal component analysis for Pinot noir wines of the exposed treatment produced**  
 348 **from grapes of different maturity levels (early harvest (EH), open symbols; late harvest (LH),**  
 349 **filled symbols) and with different berry sizes (data expressed as mean values of n = 3**  
 350 **experimental replicates). PCA space was calculated for the 16 displayed phenolic parameters**  
 351 **(\* significant at  $p < 0.05$ , \*\* significant at  $p < 0.01$ , \*\*\* significant at  $p < 0.001$ ).**

352 Anthocyanin concentrations were significantly higher in wines produced from smaller berries,  
 353 irrespective of the exposed treatment or harvest date. This observation is in agreement with the  
 354 findings of Roby *et al.* (2004) and Lafontaine *et al.* (2013). A later harvest date, however, did not  
 355 cause a significant change in the anthocyanin content. Non-precipitable polymeric pigments, which  
 356 consist mostly of anthocyanins and to a lesser extent flavan-3-ols, are formed during alcoholic  
 357 fermentation and wine aging (Harbertson *et al.*, 2003; Somers, 1968). Therefore, the higher  
 358 concentration of anthocyanins in wine produced from smaller berries is also reflected in significantly  
 359 higher np-PP. Although the later harvest did not enhance the monomeric anthocyanin concentration  
 360 in the wine, np-PP increased significantly suggesting that the anthocyanin content indeed was higher  
 361 after alcoholic fermentation, but a large portion of it already reacted to form polymeric pigments.  
 362 Concentrations of skin-associated phenolics such as epigallocatechin and B-prodelphinidins were  
 363 higher in wines produced from smaller berries, which can be explained by the higher ratio of skin  
 364 surface to the volume of the berries (Singleton, 1972). B- and C-procyanidin concentrations were  
 365 significantly higher in wines produced from smaller berries, but tannin concentrations were not  
 366 significantly different between the berry sizes. Tannin content was more affected by a later harvest

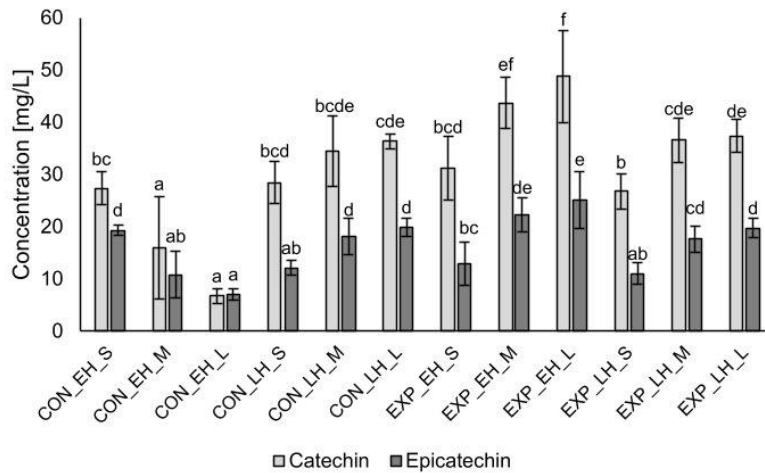
15

367 date, showing a significant decrease which is in agreement with the findings of Adams (2006).  
368 Previous studies have suggested that changes in the polysaccharide structure have a great impact on  
369 the extractability of tannins and that the selective adsorption of tannins with high proportions of  
370 gallates into grape skins and flesh affects the concentration of tannins in wine (Abi-Habib *et al.*, 2023;  
371 Hensen *et al.*, 2022). Abi-Habib *et al.* (2023) also observed a selective precipitation of tannins with  
372 skin-soluble polymers. This suggests that tannins have stronger interactions with polysaccharides in  
373 the cell walls with progressing berry ripening, which decreases the extractability and hence the  
374 concentration in the wine (Hanlin *et al.*, 2011).

375 Gallic acid and B-procyanidin gallates are mostly present in the seeds (Downey *et al.*, 2003; Obrequé-  
376 Slier *et al.*, 2010). Their content was significantly higher in wine produced from smaller berries and  
377 a later harvest resulted in significantly lower B-procyanidin gallate concentrations than early harvest.  
378 One explanation could be that the ratio of seed mass to juice is higher in smaller berries compared to  
379 larger berries resulting in higher concentrations of seed phenolics (Singleton, 1972). Another  
380 explanation could be that the extractability of seed-associated phenolics in smaller berries is still high,  
381 which could be explained by a lower conjugation of these proanthocyanidins with other cellular  
382 components (Cheynier *et al.*, 1997; Saint-Cricq de Gaulejac *et al.*, 1997) or a lesser extent of oxidative  
383 cross-linking (Kennedy *et al.*, 2000b). If the extractability of seed-associated phenolics is indeed still  
384 high, it would indicate that the seeds are lacking maturity.

385

386



387

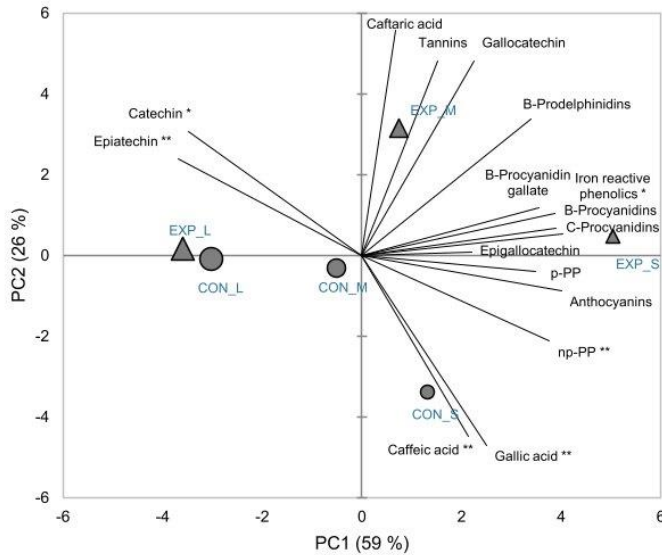
388 **Figure 6. Catechin and epicatechin concentration in Pinot noir wines of the control (CON) and**  
 389 **the exposed treatment (EXP) produced from grapes at early harvest (EH) and late harvest (LH)**  
 390 **and with different berry sizes (data expressed as mean values  $\pm$  standard deviation of  $n = 3$**   
 391 **experimental replicates; values sharing the same letter are not significantly different according**  
 392 **to Fisher's LSD test at  $p \leq 0.05$ ).**

393 Figure 6 shows the catechin and epicatechin concentrations in the wines of the control and the exposed  
 394 treatment. Flavan-3-ol concentrations of both harvest dates were higher with increasing berry size for  
 395 the exposed treatment, which is in contrast to the findings of Gil *et al.* (2015). Previous studies have  
 396 shown that the number of seeds is related to the size of a berry and can be regarded as a factor  
 397 determining the size of a mature berry (Walker *et al.*, 2005). Catechin and epicatechin concentrations  
 398 in wines of the exposed treatment decreased from early to late harvest (Figure 6) which suggests that  
 399 either the concentration of the flavan-3-ols in the berries were lower or the extractability of them  
 400 decreased. Hanlin *et al.* (2010) suggest that the lower extractability of tannins and monomeric flavan-  
 401 3-ols from late harvest seeds might be due to hardening of the seed coat and stronger interactions of  
 402 the phenolics with other cell wall material. Monomeric flavan-3-ol concentrations were also shown  
 403 to decrease in berries with progressing maturity (Kennedy *et al.*, 2000a), which can be explained by  
 404 oxidation processes resulting in polymerization reactions to form proanthocyanidins (Kennedy *et al.*,  
 405 2000a). In contrast to the wines of the exposed treatment, control wines showed higher catechin and  
 406 epicatechin concentrations in smaller berries at early harvest (Figure 6) suggesting a lack of phenolic  
 407 maturity in smaller berries. For late harvest wines, however, concentrations were higher in larger  
 408 berries, which is in agreement with the observation for wines of the exposed treatment.

409

410

### 3.2 Cluster exposure



411

412 **Figure 7. Principal component analysis for Pinot noir wines of the exposed treatment (EXP,**  
 413 **triangle) and the control (CON, circle) produced from late harvest grapes with different berry**  
 414 **sizes (data expressed as mean values of n = 3 experimental replicates). PCA space was calculated**  
 415 **on the 16 displayed phenolic parameters. (\*significant at  $p < 0.05$ , \*\*significant at  $p < 0.01$ ,**  
 416 **\*\*\*significant at  $p < 0.001$ ).**

417 Figure 7 shows the PCA for Pinot noir wines of the exposed treatment and the control produced from  
 418 late harvest grapes with different berry sizes. The PCA space was calculated on the 16 displayed  
 419 phenolic parameters. The first two PCs with eigenvalues  $> 1$  explained 88% of the cumulative  
 420 variability, with PC1 accounting for 65% of the total variance. Berry sizes were separated on PC 1  
 421 and cluster exposure on PC 2. Small berries were negatively loaded on PC1 which is explained by  
 422 higher concentrations of anthocyanins, polymeric pigments, B- and C-procyanidins, B-procyanidin  
 423 gallate and iron reactive phenolics. Large berries were positively loaded on PC1 which is explained  
 424 by higher catechin and epicatechin concentrations. Wines of the exposed treatment were positively  
 425 loaded on PC2 which is explained by higher tannin, gallo catechin and caftaric acid concentrations,  
 426 and lower gallic acid and caffeic acid concentrations. Interestingly, the shift on PC2 due to cluster  
 427 exposure decreased with increasing berry size suggesting that at a late harvest the treatment only  
 428 affected the phenolic composition of small and medium sized berries.

429 Wines with significantly higher concentrations of caftaric acid, gallic acid and tannins were  
430 produced from the exposed treatment. Caftaric acid and gallic acid concentrations in wine were  
431 not significantly affected by harvest date or berry size suggesting that cluster exposure influences the  
432 phenolic composition in grapes differently than a later harvest and hence produces a different wine  
433 style. The significantly higher tannin concentrations in the wines of the exposed treatment were also  
434 reflected in higher p-PP, while np-PP and anthocyanin content were not affected by the treatment.

435 Overall, the exposed treatment enhanced the concentrations of monomeric flavan-3-ols and  
436 proanthocyanidins significantly. This might be due to higher sun exposure – light and temperature –  
437 compared to the control (Jones, 2014; Wicks and Kiewer, 1983).

438 The combined effect of cluster exposure and small berries produced wines with the highest content  
439 of anthocyanins and polymeric pigments. This suggests that they might have the highest ageing  
440 potential in terms of colour stability. With a standard winemaking protocol, earlier harvested berries  
441 would produce wine with a higher tannin content and lower potential alcohol while a later harvest  
442 would produce wine with less tannins, but a higher potential alcohol (del Llaudy *et al.*, 2008; Del Rio  
443 and Kennedy, 2006). Winemakers should take this into consideration when choosing winemaking  
444 procedures to achieve a certain wine style. Cluster exposure and smaller berries are suitable to  
445 produce wines with higher concentrations of phenolics and potentially improve longevity and colour  
446 stability.

447

## 448 **Conclusion**

449 Table 4 summarizes the effects on chemical parameters in Pinot noir wine. Cluster exposure, harvest  
450 timing, and berry sizes significantly affected the phenolic composition of Pinot noir wine. Late  
451 harvest increased the content of skin-associated phenolics, such as epigallocatechin and np-PP. The  
452 exposed treatment enhanced the content of seed-associated phenolics, such as catechin, epicatechin,  
453 B-procyanidins, B-procyanidin gallates, C-procyanidins, and tannins. Wines produced from small  
454 berries were richer in phenolics than those made from large berries. This holds true for polymeric  
455 pigments, anthocyanins and tannins which are considered as quality indicators of red wine. Further  
456 studies should focus on different leaf removal intensities, timing of leaf removal, additional harvest  
457 dates to extend the investigation period, and other viticultural measures to modulate berry size or  
458 berry size heterogeneity. Also, a sensory assessment of Pinot noir wines produced from different  
459 berry sizes could be helpful to further assess the influence of berry sizes on wine quality.

460

461 **Table 4. Summary of the observed effects on different chemical parameters in Pinot noir wine.**  
 462 **Increasing values are indicated by “+”, decreasing values by “-“, and non-significant changes**  
 463 **by “O”. Significance testing was performed using Fisher’s LSD test at  $p \leq 0.05$ .**

	Cluster exposure (CON → EXP) <sup>a</sup>	Harvest date (EH → LH) <sup>b</sup>	Berry size (L → S) <sup>c</sup>
Anthocyanins	○	○	+
Gallocatechin	+	○	○
Epigallocatechin	○	+	+
B-Prodelfinidins	+	-	+
Caftaric acid	+	○	○
Caffeic acid	○	+	+
Gallic acid	○	○	+
Catechin	+	+	○
Epicatechin	+	○	-
B-Procyanidins	+	○	+
B-Procyanidin gallate	+	-	+
C-Procyanidins	+	○	+
Tannins	+	-	○
np-PP	○	+	+
p-PP	+	○	+
Iron reactive phenolics	+	○	+
Alcohol	○	+	+
Titratable acidity	+	-	+
pH	-	○	-

464 <sup>a</sup> control (CON), exposed (EXP)

465 <sup>b</sup> early harvest (EH), late harvest (LH)

466 <sup>c</sup> large (L), medium (M), small berries (S)

467

468

## 469 Acknowledgements

470 The authors would like to thank Daniel Zimmermann and Luise Müller for their support.

471 This IGF Project of the FEI was supported within the program for promoting the Industrial Collective  
472 Research (IGF) of the Federal Ministry of Economic Affairs and Climate Action (BMWK), based on  
473 a resolution of the German Parliament; grant number AiF 20024N. Additional funding was provided  
474 by the University of Applied Sciences Kaiserslautern.

475

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**Supplemental Table 1. Total soluble solids (TSS) of Pinot noir juice. Average values followed by SD (n = 3 experimental replicates).**

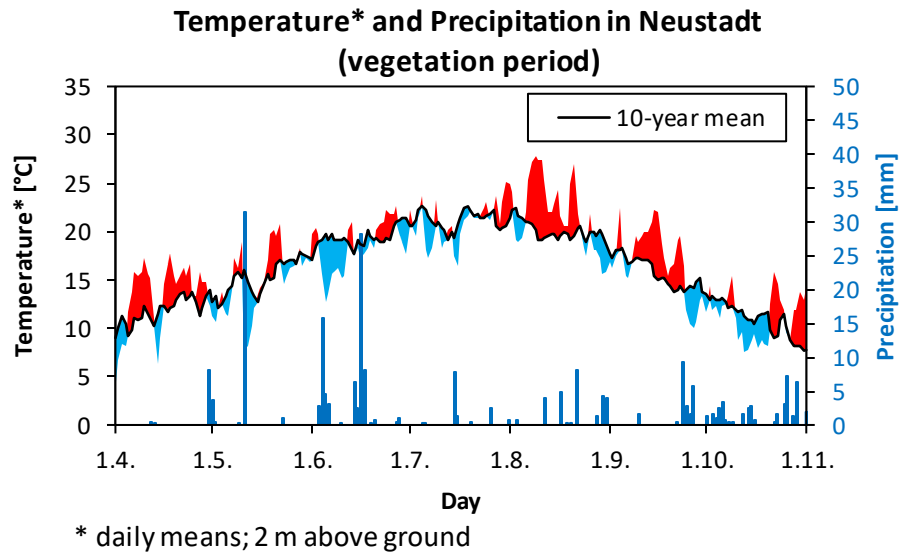
<b>Harvest date</b>	<b>Cluster exposure</b>	<b>Berry size</b>	<b>TSS [Brix]</b>
Early harvest	Control	S	21.2 ± 0.4 cd <sup>a</sup>
		M	19.5 ± 0.2 ab
		L	18.7 ± 0.4 a
	Exposed	S	21.6 ± 0.6 de
		M	20.1 ± 1.0 bc
		L	19.6 ± 0.5 ab
Late harvest	Control	S	25.4 ± 1.1 h
		M	24.0 ± 1.0 g
		L	22.6 ± 1.2 ef
	Exposed	S	25.5 ± 0.5 h
		M	23.9 ± 0.3 g
		L	22.9 ± 0.2 fg
<i>p</i>			<i>&lt;0.0001</i>

<sup>a</sup> Within a column, values followed by the same letter are not significantly different according to Fisher's LSD test at  $p \leq 0.05$ . Significant  $p$  values ( $p \leq 0.05$ ) are shown in italic letters. The data consisted of three experimental replicates.

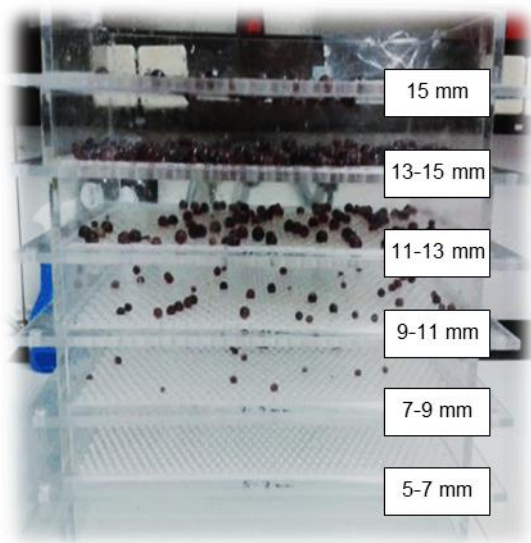
**Supplemental Table 2. Chemical analyses of Pinot noir wine. Average values followed by SD (n = 3 experimental replicates).**

Cluster exposure	Harvest date	Berry size	Anthocyanins [mg/L, Mv <sup>-3</sup> -gl <sup>1</sup> eq.]	Gallic acid [mg/L]	Caffeic acid [mg/L]	Gallic acid [mg/L]	Catechin [mg/L]	Epicatechin [mg/L]	C-Procyanidins [mg/L, Proc. C1 eq.]	B-Prodaphnidins [mg/L, Proc. B2 eq.]	Galocatechin [mg/L]	B-Procyanidins [mg/L, Proc. B2 eq.]	Epigallocatechin [mg/L]	B-Procyanidin gallic [mg/L, Proc. B2 eq.]	np-PP [AU]	p-PP [AU]	Tannins [mg/L, Cat. eq.]	Iron reactive phenolics [mg/L, Cat. eq.]
CON	EH	S	262.0 ± 10.8 cde	87.9 ± 8.0 d	0.9 ± 0.1 c	7.4 ± 0.5 cf	27.3 ± 3.2 bc	19.3 ± 0.9 d	15.5 ± 0.2 cd	4.9 ± 0.3 de	0.92 ± 0.03 c	27.6 ± 0.4 cd	0.35 ± 0.01 d	0.45 ± 0.04 ede	0.69 ± 0.03 def	0.25 ± 0.06 a	105.5 ± 13.6 abc	1066.7 ± 15.0 def
		M	176.1 ± 19.0 ab	55.8 ± 1.7 a	0.5 ± 0.0 ab	4.7 ± 0.5 ab	15.9 ± 9.7 a	10.8 ± 4.5 ab	8.9 ± 0.7 a	2.7 ± 0.2 a	0.55 ± 0.03 a	15.7 ± 1.3 a	0.19 ± 0.01 a	0.37 ± 0.02 abode	0.48 ± 0.04 abc	0.18 ± 0.06 a	90.1 ± 20.7 ab	791.6 ± 41.7 a
		L	144.1 ± 25.1 a	61.0 ± 9.1 ab	0.4 ± 0.1 a	5.4 ± 0.9 abcd	6.7 ± 1.5 a	7.0 ± 1.0 a	10.9 ± 3.4 ab	3.0 ± 0.8 ab	0.65 ± 0.18 ab	18.3 ± 5.1 ab	0.20 ± 0.05 a	0.44 ± 0.08 bcde	0.35 ± 0.03 a	0.27 ± 0.05 a	108.4 ± 8.9 bc	826.8 ± 118.8 ab
	LH	S	260.5 ± 33.6 cde	63.0 ± 14.4 ab	1.6 ± 0.5 d	7.6 ± 0.8 f	28.4 ± 4.0 bcd	12.1 ± 1.4 ab	13.5 ± 2.4 bcd	3.3 ± 0.5 abc	0.84 ± 0.10 bc	22.9 ± 4.4 abc	0.32 ± 0.03 bcd	0.34 ± 0.03 abc	0.84 ± 0.07 fg	0.31 ± 0.11 a	80.9 ± 21.2 ab	1063.1 ± 41.9 def
		M	215.2 ± 69.4 bc	65.7 ± 8.4 ab	0.8 ± 0.1 bc	5.9 ± 0.8 cd	34.4 ± 6.7 bode	18.1 ± 3.5 d	12.7 ± 3.3 abcd	3.5 ± 0.8 abc	0.91 ± 0.15 c	20.8 ± 6.0 abc	0.35 ± 0.08 cd	0.33 ± 0.15 ab	0.72 ± 0.12 cf	0.26 ± 0.03 a	94.3 ± 27.9 ab	977.5 ± 120.5 ede
		L	183.7 ± 23.8 ab	68.6 ± 5.9 abc	0.6 ± 0.0 ab	5.1 ± 0.2 abc	36.3 ± 1.5 cde	19.8 ± 1.8 d	11.5 ± 0.9 abc	3.3 ± 0.1 abc	0.88 ± 0.01 bc	19.6 ± 1.2 abc	0.31 ± 0.02 bcd	0.33 ± 0.03 ab	0.54 ± 0.02 bcde	0.12 ± 0.06 a	70.8 ± 22.0 a	897.3 ± 37.6 abc
EXP	EH	S	285.7 ± 13.4 de	91.3 ± 10.3 d	0.9 ± 0.0 c	7.4 ± 0.5 cf	31.2 ± 6.1 bcd	12.9 ± 4.1 bc	20.4 ± 3.4 e	5.7 ± 1.0 e	1.03 ± 0.16 c	36.9 ± 5.6 e	0.34 ± 0.04 bcd	0.67 ± 0.05 f	0.90 ± 0.13 g	0.48 ± 0.17 a	161.2 ± 29.0 de	1253.6 ± 56.5 g
		M	208.1 ± 37.3 bc	87.3 ± 2.2 d	0.7 ± 0.1 abc	6.0 ± 0.4 cd	43.7 ± 5.0 cf	22.3 ± 3.3 de	15.2 ± 0.3 cd	4.9 ± 0.4 de	0.97 ± 0.02 c	26.1 ± 1.0 bcd	0.28 ± 0.02 bcd	0.46 ± 0.03 de	0.51 ± 0.10 abc	0.29 ± 0.21 a	203.4 ± 19.8 f	1066.3 ± 67.4 def
		L	181.9 ± 25.9 ab	85.0 ± 5.8 cd	0.6 ± 0.0 ab	5.8 ± 0.8 bcd	48.7 ± 8.9 f	25.0 ± 5.5 e	14.3 ± 1.5 bcd	4.3 ± 0.5 cd	0.94 ± 0.10 c	24.3 ± 2.0 bcd	0.26 ± 0.04 ab	0.46 ± 0.05 ede	0.43 ± 0.07 ab	0.27 ± 0.02 a	178.2 ± 11.0 ef	993.3 ± 7.0 ede
	LH	S	294.8 ± 52.9 e	76.8 ± 16.5 bcd	0.9 ± 0.3 c	6.4 ± 0.9 de	26.7 ± 3.3 b	11.0 ± 2.0 ab	16.7 ± 4.5 de	4.3 ± 1.4 cd	0.97 ± 0.27 c	31.8 ± 11.6 de	0.32 ± 0.09 bcd	0.47 ± 0.13 e	0.98 ± 0.25 g	0.35 ± 0.12 a	112.1 ± 29.7 bc	1193.9 ± 161.2 fg
		M	232.3 ± 35.0 bcd	91.1 ± 16.8 d	0.7 ± 0.0 abc	4.8 ± 0.3 ab	36.5 ± 4.3 cde	17.5 ± 2.5 cd	13.6 ± 2.1 bcd	4.1 ± 1.0 bcd	1.02 ± 0.24 c	23.3 ± 4.0 abc	0.32 ± 0.08 bcd	0.36 ± 0.01 abcd	0.61 ± 0.03 ede	0.28 ± 0.04 a	136.1 ± 14.1 ed	1097.7 ± 71.1 ef
		L	181.4 ± 17.0 ab	75.8 ± 16.4 bcd	0.5 ± 0.1 ab	4.5 ± 0.2 a	37.4 ± 3.1 de	19.7 ± 1.8 d	10.7 ± 0.9 ab	3.1 ± 0.4 abc	0.83 ± 0.09 bc	18.2 ± 2.0 ab	0.27 ± 0.04 abc	0.32 ± 0.01 a	0.52 ± 0.06 abcd	0.23 ± 0.08 a	104.2 ± 9.2 abc	951.3 ± 18.7 bcd
<i>p</i>		<i>0.000</i>	<i>0.002</i>	<i>&lt;0.0001</i>	<i>&lt;0.0001</i>	<i>&lt;0.0001</i>	<i>&lt;0.0001</i>	<i>0.001</i>	<i>0.001</i>	<i>0.010</i>	<i>0.001</i>	<i>0.005</i>	<i>0.000</i>	<i>&lt;0.0001</i>	<i>0.061</i>	<i>&lt;0.0001</i>	<i>&lt;0.0001</i>	
<b>Cluster exposure</b>																		
CON			206.9 ± 54.0 a	67.0 ± 12.8 a	0.8 ± 0.4 a	6.0 ± 1.3 a	24.9 ± 11.6 a	14.5 ± 5.4 a	12.2 ± 2.8 a	3.5 ± 0.9 a	0.79 ± 0.17 a	20.8 ± 5.0 a	0.29 ± 0.08 a	0.38 ± 0.08 a	0.60 ± 0.18 a	0.23 ± 0.08 a	91.7 ± 21.6 a	937.2 ± 127.2 a
EXP			230.7 ± 54.5 a	84.5 ± 12.4 b	0.7 ± 0.2 a	5.8 ± 1.1 a	37.4 ± 8.8 b	18.1 ± 5.9 b	15.1 ± 3.7 b	4.4 ± 1.1 b	0.96 ± 0.16 b	26.8 ± 7.8 b	0.30 ± 0.06 a	0.46 ± 0.13 b	0.66 ± 0.24 a	0.32 ± 0.14 b	149.2 ± 40.2 b	1092.7 ± 128.0 b
<i>p</i>			0.059	<i>&lt;0.0001</i>	0.222	0.313	<i>&lt;0.0001</i>	0.002	0.001	0.001	0.002	0.002	0.441	0.002	0.139	0.025	<i>&lt;0.0001</i>	<i>&lt;0.0001</i>
<b>Harvest date</b>																		
	EH		209.7 ± 54.7 a	78.0 ± 15.7 a	0.7 ± 0.2 a	6.1 ± 1.1 a	28.9 ± 16.0 a	16.2 ± 7.3 a	14.2 ± 4.1 a	4.2 ± 1.2 b	0.85 ± 0.20 a	24.8 ± 7.6 a	0.27 ± 0.07 a	0.48 ± 0.10 b	0.56 ± 0.20 a	0.29 ± 0.14 a	141.1 ± 45.9 b	999.7 ± 169.4 a
	LH		228.0 ± 54.8 a	73.5 ± 15.0 a	0.8 ± 0.4 b	5.7 ± 1.2 a	33.3 ± 5.5 b	16.4 ± 4.1 a	13.1 ± 3.0 a	3.6 ± 0.8 a	0.91 ± 0.16 a	22.8 ± 6.7 a	0.31 ± 0.06 b	0.36 ± 0.09 a	0.70 ± 0.20 b	0.26 ± 0.10 a	99.7 ± 28.6 a	1030.2 ± 127.3 a
<i>p</i>			0.138	0.207	<i>0.011</i>	0.097	<i>0.026</i>	<b>0.890</b>	0.192	<i>0.013</i>	0.186	0.240	<i>0.017</i>	<i>&lt;0.0001</i>	<i>0.000</i>	0.374	<i>&lt;0.0001</i>	0.258
<b>Berry size</b>																		
		S	275.8 ± 31.7 c	79.8 ± 15.8 a	1.1 ± 0.4 b	7.2 ± 0.8 b	28.4 ± 4.1 a	13.8 ± 4.0 a	16.5 ± 3.7 b	4.5 ± 1.2 b	0.94 ± 0.16 a	29.8 ± 7.9 b	0.33 ± 0.05 b	0.48 ± 0.14 b	0.85 ± 0.17 c	0.35 ± 0.14 b	114.9 ± 36.8 a	1144.3 ± 114.2 b
		M	207.9 ± 43.2 b	75.0 ± 17.4 a	0.6 ± 0.1 a	5.4 ± 0.8 a	32.6 ± 12.1 a	17.2 ± 5.3 b	12.6 ± 3.0 a	3.8 ± 1.0 a	0.86 ± 0.23 a	21.5 ± 5.1 a	0.29 ± 0.08 a	0.38 ± 0.08 a	0.58 ± 0.12 b	0.25 ± 0.10 a	131.0 ± 50.8 a	983.3 ± 142.1 a
		L	172.8 ± 26.3 a	72.6 ± 12.8 a	0.5 ± 0.1 a	5.2 ± 0.7 a	32.3 ± 16.8 a	17.9 ± 7.4 b	11.8 ± 2.3 a	3.4 ± 0.7 a	0.83 ± 0.15 a	20.1 ± 3.6 a	0.26 ± 0.05 a	0.39 ± 0.08 a	0.46 ± 0.09 a	0.22 ± 0.08 a	115.4 ± 42.5 a	917.2 ± 84.4 a
<i>p</i>			<i>&lt;0.0001</i>	0.255	<i>&lt;0.0001</i>	<i>&lt;0.0001</i>	0.139	<i>0.008</i>	<i>0.000</i>	<i>0.003</i>	0.143	<i>0.000</i>	<i>0.004</i>	<i>0.002</i>	<i>&lt;0.0001</i>	<i>0.018</i>	<i>0.121</i>	<i>&lt;0.0001</i>
<b>Cluster exposure × harvest date</b>																		
<i>p</i>			0.540	0.557	<i>0.003</i>	<i>0.002</i>	<i>&lt;0.0001</i>	<i>0.001</i>	<i>0.033</i>	<i>0.049</i>	<i>0.034</i>	0.129	0.060	0.202	0.147	0.442	<i>0.004</i>	0.052
<b>Harvest date × berry size</b>																		
<i>p</i>			0.639	<i>0.015</i>	0.207	0.339	<i>0.067</i>	<i>0.009</i>	0.161	0.052	0.071	0.343	<i>0.018</i>	0.378	0.836	0.319	0.341	0.106
<b>Cluster exposure × berry size</b>																		
<i>p</i>			0.928	0.085	<i>0.039</i>	0.409	<i>0.001</i>	<i>0.000</i>	0.365	0.421	0.364	0.257	0.613	<i>0.014</i>	<i>0.049</i>	0.556	0.134	0.410
<b>Cluster exposure × harvest date × berry size</b>																		
<i>p</i>			0.690	0.294	0.190	0.405	<i>0.003</i>	<i>0.000</i>	0.645	0.245	0.250	0.594	0.194	0.876	0.925	0.230	0.370	0.747

<sup>a</sup> Within a column, values followed by the same letter are not significantly different according to Fisher's LSD test at  $p \leq 0.05$ . Significant  $p$  values ( $p \leq 0.05$ ) are shown in italic letters. The data consisted of three experimental replicates.



**Supplemental Figure 1.** Temperature and precipitation data for the 2020 vintage (vegetation period) in Neustadt, Germany.



**Supplemental Figure 2.** Berry sorting trays.

## **6 Influence of Grape Maturity and Maceration Time on Sensory Characteristics and Phenolics in Pinot noir and Cabernet Sauvignon Wines**

During grape ripening, anthocyanin and sugar accumulation do not necessarily occur at the same pace, which is a challenge for winemakers to determine the optimal grape maturity. Maceration time is another important factor for wine quality and style by modulating the extraction of polyphenols into the wine. This study shows how maceration time affects the phenolic composition and sensory characteristics of wines produced from grapes of different harvest time points. Pinot noir and Cabernet Sauvignon grapes were harvested at three different stages of grape maturity and processed using 7- and 14-day maceration. Sensory evaluation and analysis of phenolic composition were carried out approximately three months after bottling. The effect of maceration time was found to be different from the influence of grape maturity as shown by sensory evaluation and phenolic composition. Extended maceration neither compensated for grape immaturity nor enhanced the effects of immaturity. Changes in the phenolic composition with progressing grape maturity and extending maceration time were reflected in the wine color as well as in taste and tactile ratings in the sensory evaluation of the wines. Skin-associated phenolics such as anthocyanins and polymeric pigments positively correlated with grape maturity, while extending maceration time enhanced the extraction of seed-associated phenolics such as monomeric catechins, procyanidins and procyanidin gallates. It is suggested that concentrations of anthocyanins and polymeric pigments are suitable indicators for grape maturity perceived in red wine, whereas concentrations of monomeric catechins and procyanidins are good indicators for maceration time.

## Influence of grape maturity and maceration time on sensory characteristics and phenolics in Pinot noir and Cabernet Sauvignon wines

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Disclaimer:

The presented work was performed exclusively by Weincampus Neustadt/DLR Rheinpfalz (Sandra Feifel and master thesis Melanie Schaub). For more information, refer to the author contribution section.

Author Contributions:

**Sandra Feifel:** Conceptualization, Methodology, Investigation, Chemical analyses, Sensory analysis, Writing – Original draft, Review and Editing. **Daniel Zimmermann:** Winemaking, Chemical analyses. **Melanie Schaub:** Methodology, Investigation, Chemical analyses, Sensory analysis. **Pascal Wegmann-Herr:** Conceptualization, Winemaking, Writing - Review and Editing. **Elke Richling:** Supervision, Writing - Review and Editing. **Dominik Durner:** Conceptualization, Supervision, Writing – Original draft, Review and Editing, Funding acquisition.

All authors have read and agreed to the submitted version of the manuscript.

Citation:

Feifel, S., Zimmermann, D., Schaub, M., Wegmann-Herr, P., Richling, E., and Durner, D. (2025), "Influence of grape maturity and maceration time on sensory characteristics and phenolics in Pinot noir and Cabernet-Sauvignon wines", *OENO One*, Vol. 59 No. 2. <https://doi.org/10.20870/oeno-one.2025.59.2.8446>.

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**ENOLOGY ORIGINAL RESEARCH ARTICLES**

# Influence of grape maturity and maceration time on sensory characteristics and phenolics in Pinot noir and Cabernet-Sauvignon wines

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**Article number: 8446**



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Associate editor:  
Victor de Freitas



Received:  
19 December 2024

Accepted:  
20 May 2025

Published:  
24 June 2025



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## ABSTRACT

Measures of grape phenolics have become important indicators to determine grape maturity, though varietal differences must be considered. The objective of this study was to determine whether maceration time can modulate the phenolic profile and sensory characteristics of wines produced from grapes of different maturities. Pinot noir and Cabernet-Sauvignon grapes were harvested at three different stages of grape maturity, in a range of 20–25 Brix, and vinified using 7-day and 14-day maceration times in experimental fermenters. Sensory evaluation and analysis of phenolic composition were carried out after bottling. The effect of maceration time was found to be different from the influence of grape maturity as shown by sensory evaluation and phenolic composition. Extended maceration neither compensated for grape immaturity nor enhanced the effects of immaturity. Changes in the phenolic composition with progressing grape maturity and extending maceration time were reflected in the wine colour as well as in taste and tactile ratings in the sensory evaluation of the wines. Skin-associated phenolics such as anthocyanins and polymeric pigments correlated with grape maturity, while extended maceration enhanced the extraction of seed-associated phenolics such as monomeric catechins, procyanidins and procyanidin gallates. The influences of grape maturity and maceration time on gallic catechins and prodelphinidins were different for Pinot noir and Cabernet-Sauvignon. The concept of “phenolic maturity” therefore requires a differentiated view on individual phenolic compounds, seed- and skin-associated phenols, and must take varietal differences into account. It is suggested that concentrations of anthocyanins and polymeric pigments are suitable indicators for grape maturity, whereas concentrations of monomeric catechins and procyanidins are good indicators for maceration time.

**KEYWORDS:** grape maturity, phenolics, extended maceration, sensory, tannins, anthocyanins, polymeric pigments

## INTRODUCTION

Winemakers aim to adapt winemaking operations to grape maturity. Recent research suggests that the phenolic composition or individual phenolic compounds might be used as more accurate indicators to monitor grape maturity than the traditionally used total soluble solids (TSS) and titratable acidity, but also to predict the composition in wine (Feifel *et al.*, 2024; Kontoudakis *et al.*, 2010; Pérez-Magariño & González-San José, 2006). Ristic *et al.* (2010) concluded that higher concentrations of anthocyanins and skin tannins in Shiraz grape berries coupled with a lower concentration of seed tannins were associated with higher wine quality. Gil *et al.* (2015) observed that the total polysaccharide concentration in Cabernet-Sauvignon wine increased with both grape maturity and maceration time, but the effect of maceration time was more pronounced. Vidal *et al.* (2004b) showed that polysaccharides contribute the sensation of “fullness” to wine while decreasing astringency, dryness, and roughness. However, studies by del Llaudy *et al.* (2008) showed, for immature Cabernet-Sauvignon grapes, that an extended maceration produced wines that were too astringent, due to an excess of seed tannin extraction. For more mature grapes, de-pectination during ripening otherwise decreases the extractability of seed tannins into the wine while increasing the extractability of skin tannins (del Llaudy *et al.*, 2008; Garrido-Bañuelos *et al.*, 2019). An extended maceration favours the formation of polymeric pigments which contribute to colour stability and reduce the puckering sensation and astringency in wines (Vidal *et al.*, 2004a).

The effect of grape maturity on the sensory characteristics of red wines has been the focus of many studies. Sherman *et al.* (2017) harvested Merlot grapes at three different harvest dates with TSS in the range of 20.7–27.4 Brix. They observed that a later harvest resulted in fruitier, sweeter wines with a fuller body which were less vegetal than wines from earlier harvested grapes. These findings are in agreement with the study conducted by Casassa *et al.* (2013b) who harvested Merlot grapes at two different harvest dates with a TSS range of  $20.35 \pm 0.16$  Brix to  $24.91 \pm 0.46$  Brix. Their late-harvest wines were also rated higher in sweetness, viscosity, and fruit-derived aromas, while early-harvest wines were described as having a sour taste, low colour intensity, and the aroma of fresh vegetables. A decrease in green flavour attributes with progressing maturity was also observed by Schelezki *et al.* (2018) for Cabernet-Sauvignon wine, which was explained by higher 2-isobutyl-3-methoxy-pyrazine IBMP concentrations at early-harvest that decreased towards late-harvest (Dunlevy *et al.*, 2010; Schelezki *et al.*, 2018). Late-harvest Cabernet-Sauvignon wine received higher ratings in port wine aroma and hotness (Schelezki *et al.*, 2018) which is in agreement with the findings of Bindon *et al.* (2014a) for late-harvest Cabernet-Sauvignon wine that was rated lower for red colour, red fruit, and fresh green aroma, and higher for dark fruit aroma, overall fruitiness, hotness, pungency, opacity, earthiness, and bitterness. A consumer preference rating for wine quality was performed to evaluate whether

measures of phenolic maturity matched with a targeted “optimal ripeness”, which was not necessarily the case (Bindon *et al.*, 2014a).

The cited studies have been carried out in warm climate conditions with Cabernet-Sauvignon, Cabernet franc, Merlot, and Tempranillo. The studies of Bindon *et al.* (2014a) and Schelezki *et al.* (2018) were conducted in Langhorne Creek, South Australia, and McLaren Vale, South Australia, respectively, at a subtropical climate zone (lat.  $35^{\circ} 16' S$ ). The studies of del Llaudy *et al.* (2008) and Gil *et al.* (2012) were carried out in Tarragona, Spain, in a temperate climate zone (lat.  $41^{\circ} 07' N$ ). The studies of Casassa *et al.* (2013a) and Sherman *et al.* (2017) took place in Paterson, WA, USA (lat.  $45^{\circ} 5' N$ ) which is also categorised as temperate.

The experimental vineyards in our study were further north in the Pfalz region in Germany (lat.  $49^{\circ} 24' N$ ) than the aforementioned vineyards. Our study aims to investigate grape maturity in Pinot noir, which is considered an important cool climate variety, and Cabernet-Sauvignon, a well-studied warm climate variety. The Pfalz region is located in the winegrowing zone A which is considered a “cool climate”. The 10-year average growing degree days GDD at the vineyard location is 1548 (20-year average 1540) which is within Winkler region II (cool). The year 2018 was an extremely hot vintage with 1884 GDD (Winkler region III, temperate) and it may be considered as an example of what we can expect from progressing climate change. The objective of our study is to explore the extent to which different maceration times can modulate the phenolic profile and sensory characteristics of wine made from grapes of different maturities. In particular, the question is raised whether extending maceration can improve the sensory characteristics of wine from immature grape material and if sensory effects can be explained by chemical data of phenols. The study also aims to contribute to the understanding of how grape maturity is related to the phenolic composition and the sensory characteristics of Pinot noir and Cabernet-Sauvignon.

## MATERIALS AND METHODS

### 1. Wine production

*Vitis vinifera* L. cv. Pinot noir (PN; clone Mariafeld on SO4 rootstock) grapes and *Vitis vinifera* L. cv. Cabernet-Sauvignon (CS; clone 1Gm on Binova 1Opp rootstock) grapes were harvested in the 2018 vintage from vines grown in the experimental vineyards of the DLR Rheinpfalz located in the Pfalz region of Germany ( $49^{\circ} 24' N$   $8^{\circ} 11' E$ ). The growing degree days (GDD) were calculated with data from an on-site weather station yielding 1884 GDD according to Method 1 by McMaster and Wilhelm (1997). A Huglin Index (HI) of 2412 was calculated according to Huglin (1978). The yield was 8 t/ha for both grape varieties.

The grape bunches were harvested manually at three different stages of grape maturity in a range which is realistic for winemaking in Germany (20–25 Brix). Harvest dates were two weeks apart, starting with “early-harvest” at 20.7 Brix

for Pinot noir and 20.2 Brix for Cabernet-Sauvignon, “commercial harvest” at 22.5 Brix for Pinot noir and 23.0 Brix for Cabernet-Sauvignon, and “late-harvest” at 24.8 Brix for Pinot noir and 24.0 Brix for Cabernet-Sauvignon. After destemming, grapes were crushed and transferred into 100 L experimental stainless-steel fermenters. Per grape variety and harvest date, four lots, each with 70 kg, were processed.

Potential alcohol adjustment to achieve a set potential alcohol for all experimental treatments was not performed, because previous studies have shown that the enhanced potential alcohol has a varying influence on the extraction of phenolics depending on the phenolic maturity of the grapes (Feifel *et al.*, 2024). All musts were inoculated with 200 mg/L ZYMAFLORE® RB2 yeast (LAFFORT®, Bordeaux, France) and allowed to warm up to 28 °C within 24 hours. Once fermentation commenced, fermenting wines were maintained at 24 °C and manually punched down six times per day for two days. From the third day until the end of alcoholic fermentation (< 1 g/L residual sugar), three punch downs per day were conducted. Afterwards, until the end of maceration, no punch downs were performed. Fermentations were monitored with a handheld density meter (DMA 35 Basic, Anton Paar, Graz, Austria).

Two of the four lots per grape variety and harvest date (experimental replicates) were kept on skins and seeds for a total maceration time of either 7 days or 14 days. After maceration, all wines were < 1 g/L residual sugar. Wines were pressed off (1.6 bar) with a tank press (T1, Scharfenberger, Bad Dürkheim, Germany). The wines were transferred into 50 L carboys and inoculated with 10 mg/L *Oenococcus oeni* VP41 (ZEFÜG GmbH & Co. KG, Bingen, Germany). After completion of malolactic fermentation, 60 mg/L SO<sub>2</sub> (IOC Sulfinvin K150, ZEFÜG GmbH & Co. KG, Bingen, Germany) was added. Wines were kept for two months at 15 °C until filtration and bottling. Before bottling, SO<sub>2</sub> was adjusted to obtain 35 mg/L free SO<sub>2</sub>. Wines were filled into 0.75 L Alsace-style wine bottles, closed with screw caps and stored for two months at 16 °C until chemical analyses and sensory evaluation.

## 2. Chemicals

Hydrochloric acid, propan-2-ol, caffeine anhydrous, Folin–Ciocalteu reagent, and sodium chloride were purchased from AppliChem (Darmstadt, Germany). Ethanol, *ortho*-phosphoric acid, acetic acid, and potassium metabisulfite were purchased from ORG Laborchemie (Bunde, Germany). Procyanidin B<sub>2</sub> and procyanidin C<sub>1</sub> were purchased from PhytoLab (Vestenbergsgreuth, Germany). Catearic acid, cyanidin-3-*O*-rutinoside, (–)-epicatechin-3-*O*-gallate, (–)-epigallocatechin, (+)-gallocatechin, and oenin chloride were purchased from PhytoPlan (Heidelberg, Germany). L(+)-tartaric acid and sodium carbonate were purchased from Roth (Karlsruhe, Germany). Caffeic acid, (+)-catechin hydrate, (–)-epicatechin, gallic acid monohydrate, sodium hydroxide, maleic acid, sodium dodecyl sulphate, tannic acid, aluminium sulphate, 3-isobutyl-2-methoxypyrazine, triethanolamine, and iron(III) chloride

were purchased from Sigma Aldrich (Steinheim, Germany). Acetonitrile and formic acid were purchased from Bernd Kraft (Duisburg, Germany) and Honeywell (Morris Plains, New Jersey), respectively. Albumin fraction V (BSA), acetic acid, and potassium dihydrogen phosphate were purchased from Merck (Darmstadt, Germany) and VWR International (Darmstadt, Germany), respectively. Ultrapure water was obtained from a PURELAB flex (ELGA LabWater, Celle, Germany) water purification system. All chromatographic solvents were HPLC grade.

## 3. Basic analytical parameters

Grape juice parameters (relative density, titratable acidity, pH, glucose, and fructose) were measured by Fourier transform infrared spectroscopy (FT-IR) (WineScan™ FT120 Basic, FOSS GmbH, Hamburg, Germany) using a calibration method provided by the manufacturer. Each harvest date, early-, commercial and late-harvest, including experimental replicates, was analysed in duplicate. Wine parameters (alcohol, titratable acidity, tartaric acid, malic acid, lactic acid, pH, acetic acid, and residual sugars) were measured by FT-IR (WineScan™ Auto, FOSS GmbH, Hamburg, Germany) using a calibration method provided by the manufacturer. Samples were centrifuged for 5 min at 4,500 rpm (4,754 rcf) and degassed prior to FT-IR analysis. For each experimental treatment, the sample was analysed in duplicate.

## 4. Spectrophotometric analysis

The photometric analyses were scheduled to coincide with the sensory evaluation. Iron reactive phenolics, tannins, protein-precipitable polymeric pigments (p-PP) and non-precipitable polymeric pigments (np-PP) were measured by the assay described by Harbertson *et al.* (2003). A Varian Cary 100 scan UV-visible spectrophotometer (Agilent Technologies, Waldbronn, Germany) was used for all measurements. Oxidisable phenolics were determined by the Folin–Ciocalteu assay as described by Singleton and Rossi (1965) with an automated Konelab 20i (Thermo Fisher Scientific, Waltham, USA). For each experimental treatment, the sample was analysed in duplicate.

## 5. HPLC-DAD/FD analysis

The analysis was performed using a HPLC-DAD/FD (Jasco, Pfungstadt, Germany) comprising a PU-2080 Plus Intelligent HPLC Pump, a DG-2080-53 3-Line Degasser, a LG-2080-02 Ternary Gradient Unit, an AS-2055 Plus Intelligent Sampler, a MD-2010 Plus Multiwavelength Detector, and a FP-2020 Plus Intelligent Fluorescence Detector.

Separation of phenolic compounds was achieved with a reversed-phase Gemini® NX-C18 (110 Å, 250 × 4.6 mm, 5 µm; Phenomenex, Torrance, USA) column, protected by a guard column containing the same material (Security Guard Cartridge System C18; Phenomenex, Torrance, USA) at a temperature of 40 °C (CO-2067 Plus Intelligent Column Oven; Jasco, Pfungstadt, Germany). A gradient consisting of eluent A (phosphate buffer (1.36 g/L KH<sub>2</sub>PO<sub>4</sub>,

pH = 1.5 (85 % H<sub>3</sub>PO<sub>4</sub>)/acetonitrile 95/5 (v/v)) and eluent B (phosphate buffer (1.36 g/L KH<sub>2</sub>PO<sub>4</sub>, pH = 1.5 (85 % H<sub>3</sub>PO<sub>4</sub>)/acetonitrile 50/50 (v/v)) was applied at a flow rate of 1.2 mL/min as follows: 0 % B at 0 min, 12 % B at 12 min (6 min hold), 51 % B at 37.5 min, 75 % B at 38 min (3 min hold), 0 % B at 42 min (1 min hold). The injection volume was 20 µL. The phenolic compounds were identified according to their order of elution and the retention times of reference compounds. The compounds were: malvidin-3-*O*-glucoside (mv-3-gl), caffeic acid, caftaric acid, gallic acid, (+)-catechin and (–)-epicatechin. Detection took place at 520 nm for anthocyanins, 320 nm for hydroxycinnamic acids and 280 nm for gallic acid. Flavan-3-ols were quantified by fluorescence with excitation and emission wavelengths of 300 nm and 325 nm, respectively, to avoid interferences with the other compounds. External standards were used for quantification in the ranges of 0.5–125 mg/L for (–)-epicatechin and caftaric acid, 1.0–250 mg/L for gallic acid and (+)-catechin, and 1–450 mg/L for anthocyanins. Anthocyanins were quantified as mv-3-gl equivalents. (–)-epicatechin and (+)-catechin were expressed as “monomeric catechins”.

Samples were filtered through 0.45 µm cellulose acetate membranes (Graphic Controls, Totnes, UK) and then analysed directly. Galaxy Chromatography Software (version 1.10.0.5590, Agilent Technologies, Waldbronn, Germany) was used for HPLC data acquisition and evaluation. Analysis was performed in duplicate.

## 6. LC-QToF-MS analysis

The analysis was performed using an LC-QToF-MS (Agilent Technologies, Waldbronn, Germany) comprising an Agilent 1260 Infinity Binary Pump, an Agilent 1260 Infinity HiP Degasser, an Agilent 1260 Infinity HiP ALS Autosampler, an Agilent Jet Stream Technology Ion Source (AJS) G-1958-65138, an Agilent 1260 DAD, and a 6530 Accurate-Mass Q-ToF LC/MS.

Separation of phenolics was achieved with a reversed-phase Kinetex® C18 (100 Å, 150 × 2.10 mm, 2.6 µm; Phenomenex, Torrance, USA) column at a temperature of 40 °C (Agilent 1260 TCC; Agilent Technologies, Waldbronn, Germany). A gradient consisting of eluent A (H<sub>2</sub>O/acetonitrile/formic acid 93/5/2 (v/v/v)) and eluent B (H<sub>2</sub>O/acetonitrile/formic acid 5/93/2 (v/v/v)) was applied at a flow rate of 0.4 mL/min as follows: 0 % B at 0 min (2 min hold), 30 % B at 18 min, 100 % B at 22 min (1 min hold), 0 % B at 24 min (8 min hold). The injection volume was 5 µL.

MS analysis was performed in the positive ionisation mode. The MS parameters were as follows: nebuliser pressure 35 psig, fragmentation at 170 V, skimming at 65 V, VCap at 3000 V, gas flow 8 L/min, gas temperature 320 °C, sheath gas temperature 380 °C, rate 5 spectra/sec, scan at *m/z* 100–1700.

B-prodelphinidins and B-procyanidin gallates were identified according to their *m/z*. Gallocatechin, epigallocatechin, B-procyanidins, and C-procyanidins were identified according to the retention times of reference compounds. Cyanidin-3-*O*-rutinoside was used as internal standard

and external standards were used for quantification. B-procyanidins, B-prodelphinidins, and B-procyanidin gallates were quantified as procyanidin B<sub>2</sub> equivalents. C-procyanidins were quantified as procyanidin C<sub>1</sub> equivalents. A calibration range of 0.1–15 mg/L was used for all reference compounds.

Samples were filtered through 0.45 µm cellulose acetate membranes (Graphic Controls, Totnes, UK) and then analysed directly. MassHunter Workstation Software (version B.05.00, Build 5.0.291; Agilent Technologies, Waldbronn, Germany) was used for MS data acquisition and evaluation. Analysis was performed in duplicate.

## 7. HS-SPME-H/C-MDGC-MS-MS analysis

Analyses of 2-isobutyl-3-methoxypyrazine (IBMP) were performed using an established method (Slabizki *et al.*, 2015; Slabizki *et al.*, 2014). For each experimental treatment, the sample was analysed in duplicate.

## 8. Sensory evaluation

Descriptive analysis (DA) was performed three months after bottling (eight months after alcoholic fermentation) by a trained sensory panel. The panel was composed of 16 judges for Pinot noir wines (8 females, 8 males) and 19 judges for Cabernet-Sauvignon wines (9 females, 10 males) with a range between 22 to 64 years of age. Panellists attended three training sessions (each 90 min) before evaluating Pinot noir wines and another two training sessions before evaluating Cabernet-Sauvignon wines. During the first training session for each grape variety, random wines from this study of the respective grape variety were presented to the panellists. They were asked to describe the aroma of these wines in their own words and then reach a consensus on five aroma descriptors per grape variety that differentiate the wines (Table 1). Training for blind recognition and intensity of aroma, taste, and tactile attributes was carried out to create a common sense for these attributes. Other training sessions were carried out presenting the base wine and the reference standards (Table 1), undiluted and 1:2 diluted with wine, to the judges to familiarise them with the use of unstructured line scales. For training sessions and evaluation sessions, the unstructured line scales were anchored at the left with low intensity and at the right end with high intensity unless stated otherwise in Table 1. Also part of the training was a trial-run DA, which entailed the evaluation of random wines from this study for their appearance, aroma, taste, and mouthfeel on the unstructured line scales, to familiarise panellists with the wines and the evaluation process under the same conditions as those for the formal sessions.

Formal evaluation sessions were held repeatedly for Pinot noir and Cabernet-Sauvignon on separate days. Each flight consisted of six samples. For aroma, taste, and tactile attributes, samples were presented to panellists (30 mL per sample at 16 °C) in black Sensus glasses (DIN 10960 wine-tasting glasses; Schott Zwiesel, Germany) covered with plastic lids. For appearance attributes, the same samples were presented in clear glasses (DIN 10960 wine-tasting

**TABLE 1.** Descriptors, definitions, reference standards, and anchor terms for scales used in the sensory descriptive analysis.

Descriptor	Definition	Reference Standard <sup>a</sup>
<b>Appearance attributes</b>		
Colour intensity	How much the wine blocks the passage of light	-
Colour hue <sup>b</sup>	Wine colour from brown to red to purple	-
<b>Aroma attributes for Pinot noir</b>		
Berries	Aroma of sour cherry and blackcurrant	100 mL sour cherry juice (REWE Beste Wahl) and 100 mL blackcurrant juice (REWE Beste Wahl) to 1 L base wine
Jammy	Aroma of sweet, cooked fruits like plum puree	1.5 soft prunes (Seeberger) to 100 mL base wine, pureed and stirred for 10 min and centrifuged. Supernatant collected. 2 µL acetaldehyde (Sigma Aldrich, certified reference material, pharmaceutical secondary standard) added to supernatant. 100 mL of this mixture and 80 mL juice from canned plums (Jütro) to 1 L base wine
Herbaceous	Fresh, essential oil aromas with Mediterranean herbs	20 mL eucalyptus mixture (1 cough drop eucalyptus (Wick) to 100 mL base wine) and 100 mL herbs mixture (in 100 mL base wine: 0.1 g dried oregano (Ostmann), 1 juniper berry (0.15–0.2 g) (Ostmann), 0.35 g/L fresh rosemary, 1.5 g green tea (Westminster), 1.5 g black tea (Lipton), a pinch of black pepper (Ostmann), a pinch of cloves (Ostmann), stirred for 10 min, then strained)
Spices	Spicy or savoury aroma	In 100 mL base wine: a pinch of black pepper (Ostmann), a pinch of cloves (Ostmann), 20 mL wood mixture (in 50 mL base wine 1 wooden chip, stirred for 3 min), stirred for 10 min, then strained
Oxidised	Aroma of nail polish remover	75 µL ethyl acetate (Sigma Aldrich, EMPROVE® ESSENTIAL) and 1.5 mL acetic acid (Merck, food grade) to 1 L base wine
<b>Aroma attributes for Cabernet-Sauvignon</b>		
Sour cherry	Aroma of sour cherry	200 mL sour cherry juice (REWE Beste Wahl) to 1 L base wine
Dark fruit	Aroma of dark fruits such as blackberry and blackcurrant	200 mL blackcurrant juice (REWE Beste Wahl) to 5 g blackberry tea (Westcliff) in 1 L base wine, stirred for 5 min, then strained
Green bell pepper	Aroma of green bell pepper, unripe	2 mL 3-Isobutyl-2-methoxy-pyrazine (c = 10 mg/L) (Sigma Aldrich, food grade) to 1 L base wine
Eucalyptus	Fresh, cooling eucalyptus aroma	4 cough drop eucalyptus (Wick) finely crushed and 50 mL ethanol to 1 L base wine
Spices	Spicy or savoury aroma	In 100 mL base wine: a pinch of black pepper (Ostmann), a pinch of cloves (Ostmann), ¼ soft liquorice (Katjes), stirred for 10 min, then strained
<b>Taste and tactile attributes</b>		
Bitter	The intensity of bitter taste perceived in the back part of the palate	0.8 g/L caffeine (AppliChem, food grade)
Sour	The intensity of sour taste/acidity perceived in the mouth	2.0 g/L tartaric acid (Carl Roth, food grade)
Sweet	The intensity of sweetness perceived in the mouth	-
Dry astringency	Reduction of the natural lubrication in the mouth, giving a drying feeling	0.8 g/L tannic acid (Sigma Aldrich, reference material, pharmaceutical secondary standard)
Smooth astringency <sup>c</sup>	Mouth feeling from rough to smooth	1 g/L aluminium sulphate (Sigma Aldrich, United States Pharmacopeia Reference Standard)
Green astringency	Palate drying effect with an unripe aftertaste	1.2 g/L catechin (Sigma Aldrich, Pharmaceutical Secondary Standard, Certified Reference Material)
Full body <sup>d</sup>	Perception of the body, weight or thickness of the wine from light to full	-
Harsh mouthfeel <sup>e</sup>	Mouthfeel from harmonious to harsh	-

<sup>a</sup> Standards were produced with a red base wine, grape variety: Regent, 2018 vintage. A volume of 1 L was used unless otherwise stated.

<sup>b</sup> Scale was anchored on the left with brown colour hue, in the middle with red colour hue and on the right with a purple colour hue.

<sup>c</sup> Scale was anchored on the left with rough and on the right with smooth.

<sup>d</sup> Scale was anchored on the left with light and on the right with full.

<sup>e</sup> Scale was anchored on the left with harmonious and on the right with harsh.

glasses; Schott Zwiesel, Germany) covered with plastic lids. All samples were coded with a three-digit number. The wines were presented in a randomised order. Formal evaluations were held in an air-conditioned sensory laboratory with individual booths at 20 °C. Data were acquired using FIZZ software (Version 2.51 c 02, Biosystèmes, Couternon, France). All samples were expectorated. To avoid sensory fatigue, panellists were required to rest for 45 sec while cleansing their palate with water after each sample.

## 9. Statistical analysis

An analysis of variance (ANOVA) was performed for the chemical data. The analytical repetitions and the experimental replicates were additionally regarded as separate factors. The sensory data was processed by a three-way mixed model ANOVA considering panellist as a random effect and wine and tasting replication as fixed effects. Fisher's least significant difference (LSD) post-hoc test ( $p \leq 0.05$ ) was carried out for all data. Principal component analysis (PCA) was performed for chemical as well as sensory evaluation data using Pearson correlation ( $n-1$ ). All statistical analyses were performed using XLSTAT SENSORY (Version 2021.2.2.1147 (32 bit), Addinsoft SARL, Paris, France).

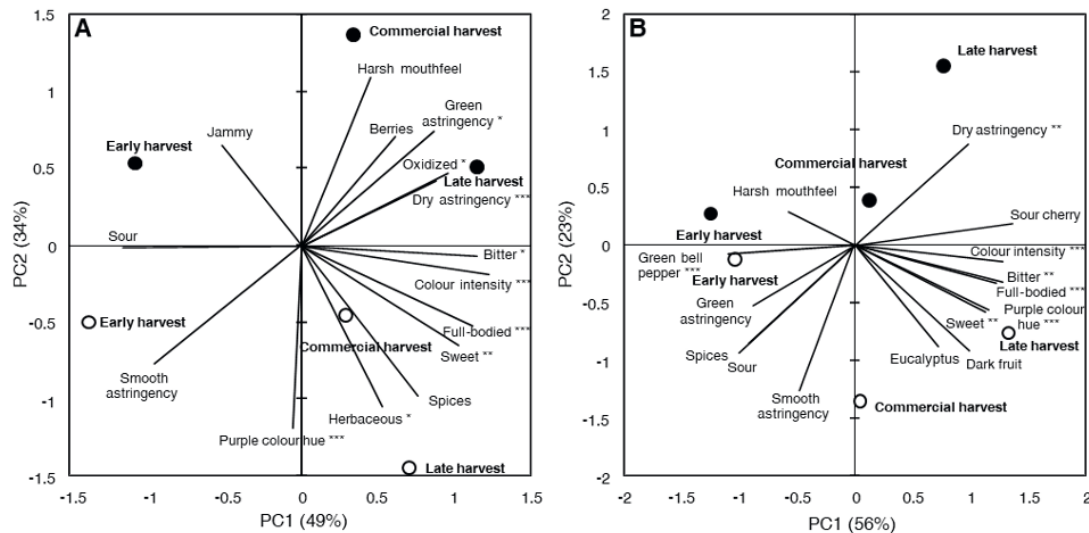
## RESULTS AND DISCUSSION

### 1. Sensory evaluation

Figure 1 shows the principal component analysis (PCA) for Pinot noir (Figure 1A) and Cabernet-Sauvignon (Figure 1B) wines produced from grapes of different maturity levels and with 7 d maceration and 14 d maceration. PCA space was calculated for 15 sensory attributes. For both grape

varieties, the different maturity levels were separated on PC1, the maceration time was separated on PC2. This shows that the effect of maceration time is different from the influence of grape maturity suggesting that sensory characteristics of wine made from immature grape material cannot be driven towards those associated with a higher grape maturity by manipulating maceration time. Results for Cabernet franc in a study by Cadot *et al.* (2012), who investigated two different maturity levels and two different maceration times, indicated similar findings. It is thought that extended maceration cannot compensate for grape immaturity because of the lack of desired substances in grape skins (Ribéreau-Gayon *et al.*, 2006) and/or their low extractability caused by pectin (Garrido-Bañuelos *et al.*, 2019). Extended maceration is known to enhance seed extraction (del Llaudy *et al.*, 2008; Gil *et al.*, 2012) and is therefore not practical. For both grape varieties, the list of sensory attributes could discriminate grape maturity better than maceration times. This is in agreement with Casassa *et al.* (2013b) who concluded that the harvest date defines the sensory profile of the wines and outweighs sensory effects arising from extended maceration.

Both grape varieties showed a slight tendency in the effect of maceration time being more pronounced at higher grape maturity. Tables S1 and S2 reveal this for the sensory descriptors herbaceous and green astringency in Pinot noir as well as for sweetness and full body in Cabernet-Sauvignon. The higher susceptibility of late-harvest wines to be influenced by maceration time can be explained by a greater de-pectination and de-esterification of the grape berry cell walls with increasing grape maturity (Garrido-Bañuelos *et al.*, 2019).



**FIGURE 1.** Principal component analysis for Pinot noir (A) and Cabernet-Sauvignon (B) wines produced from grapes of different maturity levels and with 7 d maceration (open symbols) and 14 d maceration (filled symbols). PCA space was calculated for the fifteen displayed sensory attributes. \* significant at  $p < 0.05$ , \*\* significant at 0.01, \*\*\* significant at 0.001.

Also, another explanation for the effect of maceration time being more pronounced at higher grape maturity is that higher alcohol concentrations are present. Accordingly, longer maceration times can potentially lead to an enhanced extraction.

The observation of higher ratings in acidity/sourness and green bell pepper/vegetal aroma in early-harvest wines is in agreement with the results of Gil *et al.* (2012), Cadot *et al.* (2012), and Casassa *et al.* (2013b). Increasing grape maturity resulted in higher ratings in colour intensity, fruity aroma attributes, sweetness, dry astringency, bitterness, and a full body, which is in agreement with the findings of Cadot *et al.* (2012). The higher ratings in bitterness for late-harvest grapes of both varieties are in contrast with the observations of Gil *et al.* (2012) who showed a decrease in bitterness and astringency with increasing grape maturity. However, the increased bitterness observed here in late-harvest wines may be explained by their higher ethanol content, which is known to enhance bitterness (Fischer & Noble, 1994; Fontoin *et al.*, 2008).

The green bell pepper aroma observed in Cabernet-Sauvignon early-harvest wine is most likely elicited by its concentration of IBMP ( $5.90 \pm 0.71$  ng/L), significantly higher than that of late-harvest wine ( $3.00 \pm 0.32$  ng/L). Roujou de Boubée *et al.* (2000) suggested IBMP as a marker for grape unripeness. A study by Bindon *et al.* (2013) observed a decline in IBMP from  $14.3 \pm 0.4$  ng/L to  $7.03 \pm 0.6$  ng/L during ripening in Cabernet-Sauvignon wine made from grapes with five levels of grape maturity ranging from  $20.3 \pm 0.12$  Brix to  $26.0 \pm 0.00$  Brix.

Extending maceration resulted in wines with higher ratings in astringency as well as tendencies to a lighter colour and yellow hue. Such effects of maceration time have been reported across different grape varieties such as Merlot, Cabernet-Sauvignon, Cabernet franc, and Tempranillo (Cadot *et al.*, 2012; Casassa *et al.*, 2013b; Gil *et al.*, 2012). The findings here extend such observations to Pinot noir.

For both grape varieties, ratings for green astringency were significantly higher in early-harvest wines. This might be due to an enhanced astringency perception of catechins in wines with a lower pH (Peleg *et al.*, 1998). Previous studies have also shown that astringency perception decreases with increasing ethanol concentration (Fontoin *et al.*, 2008; Noble, 1998).

However, only in Pinot noir wine green astringency was also significantly higher with the 14 d maceration than with the 7 d maceration, outweighing the effect of immaturity. The effect of extended maceration on catechin extraction was likely more pronounced in Pinot noir than in Cabernet-Sauvignon. For both grape varieties ratings for purple hue were significantly higher in 7 d maceration wines than in 14 d maceration wines, which is in agreement with the findings of Casassa *et al.* (2013b). Longer maceration may allow browning reactions to take place, such as the oxidation of caftaric acid (Singleton *et al.*, 1985). At the same time, monomeric anthocyanins might undergo polymerisation reactions (Romero-Cascales *et al.*, 2005).

Ratings of purple hue were significantly higher in Cabernet-Sauvignon commercial harvest and late-harvest wines for both maceration treatments relative to early-harvest wine, which is in agreement with the findings of Bindon *et al.* (2014a). More monomeric and polymeric pigments likely increase in Cabernet-Sauvignon wine with progressing grape maturity. In contrast, the effect of progressing maturity on purple hue was only observed for 7 d maceration Pinot noir wines.

## 2. Basic juice and wine parameters

With increasing grape maturity, glucose and fructose as well as the pH increased, and titratable acidity decreased in grape juice (Table 2). Tables 3 and 4 show the basic wine parameters of the resulting Pinot noir and Cabernet-Sauvignon wines. Late-harvest wines had significantly lower titratable acidity and higher pH than commercial harvest and early-harvest wines. Extended

**TABLE 2.** Basic chemical analyses of the juice. Average values followed by SD.

	Brix	Titratable acidity [g/L TAE]	pH	Glucose [g/L]	Fructose [g/L]
<b>Pinot noir</b>					
Early-harvest	20.68 ± 0.00 c <sup>a</sup>	9.1 ± 0.1 a	3.3 ± 0.0 b	101.8 ± 0.4 c	101.5 ± 0.4 c
Commercial harvest	22.46 ± 0.00 b	6.6 ± 0.0 b	3.4 ± 0.0 a	111.5 ± 0.2 b	114.2 ± 0.4 b
Late-harvest	24.87 ± 0.00 a	6.2 ± 0.0 c	3.4 ± 0.0 a	125.2 ± 0.3 a	128.8 ± 0.6 a
<i>p</i>	< 0.0001	< 0.0001	0.001	< 0.0001	< 0.0001
<b>Cabernet-Sauvignon</b>					
Early-harvest	20.01 ± 0.00 c	9.3 ± 0.0 a	3.2 ± 0.0 c	97.6 ± 0.1 c	99.0 ± 0.8 c
Commercial harvest	22.68 ± 0.00 b	6.9 ± 0.1 b	3.4 ± 0.0 b	114.0 ± 0.4 b	117.7 ± 0.9 b
Late-harvest	23.56 ± 0.00 a	4.2 ± 0.1 c	3.6 ± 0.0 a	117.6 ± 0.7 a	123.9 ± 0.5 a
<i>p</i>	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001

<sup>a</sup> Within a column, values followed by the same letter are not significantly different according to Fisher's LSD test at  $p \leq 0.05$ . Significant  $p$  values ( $p \leq 0.05$ ) are shown in italic letters. Data consisted of four experimental replicates.

**TABLE 3.** Basic chemical analyses of Pinot noir wine. Average values followed by SD.

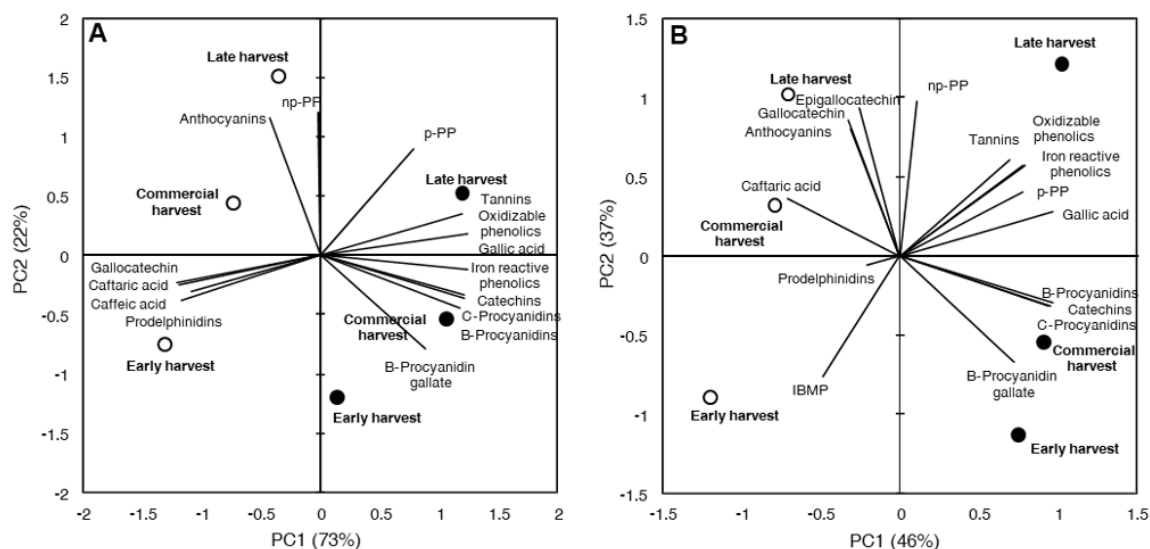
	Alcohol [g/L]	Alcohol [% vol.]	Residual sugar [g/L]	pH	Titritable acidity [g/L TAE]	Tartaric acid [g/L]	Malic acid [g/L]	Lactic acid [g/L]	Acetic acid [g/L]	
<b>Maturity</b>										
<b>Maceration</b>										
Early-harvest	7 d	89.9 ± 0.6 e <sup>a</sup>	11.36 ± 0.08 e	0.9 ± 0.1 b	3.43 ± 0.01 d	5.4 ± 0.0 a	2.8 ± 0.0 a	0.1 ± 0.0 c	1.8 ± 0.0 a	0.4 ± 0.0 c
	14 d	88.3 ± 0.8 f	11.16 ± 0.10 f	0.9 ± 0.0 b	3.46 ± 0.04 d	5.3 ± 0.1 b	2.5 ± 0.0 b	0.3 ± 0.0 a	1.5 ± 0.0 b	0.5 ± 0.0 b
Commercial harvest	7 d	100.3 ± 0.2 c	12.68 ± 0.03 c	1.2 ± 0.1 a	3.55 ± 0.01 c	4.9 ± 0.1 c	2.3 ± 0.1 c	0.0 ± 0.0 d	1.5 ± 0.0 b	0.5 ± 0.0 b
	14 d	96.2 ± 1.0 d	12.16 ± 0.13 d	0.9 ± 0.1 b	3.67 ± 0.04 b	4.5 ± 0.2 e	2.1 ± 0.0 d	0.2 ± 0.1 b	1.4 ± 0.0 c	0.6 ± 0.0 a
Late-harvest	7 d	112.6 ± 0.1 a	14.23 ± 0.01 a	1.3 ± 0.1 a	3.66 ± 0.02 b	4.7 ± 0.1 d	2.1 ± 0.1 d	0.0 ± 0.0 d	1.4 ± 0.0 c	0.5 ± 0.0 b
	14 d	110.1 ± 1.0 b	13.91 ± 0.13 b	1.2 ± 0.2 a	3.74 ± 0.02 a	4.6 ± 0.0 de	1.8 ± 0.1 e	0.1 ± 0.0 c	1.3 ± 0.0 d	0.6 ± 0.0 a
P	< 0.0001	< 0.0001	0.002	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001
<b>Maturity</b>										
Early-harvest	7 d	89.1 ± 1.1 c	11.26 ± 0.14 c	0.9 ± 0.1 b	3.45 ± 0.03 c	5.3 ± 0.1 a	2.7 ± 0.2 a	0.2 ± 0.1 a	1.6 ± 0.1 a	0.5 ± 0.0 b
	Commercial harvest	98.2 ± 2.4 b	12.41 ± 0.30 b	1.0 ± 0.2 b	3.61 ± 0.08 b	4.7 ± 0.2 b	2.2 ± 0.2 b	0.1 ± 0.1 b	1.5 ± 0.1 b	0.5 ± 0.1 b
Late-harvest	7 d	111.4 ± 1.5 a	14.08 ± 0.19 a	1.2 ± 0.2 a	3.70 ± 0.05 a	4.6 ± 0.1 c	2.0 ± 0.2 c	0.1 ± 0.1 b	1.4 ± 0.1 c	0.6 ± 0.1 a
	P	< 0.0001	< 0.0001	0.001	< 0.0001	< 0.0001	< 0.0001	0.002	< 0.0001	< 0.0001
<b>Maceration</b>										
7 d	7 d	100.9 ± 10.2 a	12.75 ± 1.29 a	1.1 ± 0.2 a	3.55 ± 0.10 b	5.0 ± 0.3 a	2.4 ± 0.3 a	0.0 ± 0.0 b	1.6 ± 0.2 a	0.5 ± 0.0 b
	14 d	98.2 ± 9.9 b	12.41 ± 1.25 b	1.0 ± 0.2 a	3.62 ± 0.13 a	4.8 ± 0.4 b	2.2 ± 0.3 b	0.2 ± 0.1 a	1.4 ± 0.1 b	0.6 ± 0.0 a
P	< 0.0001	< 0.0001	0.056	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001
<b>Maturity x Maceration</b>										
P	0.002	0.002	0.142	0.004	0.001	0.670	0.506	0.003	0.139	0.139

<sup>a</sup> Within a column, values followed by the same letter are not significantly different according to Fisher's LSD test at  $p \leq 0.05$ . Significant  $p$  values ( $p \leq 0.05$ ) are shown in italic letters. Data consisted of two experimental replicates.

**TABLE 4.** Basic chemical analyses of Cabernet-Sauvignon wine. Average values followed by SD.

	Alcohol [g/L]	Alcohol [% vol.]	Residual sugar [g/L]	pH	Titratable acidity [g/L TAE]	Tartaric acid [g/L]	Malic acid [g/L]	Lactic acid [g/L]	Acetic acid [g/L]
<b>Maturity</b>									
<b>Maceration</b>									
Early-harvest	7d	87.4 ± 0.7 e <sup>a</sup>	11.05 ± 0.09 e	3.59 ± 0.00 f	5.4 ± 0.0 a	2.6 ± 0.1 a	0.4 ± 0.1 c	2.0 ± 0.0 a	0.4 ± 0.0 b
	14d	85.1 ± 0.5 f	10.75 ± 0.06 f	0.9 ± 0.1 c	3.64 ± 0.00 e	2.5 ± 0.0 b	0.6 ± 0.0 a	1.9 ± 0.0 b	0.5 ± 0.0 a
Commercial harvest	7d	101.6 ± 0.1 c	12.84 ± 0.01 c	1.2 ± 0.0 bc	3.78 ± 0.01 d	2.3 ± 0.0 c	0.5 ± 0.0 b	2.0 ± 0.0 a	0.4 ± 0.0 b
	14d	98.7 ± 0.0 d	12.47 ± 0.00 d	1.4 ± 0.1 ab	3.86 ± 0.01 c	1.9 ± 0.0 e	0.6 ± 0.0 a	2.0 ± 0.0 a	0.5 ± 0.0 a
Late-harvest	7d	107.2 ± 0.4 a	13.55 ± 0.05 a	1.5 ± 0.2 a	3.90 ± 0.01 b	2.2 ± 0.1 d	0.3 ± 0.0 c	1.6 ± 0.0 c	0.4 ± 0.0 b
	14d	106.1 ± 0.7 b	13.41 ± 0.09 b	1.0 ± 0.1 bc	3.99 ± 0.02 a	1.9 ± 0.1 e	0.5 ± 0.0 b	1.5 ± 0.1 d	0.4 ± 0.0 b
P	< 0.0001	< 0.0001	0.002	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001
<b>Maturity</b>									
Early-harvest		86.3 ± 1.4 c	10.91 ± 0.18 c	0.9 ± 0.0 b	3.62 ± 0.03 c	2.6 ± 0.1 a	0.5 ± 0.1 a	1.9 ± 0.0 b	0.5 ± 0.1 a
		100.6 ± 1.7 b	12.71 ± 0.21 b	1.2 ± 0.1 a	3.81 ± 0.05 b	2.2 ± 0.3 b	0.5 ± 0.1 a	2.0 ± 0.0 a	0.5 ± 0.1 a
Late-harvest		106.6 ± 0.8 a	13.47 ± 0.10 a	1.3 ± 0.3 a	3.95 ± 0.05 a	2.1 ± 0.1 c	0.4 ± 0.1 b	1.6 ± 0.1 c	0.4 ± 0.0 b
P	< 0.0001	< 0.0001	0.002	< 0.0001	< 0.0001	< 0.0001	0.003	< 0.0001	< 0.0001
<b>Maceration</b>									
7 d		98.7 ± 9.1 a	12.47 ± 1.15 a	1.2 ± 0.3 a	3.83 ± 0.14 a	2.4 ± 0.2 a	0.4 ± 0.1 b	1.8 ± 0.2 a	0.4 ± 0.0 b
14 d		96.2 ± 9.5 b	12.16 ± 1.20 b	1.0 ± 0.2 a	3.76 ± 0.16 b	2.1 ± 0.3 b	0.6 ± 0.1 a	1.8 ± 0.2 a	0.5 ± 0.0 a
P	< 0.0001	< 0.0001	0.237	< 0.0001	< 0.0001	< 0.0001	< 0.0001	0.627	< 0.0001
<b>Maturity x Maceration</b>									
P	0.006	0.006	0.010	0.050	< 0.0001	0.003	0.205	0.006	0.001

<sup>a</sup> Within a column, values followed by the same letter are not significantly different according to Fisher's LSD test at  $p \leq 0.05$ . Significant  $p$  values ( $p \leq 0.05$ ) are shown in italic letters. Data consisted of two experimental replicates.



**FIGURE 2.** Principal component analysis for Pinot noir (A) and Cabernet-Sauvignon (B) wines produced from grapes of different maturity levels and with 7 d maceration (open symbols) and 14 d maceration (filled symbols). PCA space was calculated on the fifteen displayed chemical attributes.

maceration wines of both grape varieties had significantly lower titratable acidity than 7 d maceration wines due to the formation of potassium bitartrate and had higher acetic acid concentrations as a result of oxidation. Irrespective of the maceration, time the acetic acid concentrations were below the sensory threshold (Corison *et al.*, 1979). Extended maceration time also caused significantly lower alcohol concentrations, which is due to the evaporation of alcohol during the post-alcoholic fermentation maceration.

### 3. Phenolic composition of wines

For both grape varieties, PC1 segregated the wines by maceration time and PC2 segregated the wines by grape maturity, indicating that the effect of maceration time on the phenolic composition of the wines outweighs the influence of grape maturity (Figure 2). This observation contrasts with the sensory data (Figure 1): the influence of grape maturity (PC1) outweighed the effect of maceration time (PC2) on the sensory profile of the wines.

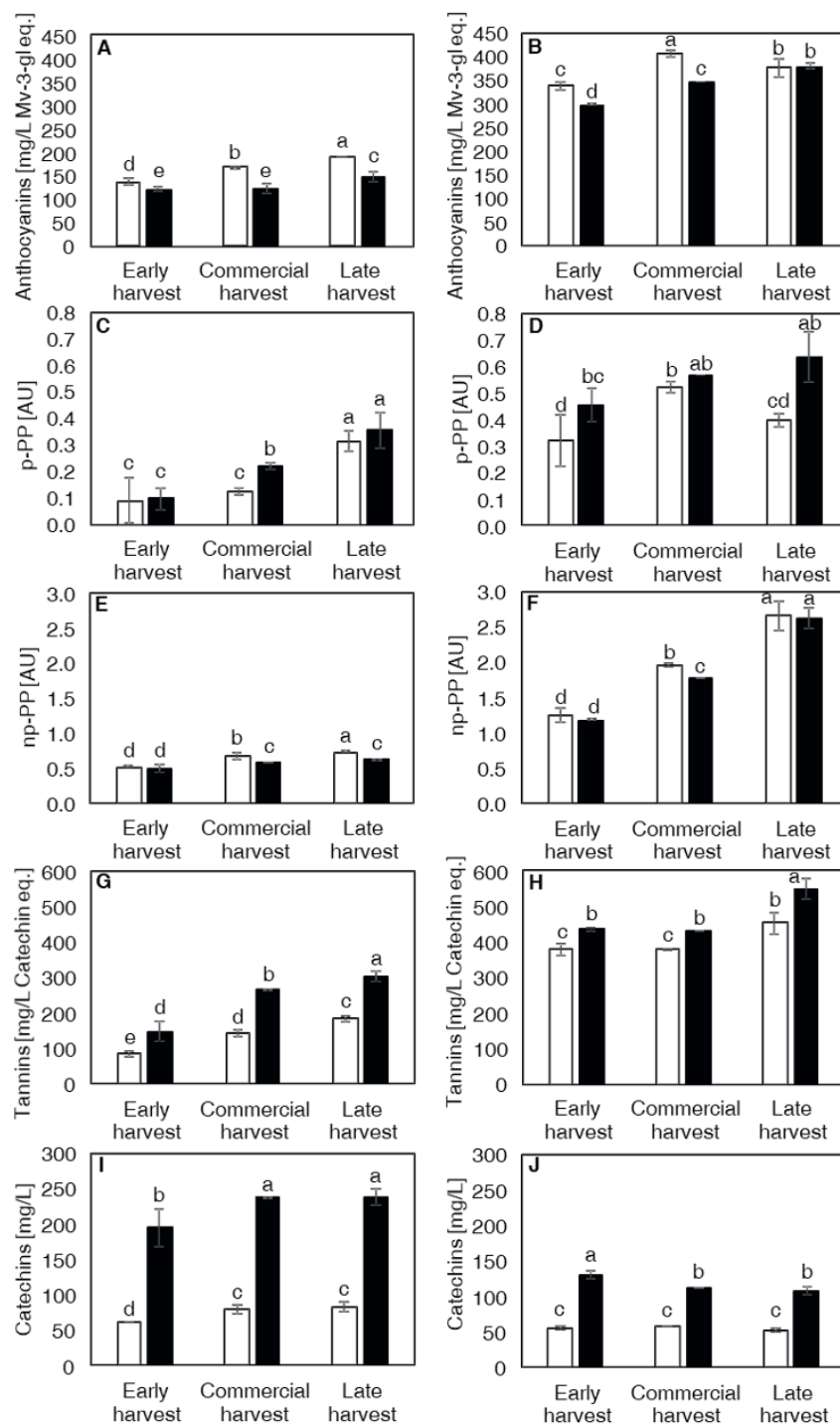
For both grape varieties, the phenolic data in Figure 2 shows that the effect of maceration time is different from the influence of grape maturity suggesting that sensory characteristics of wine made from immature grape material cannot be driven towards those associated with a higher grape maturity by manipulating maceration time. This observation supports the earlier findings with sensory data (Figure 1). However, extended maceration did not enhance the effects of grape immaturity on phenolics in wine either. Extended maceration enhanced seed extraction which is in agreement with the literature (del Llaudy *et al.*, 2008; Gil *et al.*, 2012).

Pinot noir wine (Figure 2A) showed a significant decrease in prodelphinidin dimers with extended maceration suggesting

that prodelphinidin dimers react with other phenolic compounds to form polymerised proanthocyanidins. Extended maceration also led to significantly lower gallic acid concentration irrespective of the level of grape maturity in Pinot noir wine, likely due to polymerisation or oxidation reactions. On the other hand, Cabernet-Sauvignon wine (Figure 2B) showed no significant difference in prodelphinidin concentration between 7 d and 14 d maceration treatments. Significantly higher gallic acid and epigallocatechin concentrations were observed with increasing grape maturity for Cabernet-Sauvignon wine. Extended maceration led to significantly lower concentrations of gallic acid and epigallocatechin. In Cabernet-Sauvignon wine, the parameters gallic acid and epigallocatechin had higher contributions to PC2, which segregated the wine by grape maturity, than to PC1, which segregated the wine by maceration time. Gallic acid and epigallocatechin are found in grape skins suggesting that the extractability of skin tannins into the wine increased during grape ripening due to de-pectination (del Llaudy *et al.*, 2008; Garrido-Bañuelos *et al.*, 2019).

The stronger influence of maceration time on late-harvest wines, as seen in the sensory data (Figure 1), was not evident in phenolic composition (Figure 2). Most of the sensory descriptors that contributed to a shift on the PC2 axis were aroma descriptors such as herbaceous, spices or berries (Figure 1A), and eucalyptus/ethereal or dark fruit (Figure 1B).

Figures 3A and 3B show the anthocyanin concentrations of Pinot noir and Cabernet-Sauvignon wines. In general, anthocyanin concentrations increased with progressing grape maturity and decreased with extending maceration time in both grape varieties. The difference in anthocyanin



**FIGURE 3.** Polyphenol concentrations in Pinot noir (A, C, E, G, I) and Cabernet-Sauvignon (B, D, F, H, J) wines produced from grapes of different maturity levels and with 7 d maceration (white bars) and 14 d maceration (black bars). (A, B) Anthocyanins [mg/L Mv-3-gl eq.]; (C, D) p-PP [AU]; (E, F) np-PP [AU]; (G, H) Tannins [mg/L Catechin eq.]; (I, J) Catechins [mg/L]. The data presented are the mean ( $\pm$  SD) of the experimental replicates ( $n = 2$ ); values sharing the same letter are not significantly different at  $p \leq 0.05$ .

concentrations between Point noir and Cabernet-Sauvignon outweighed the effects of grape maturity and maceration time. The increase of anthocyanins with progressing grape maturity was most pronounced for 7 d maceration in Pinot noir and 14 d maceration in Cabernet-Sauvignon. Although Pinot noir and Cabernet-Sauvignon differ in their absolute anthocyanin concentrations, the de-pectination process with progressing grape maturity (Garrido-Bañuelos *et al.*, 2019) might explain why anthocyanins are more readily extracted during winemaking for both varieties. Remarkably, 7 d maceration of Cabernet-Sauvignon reached a maximum in anthocyanins at commercial harvest and slightly decreased towards late-harvest. The peak of anthocyanin concentration of Cabernet-Sauvignon at commercial harvest indicates that anthocyanins can be broken down if the grapes become overripe (Ribéreau-Gayon *et al.*, 2006). Another explanation could be an increased adsorption of anthocyanins on the cell wall material as the surface of the cell walls increases with progressing maturity (Bindon *et al.*, 2014b).

When extending maceration time, a significant decrease in anthocyanins was observed irrespective of grape maturity for both varieties. This is in agreement with the study by Casassa *et al.* (2013b) and with the sensory results described earlier showing that purple hue decreased when extending maceration. Extended maceration promotes polymerisation reactions of monomeric anthocyanins and therefore decreases anthocyanins. Casassa *et al.* (2013a) observed a significant relationship between the decrease in anthocyanins and the increase of precipitable polymeric pigments in extended maceration wines.

Globally, p-PP increased with progressing grape maturity (Figures 3C and 3D) and with extended maceration in both grape varieties. This observation is in agreement with the findings of Casassa *et al.* (2013b). Harbertson *et al.* (2009) suggested that higher tannin concentrations due to extended maceration favour p-PP formation. However, 7 d maceration of Cabernet-Sauvignon reached a maximum in p-PP at commercial harvest and slightly decreased towards late-harvest. The same observation was made with anthocyanin concentration. It is thought that p-PP formation is dependent on anthocyanin concentration. Therefore, the assumed anthocyanin breakdown in overripe grapes (Ribéreau-Gayon *et al.*, 2006) is also visible in p-PP.

Increasing np-PP concentrations can be observed with progressing grape maturity, especially in Cabernet-Sauvignon (Figure 3F). In contrast, the influence of grape maturity in Pinot noir was very little (Figure 3E). The effect of maceration time was less distinct than the influence of grape maturity. This was also indicated in Figure 2 where np-PP had a high contribution to PC2 which segregated the wines by grape maturity. In both grape varieties extended maceration led to significantly lower np-PP. This observation is in agreement with the observations made by Casassa *et al.* (2013b). Studies by Adams *et al.* (2004) and Casassa *et al.* (2013b) assumed that np-PP is a heterogeneous mixture of anthocyanin-derived products such as acetaldehyde cross-linked oligomers or cycloaddition products, which are unable to precipitate

with proteins. On the other hand, p-PP were assumed to be pigmented tannin-anthocyanin polymers. A shorter maceration time, indicating lower tannin concentrations would, therefore, favour the formation of np-PP as the anthocyanin to tannin ratio is higher.

Figures 3G and 3H show the tannin concentrations in the wines. Tannin concentrations were significantly higher with increasing grape maturity as well as with extended maceration time, irrespective of the grape variety. These observations support the theory discussed earlier with p-PP data that higher tannin concentrations with increasing grape maturity and extending maceration time might enhance p-PP formation. It is assumed that higher tannin concentrations with increased grape maturity and extended maceration time led to higher ratings of dry astringency in the 14 d late-harvest wines of both grape varieties. Casassa *et al.* (2013b) suggested that an increased tannin concentration in late-harvest Merlot wines resulted from higher tannin extractability from seeds.

Monomeric catechins were highest in Pinot noir wine (Figure 3I). A significantly higher concentration of catechins was observed for extended maceration time and—to a smaller extent—progressing grape maturity. An enhanced seed extraction when extending maceration time was also observed by others (del Llaudy *et al.*, 2008; Gil *et al.*, 2012). Similar, but less pronounced than in Pinot noir, extending maceration time also increased monomeric catechins in Cabernet-Sauvignon (Figure 3J). No effect of grape maturity was observed in the 7 d maceration wine of Cabernet-Sauvignon. The 14 d maceration wine showed even a negative trend with progressing grape maturity. For both grape varieties, the effect of maceration time on the catechin concentrations was more distinct than the effect of grape maturity. Monomeric catechins are not suitable to indicate grape maturity because of the contrasting behaviour of different grape varieties. *Vice versa*, catechins can exhibit high resolution for the effect of maceration time.

## CONCLUSION

Maceration time affected sensory characteristics and phenolic composition of Pinot noir and Cabernet-Sauvignon wine differently than grape maturity. Extended maceration neither compensated for grape immaturity nor enhanced the effects of immaturity. Changes in the phenolic composition with progressing grape maturity and extending maceration time were reflected in the wine colour as well as in taste and tactile ratings in the sensory evaluation of the wines. Skin-associated phenolics such as anthocyanins and polymeric pigments correlated with grape maturity, while extended maceration enhanced the extraction of seed-associated phenolics such as monomeric catechins, procyanidins and procyanidin gallates. Concentrations of anthocyanins and polymeric pigments are suggested as suitable indicators for grape maturity in wine. Concentrations of monomeric catechins and procyanidins are good indicators for maceration time.

## ACKNOWLEDGEMENTS

This research project was supported by the German Federal Ministry of Economic Affairs and Climate Action (*via* AiF) and the FEI (Forschungskreis der Ernährungsindustrie e.V., Bonn) project AiF 20024N and internal funding of the University of Applied Sciences Kaiserslautern. The authors would like to thank Daniel Munder for his support in producing the wines. Participation in the sensory panel is gratefully acknowledged.

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**Supplemental Table 1. Sensory data of Pinot noir wine.**

		Colour intensity	Purple colour hue	Berries	Jammy	Herbaceous	Spices	Oxidized	Sweet	Sour	Full body	Harsh mouthfeel	Dry astringency	Smooth astringency	Green astringency	Bitterness
<b>Maturity</b>	<b>Maceration</b>															
Early harvest	7d	2.5 ± 0.4 a <sup>a</sup>	5.5 ± 0.2 c	4.2 ± 0.2 a	3.5 ± 0.4 a	2.8 ± 0.6 a	2.9 ± 0.4 a	1.9 ± 0.5 a	3.2 ± 0.4 ab	5.7 ± 0.3 bc	3.9 ± 0.5 a	4.4 ± 0.3 a	4.4 ± 0.4 a	3.8 ± 0.9 a	3.0 ± 0.4 a	2.3 ± 0.2 a
	14d	2.7 ± 0.1 a	4.5 ± 0.2 b	4.3 ± 0.3 a	3.3 ± 0.4 a	2.7 ± 0.4 a	2.8 ± 0.3 a	2.2 ± 0.6 ab	2.8 ± 0.3 a	5.8 ± 0.4 c	3.8 ± 0.6 a	5.0 ± 0.3 a	5.3 ± 0.2 b	4.2 ± 0.3 a	3.0 ± 0.2 a	2.3 ± 0.1 a
Commercial harvest	7d	5.6 ± 0.6 b	6.1 ± 0.4 d	4.5 ± 0.5 a	3.5 ± 0.8 a	3.3 ± 0.2 ab	3.2 ± 0.2 a	2.4 ± 0.4 ab	3.6 ± 0.4 bc	5.1 ± 0.4 ab	4.9 ± 0.7 b	4.6 ± 0.7 a	5.0 ± 0.3 ab	4.4 ± 0.8 a	3.6 ± 0.2 ab	2.7 ± 0.2 ab
	14d	5.2 ± 0.5 b	3.7 ± 0.2 a	4.5 ± 0.6 a	3.8 ± 0.4 a	2.7 ± 0.3 a	2.9 ± 0.0 a	3.2 ± 0.9 c	3.2 ± 0.2 ab	5.1 ± 0.7 ab	4.3 ± 0.5 a	5.3 ± 0.2 a	5.4 ± 0.3 b	4.6 ± 0.4 a	3.8 ± 0.6 b	3.2 ± 0.4 bc
Late harvest	7d	7.0 ± 1.4 c	6.5 ± 0.2 d	4.2 ± 0.5 a	2.8 ± 0.2 a	3.8 ± 0.4 b	3.3 ± 0.5 a	2.8 ± 0.5 bc	3.9 ± 0.1 c	5.2 ± 0.3 ab	5.3 ± 0.2 b	4.5 ± 0.3 a	5.4 ± 0.3 b	4.2 ± 0.5 a	3.0 ± 0.4 a	3.5 ± 0.2 c
	14d	7.5 ± 1.0 c	4.0 ± 0.4 ab	4.4 ± 0.1 a	2.9 ± 0.6 a	2.8 ± 0.7 a	3.1 ± 0.3 a	2.6 ± 0.7 abc	3.8 ± 0.4 c	5.0 ± 0.1 a	5.2 ± 0.4 b	5.3 ± 0.1 a	6.2 ± 0.4 c	4.6 ± 0.1 a	4.2 ± 0.1 b	3.4 ± 0.4 c
<i>p</i>		<0,0001	<0,0001	0.914	0.087	0.016	0.718	0.010	<0,0001	0.049	<0,0001	0.065	0.000	0.154	0.001	0.000
<b>Maturity</b>																
Early harvest		2.6 ± 0.3 a	5.0 ± 0.6 a	4.2 ± 0.3 a	3.4 ± 0.4 ab	2.7 ± 0.4 a	2.9 ± 0.3 a	2.1 ± 0.5 a	3.0 ± 0.4 a	5.7 ± 0.3 b	3.9 ± 0.5 a	4.7 ± 0.4 a	4.9 ± 0.5 a	4.0 ± 0.7 a	3.0 ± 0.3 a	2.3 ± 0.2 a
Commercial harvest		5.4 ± 0.6 b	4.9 ± 1.4 a	4.5 ± 0.5 a	3.7 ± 0.6 b	3.0 ± 0.5 a	3.0 ± 0.2 a	2.8 ± 0.8 b	3.4 ± 0.4 b	5.1 ± 0.5 a	4.6 ± 0.6 b	4.9 ± 0.6 a	5.2 ± 0.4 a	4.5 ± 0.6 a	3.7 ± 0.4 b	2.9 ± 0.4 b
Late harvest		7.2 ± 1.1 c	5.3 ± 1.4 a	4.3 ± 0.3 a	2.8 ± 0.4 a	3.3 ± 0.7 a	3.2 ± 0.4 a	2.7 ± 0.5 b	3.9 ± 0.2 c	5.1 ± 0.2 a	5.2 ± 0.3 c	4.9 ± 0.4 a	5.8 ± 0.6 b	4.4 ± 0.4 a	3.6 ± 0.7 b	3.5 ± 0.3 c
<i>p</i>		<0,0001	0.095	0.538	0.014	0.108	0.461	0.009	<0,0001	0.005	<0,0001	0.729	0.001	0.108	0.013	<0,0001
<b>Maceration</b>																
7 d		5.0 ± 2.1 a	6.0 ± 0.5 b	4.3 ± 0.4 a	3.3 ± 0.6 a	3.3 ± 0.6 b	3.2 ± 0.4 a	2.4 ± 0.6 a	3.6 ± 0.4 b	5.3 ± 0.4 a	4.7 ± 0.8 a	4.5 ± 0.4 a	4.9 ± 0.5 a	4.1 ± 0.7 a	3.2 ± 0.5 a	2.8 ± 0.6 a
14 d		5.1 ± 2.2 a	4.1 ± 0.4 a	4.4 ± 0.4 a	3.3 ± 0.6 a	2.7 ± 0.4 a	2.9 ± 0.2 a	2.7 ± 0.8 a	3.3 ± 0.5 a	5.3 ± 0.5 a	4.4 ± 0.7 a	5.2 ± 0.2 b	5.6 ± 0.5 b	4.5 ± 0.3 a	3.7 ± 0.6 b	3.0 ± 0.6 a
<i>p</i>		0.444	<0,0001	0.683	0.757	0.007	0.283	0.160	0.040	0.969	0.066	0.002	0.001	0.068	0.014	0.481
<b>Maturity x Maceration</b>		0.068	<0,0001	0.960	0.634	0.288	0.916	0.153	0.586	0.842	0.361	0.956	0.640	0.883	0.065	0.413

<sup>a</sup> Within a column, values followed by the same letter are not significantly different according to Fisher's LSD test at  $p \leq 0.05$ . Significant  $p$  values ( $p \leq 0.05$ ) are shown in italic letters. The data consisted of two panel repetitions.

**Supplemental Table 2. Sensory data of Cabernet Sauvignon wine.**

		Colour intensity	Purple colour hue	Sour cherry	Dark fruit	Green bell pepper	Spices	Eucalyptus	Sweet	Sour	Full body	Harsh mouthfeel	Dry astringency	Smooth astringency	Green astringency	Bitterness
<b>Maturity</b>	<b>Maceration</b>															
Early harvest	7d	5.0 ± 0.5 a <sup>a</sup>	6.5 ± 0.4 b	4.0 ± 0.3 a	4.3 ± 0.1 a	5.4 ± 0.1 b	3.8 ± 0.2 a	3.6 ± 0.1 a	3.4 ± 0.4 a	5.2 ± 0.5 a	4.2 ± 0.4 a	4.3 ± 0.0 a	5.4 ± 0.7 a	4.2 ± 0.6 a	4.4 ± 0.5 a	2.7 ± 0.0 a
	14d	4.8 ± 0.1 a	6.1 ± 0.1 a	4.0 ± 0.0 ab	3.9 ± 0.2 a	4.8 ± 0.0 b	4.2 ± 1.0 a	3.7 ± 0.1 a	2.9 ± 0.1 a	5.1 ± 0.2 a	4.5 ± 0.6 ab	5.2 ± 0.3 b	5.9 ± 0.0 abc	4.1 ± 0.1 a	4.4 ± 0.7 a	2.6 ± 0.1 a
Commercial harvest	7d	8.3 ± 0.0 c	8.0 ± 0.5 d	4.3 ± 0.3 abc	5.0 ± 0.0 a	3.5 ± 0.3 a	4.1 ± 0.2 a	4.0 ± 0.1 a	3.6 ± 0.6 a	5.2 ± 0.5 a	5.1 ± 0.3 bc	4.9 ± 0.4 ab	5.6 ± 0.4 ab	4.0 ± 0.5 a	4.7 ± 0.3 a	3.7 ± 0.4 b
	14d	7.1 ± 0.1 b	6.9 ± 0.0 b	4.7 ± 0.6 abc	4.6 ± 0.1 a	3.3 ± 0.2 a	3.3 ± 0.2 a	4.1 ± 0.2 a	3.3 ± 0.3 a	4.7 ± 0.1 a	4.8 ± 0.3 abc	5.0 ± 0.3 ab	6.4 ± 0.1 cd	4.3 ± 0.6 a	4.6 ± 0.2 a	3.2 ± 0.4 ab
Late harvest	7d	8.4 ± 0.2 c	7.9 ± 0.3 cd	5.4 ± 0.5 c	5.1 ± 0.8 a	3.7 ± 0.5 a	3.5 ± 0.3 a	4.5 ± 0.3 a	4.3 ± 0.2 b	4.6 ± 0.0 a	6.0 ± 0.0 d	4.2 ± 0.3 a	6.3 ± 0.3 bcd	4.1 ± 0.3 a	3.8 ± 0.5 a	3.6 ± 0.2 b
	14d	8.5 ± 0.1 c	7.4 ± 0.3 c	5.0 ± 1.9 bc	4.3 ± 0.4 a	3.4 ± 0.1 a	3.2 ± 0.5 a	3.5 ± 0.5 a	3.5 ± 0.3 a	4.3 ± 0.1 a	5.2 ± 0.1 c	4.7 ± 0.3 ab	6.7 ± 0.0 d	4.7 ± 0.1 a	3.9 ± 0.2 a	3.5 ± 0.5 b
<i>p</i>		<i>&lt;0,0001</i>	<i>&lt;0,0001</i>	<i>0.047</i>	0.132	<i>&lt;0,0001</i>	0.142	0.265	<i>0.002</i>	0.061	<i>&lt;0,0001</i>	0.089	<i>0.005</i>	0.587	0.171	<i>0.004</i>
<b>Maturity</b>																
Early harvest		4.9 ± 0.3 a	6.3 ± 0.3 a	4.0 ± 0.2 a	4.1 ± 0.2 a	5.1 ± 0.4 b	4.0 ± 0.6 a	3.7 ± 0.1 a	3.2 ± 0.3 a	5.1 ± 0.3 b	4.4 ± 0.4 a	4.8 ± 0.6 a	5.6 ± 0.5 a	4.2 ± 0.3 a	4.4 ± 0.5 ab	2.7 ± 0.0 a
Commercial harvest		7.7 ± 0.7 b	7.4 ± 0.7 b	4.5 ± 0.4 ab	4.8 ± 0.2 a	3.4 ± 0.2 a	3.7 ± 0.5 a	4.0 ± 0.1 a	3.4 ± 0.4 a	4.9 ± 0.4 ab	4.9 ± 0.3 b	5.0 ± 0.3 a	6.0 ± 0.5 ab	4.2 ± 0.5 a	4.7 ± 0.2 b	3.4 ± 0.4 b
Late harvest		8.4 ± 0.1 c	7.6 ± 0.4 b	5.2 ± 1.1 b	4.7 ± 0.7 a	3.6 ± 0.3 a	3.3 ± 0.4 a	4.0 ± 0.7 a	3.9 ± 0.5 b	4.5 ± 0.2 a	5.6 ± 0.5 c	4.4 ± 0.4 a	6.5 ± 0.3 b	4.4 ± 0.4 a	3.8 ± 0.3 a	3.6 ± 0.3 b
<i>p</i>		<i>&lt;0,0001</i>	<i>&lt;0,0001</i>	<i>0.006</i>	0.098	<i>&lt;0,0001</i>	0.140	0.526	<i>0.007</i>	<i>0.028</i>	<i>&lt;0,0001</i>	0.241	<i>0.006</i>	0.577	<i>0.023</i>	<i>0.001</i>
<b>Maceration</b>																
	7 d	7.2 ± 1.8 b	7.5 ± 0.8 b	4.6 ± 0.7 a	4.8 ± 0.5 a	4.2 ± 1.0 a	3.8 ± 0.3 a	4.0 ± 0.4 a	3.8 ± 0.5 b	5.0 ± 0.4 a	5.1 ± 0.8 a	4.5 ± 0.4 a	5.8 ± 0.6 a	4.1 a ± 0.4	4.3 ± 0.6 a	3.3 ± 0.6 a
	14 d	6.8 ± 1.7 a	6.8 ± 0.6 a	4.6 ± 1.0 a	4.3 ± 0.4 a	3.9 ± 0.7 a	3.6 ± 0.7 a	3.7 ± 0.3 a	3.3 ± 0.3 a	4.7 ± 0.4 a	4.8 ± 0.4 a	5.0 ± 0.3 b	6.3 ± 0.4 b	4.4 ± 0.4 a	4.3 ± 0.5 a	3.1 ± 0.5 a
<i>p</i>		<i>0.007</i>	<i>&lt;0,0001</i>	0.946	0.066	0.184	0.365	0.300	<i>0.006</i>	0.100	0.126	<i>0.037</i>	<i>0.014</i>	0.277	0.939	0.319
<b>Maturity x Maceration</b>		<i>0.006</i>	0.113	0.675	0.789	0.780	0.170	0.129	0.420	0.698	0.062	0.299	0.793	0.483	0.941	0.600

<sup>a</sup> Within a column, values followed by the same letter are not significantly different according to Fisher's LSD test at  $p \leq 0.05$ . Significant  $p$  values ( $p \leq 0.05$ ) are shown in italic letters. The data consisted of two panel repetitions.

**Supplemental Table 3. Chemical analyses of Pinot noir wine.**

		Iron reactive phenolics [mg/L CE]	np-PP [AU]	p-PP [AU]	Tannins [mg/L CE]	Ox. Phenolics [mg/L GAE]	Anthocyanins [mg/L Mv-3-gl]	Galic acid [mg/L]	Caftaric acid [mg/L]	C-Procyanidins [mg/L C1 eq]	B-Procyanidins [mg/L B2 eq]	B-Prodelphinidins [mg/L B2 eq]	B-Procyanidin gallates [mg/L B2 eq]	Gallo-catechin [mg/L]	Epigallo-catechin [mg/L]	Catechins [mg/L]
<b>Maturity</b>	<b>Maceration</b>															
Early harvest	7d	637.9 ± 46.5 a <sup>a</sup>	0.52 ± 0.01 ab	0.09 ± 0.08 a	85.5 ± 6.6 a	1011.7 ± 1.1 a	136.1 ± 6.5 ab	7.5 ± 0.1 a	43.9 ± 0.3 d	23.0 ± 0.5 a	39.4 ± 0.6 a	6.2 ± 0.2 d	1.2 ± 0.0 a	2.6 ± 0.1 c	0.25 ± 0.01 d	60.9 ± 0.2 a
	14d	1000.4 ± 127.3b	0.50 ± 0.07 a	0.10 ± 0.04 a	148.5 ± 29.5 b	1327.8 ± 176.5 b	120.2 ± 5.6 a	17.2 ± 2.9 c	28.0 ± 3.0 b	64.7 ± 7.9 b	74.2 ± 2.8 c	4.9 ± 0.2 c	5.3 ± 0.1 c	2.1 ± 0.0 b	0.16 ± 0.01 b	194.7 ± 26.9 b
Commercial harvest	7d	714.8 ± 3.3 a	0.67 ± 0.04 de	0.12 ± 0.01 a	143.2 ± 9.8 b	1187.5 ± 4.5 b	167.1 ± 2.2 c	11.8 ± 0.2 b	34.5 ± 2.6 c	29.1 ± 2.0 a	45.8 ± 2.4 b	5.0 ± 0.4 c	0.9 ± 0.1 a	2.1 ± 0.1 b	0.21 ± 0.01 c	79.6 ± 5.9 a
	14d	1228.8 ± 14.7 c	0.58 ± 0.00 bc	0.22 ± 0.01 ab	265.1 ± 2.6 d	1647.3 ± 1.5 c	121.5 ± 10.9 a	20.0 ± 0.4 c	21.6 ± 1.1 a	90.8 ± 0.4 c	82.9 ± 0.3 d	2.8 ± 0.2 a	6.9 ± 0.7 d	1.4 ± 0.0 a	0.06 ± 0.03 a	237.3 ± 1.0 c
Late harvest	7d	789.4 ± 46.5 a	0.73 ± 0.00 e	0.31 ± 0.04 bc	185.0 ± 7.3 c	1308.5 ± 42.4 b	189.4 ± 0.4 d	12.8 ± 0.2 b	29.6 ± 0.9 bc	29.5 ± 2.1 a	43.7 ± 3.0 ab	4.2 ± 0.1 b	0.4 ± 0.1 a	2.0 ± 0.0 b	0.19 ± 0.01 c	83.6 ± 7.0 a
	14d	1326.2 ± 33.0 c	0.62 ± 0.01 cd	0.35 ± 0.07 c	302.6 ± 14.8 e	1757.3 ± 59.2 c	147.3 ± 11.8 b	24.0 ± 0.7 d	16.3 ± 2.7 a	86.6 ± 7.8 c	79.5 ± 2.5 cd	2.8 ± 0.3 a	3.0 ± 0.1 b	1.4 ± 0.1 a	0.05 ± 0.00 a	237.2 ± 12.1 c
<i>p</i>		<i>0.000</i>	<i>0.002</i>	<i>0.014</i>	<i>&lt;0,0001</i>	<i>0.001</i>	<i>0.001</i>	<i>0.000</i>	<i>0.001</i>	<i>&lt;0,0001</i>	<i>&lt;0,0001</i>	<i>0.000</i>	<i>&lt;0,0001</i>	<i>0.000</i>	<i>&lt;0,0001</i>	<i>&lt;0,0001</i>
<b>Maturity</b>																
Early harvest		819.2 ± 223.4 a	0.51 ± 0.04 a	0.09 ± 0.05 a	117.0 ± 40.3 a	1169.7 ± 209.0 a	128.1 ± 10.5 a	12.4 ± 5.8 a	35.9 ± 9.4 c	43.9 ± 24.5 a	56.8 ± 20.2 a	5.5 ± 0.8 b	3.2 ± 2.3 b	2.3 ± 0.3 b	0.20 ± 0.05 b	127.8 ± 78.8 a
Commercial harvest		971.8 ± 296.9 b	0.63 ± 0.06 b	0.17 ± 0.05 a	204.2 ± 70.7 b	1417.4 ± 265.5 b	144.3 ± 27.1 b	15.9 ± 4.7 b	28.1 ± 7.6 b	60.0 ± 35.6 b	64.3 ± 21.5 b	3.9 ± 1.3 a	3.9 ± 3.5 c	1.7 ± 0.4 a	0.14 ± 0.09 a	158.4 ± 91.2 b
Late harvest		1057.8 ± 311.7 b	0.68 ± 0.07 c	0.33 ± 0.05 b	243.8 ± 68.6 c	1532.9 ± 262.5 c	168.4 ± 25.2 c	18.4 ± 6.5 c	22.9 ± 7.9 a	58.1 ± 33.3 b	61.6 ± 20.8 b	3.5 ± 0.8 a	1.7 ± 1.5 a	1.7 ± 0.3 a	0.12 ± 0.08 a	160.4 ± 89.0 b
<i>p</i>		<i>0.007</i>	<i>0.001</i>	<i>0.003</i>	<i>&lt;0,0001</i>	<i>0.002</i>	<i>0.001</i>	<i>0.002</i>	<i>0.001</i>	<i>0.006</i>	<i>0.014</i>	<i>0.000</i>	<i>0.001</i>	<i>0.000</i>	<i>0.000</i>	<i>0.018</i>
<b>Maceration</b>																
	7 d	714.0 ± 73.8 a	0.64 ± 0.10 b	0.17 ± 0.12 a	137.9 ± 45.1 a	1169.2 ± 134.8 a	164.2 ± 24.1 b	10.7 ± 2.5 a	36.0 ± 6.6 b	27.2 ± 3.5 a	42.9 ± 3.4 a	5.1 ± 0.9 b	0.8 ± 0.4 a	2.2 ± 0.3 b	0.22 ± 0.03 b	74.7 ± 11.6 a
	14 d	1185.1 ± 160.8 b	0.57 ± 0.06 a	0.22 ± 0.12 a	238.7 ± 73.4 b	1577.4 ± 216.2 b	129.7 ± 15.7 a	20.4 ± 3.3 b	21.9 ± 5.6 a	80.7 ± 13.5 b	78.9 ± 4.3 b	3.5 ± 1.1 a	5.0 ± 1.8 b	1.6 ± 0.3 a	0.09 ± 0.05 a	223.1 ± 25.6 b
<i>p</i>		<i>&lt;0,0001</i>	<i>0.004</i>	0.181	<i>&lt;0,0001</i>	<i>0.000</i>	<i>0.000</i>	<i>&lt;0,0001</i>	<i>0.000</i>	<i>&lt;0,0001</i>	<i>&lt;0,0001</i>	<i>0.000</i>	<i>&lt;0,0001</i>	<i>&lt;0,0001</i>	<i>&lt;0,0001</i>	<i>&lt;0,0001</i>
<b>Maturity x Maceration</b>		0.179	0.132	0.572	0.020	0.330	0.057	0.281	0.607	0.050	0.771	0.146	0.002	0.351	0.047	0.373

<sup>a</sup> Within a column, values followed by the same letter are not significantly different according to Fisher's LSD test at  $p \leq 0.05$ . Significant  $p$  values ( $p \leq 0.05$ ) are shown in italic letters. The data consisted of two experimental replicates.

**Supplemental Table 4. Chemical analyses of Cabernet Sauvignon wine.**

	Iron reactive phenolics [mg/L CE]	np-PP [AU]	p-PP [AU]	Tannins [mg/L CE]	Ox. Phenolics [mg/L GAE]	Anthocyanins [mg/L Mv-3-g]	Gallic acid [mg/L]	Caftaric acid [mg/L]	C-Procyanidins [mg/L C1 eq]	B-Procyanidins [mg/L B2 eq]	B-Prodelphinidins [mg/L B2 eq]	B-Procyanidin gallates [mg/L B2 eq]	Gallo-catechin [mg/L]	Epigallo-catechin [mg/L]	Catechins [mg/L]	
<b>Maturity</b>																
<b>Maceration</b>																
Early harvest	7d	1269.5 ± 141.4 a <sup>a</sup>	1.2 ± 0.1 a	0.3 ± 0.1 a	378.6 ± 17.0 a	1770.6 ± 88.1 a	338.2 ± 8.9 b	15.6 ± 0.5 a	38.0 ± 1.8 c	24.7 ± 2.4 a	39.3 ± 2.1 a	17.1 ± 1.4 a	2.4 ± 0.2 bc	9.3 ± 0.8 a	0.5 ± 0.0 a	54.7 ± 2.8 a
	14d	1748.6 ± 12.5 c	1.2 ± 0.0 a	0.5 ± 0.1 a	437.5 ± 6.0 bc	2077.4 ± 26.4 a	298.6 ± 1.6 a	27.2 ± 2.0 c	29.9 ± 0.1 ab	58.3 ± 1.6 c	60.7 ± 1.1 b	18.4 ± 0.4 a	6.4 ± 0.2 e	8.7 ± 0.3 a	0.4 ± 0.0 a	131.0 ± 5.0 c
Commercial harvest	7d	1431.3 ± 31.2 ab	1.9 ± 0.0 b	0.5 ± 0.0 a	381.8 ± 3.4 ab	2047.7 ± 4.0 a	405.9 ± 8.0 d	19.1 ± 0.8 ab	36.2 ± 0.8 bc	26.8 ± 0.5 a	39.1 ± 0.2 a	17.0 ± 0.5 a	1.9 ± 0.0 ab	10.5 ± 0.3 a	0.7 ± 0.0 b	57.5 ± 0.2 a
	14d	1687.9 ± 6.0 bc	1.8 ± 0.0 b	0.6 ± 0.0 a	435.2 ± 8.8 abc	2106.2 ± 0.4 a	348.1 ± 0.4 bc	30.6 ± 0.4 c	20.9 ± 0.0 a	52.0 ± 0.7 bc	57.5 ± 0.0 b	14.1 ± 0.0 a	4.4 ± 0.1 d	7.5 ± 0.1 a	0.5 ± 0.0 a	111.6 ± 0.6 b
Late harvest	7d	1744.0 ± 86.2 c	2.6 ± 0.2 c	0.4 ± 0.0 a	453.5 ± 28.4 c	2009.7 ± 218.6 a	376.6 ± 20.0 cd	21.0 ± 0.1 b	36.3 ± 5.3 bc	24.6 ± 1.2 a	37.6 ± 0.4 a	16.5 ± 1.1 a	1.3 ± 0.1 a	11.8 ± 1.0 a	0.9 ± 0.0 c	52.9 ± 2.5 a
	14d	2227.3 ± 124.0 d	2.6 ± 0.1 c	0.6 ± 0.1 a	550.4 ± 26.6 d	2381.5 ± 104.3 a	379.3 ± 4.5 cd	36.8 ± 0.1 d	34.3 ± 2.1 bc	47.9 ± 6.0 b	56.0 ± 2.8 b	17.1 ± 1.8 a	3.0 ± 0.4 c	11.3 ± 1.5 a	0.7 ± 0.0 b	107.9 ± 5.9 b
<i>p</i>	<i>0.006</i>	<i>0.001</i>	<i>0.090</i>	<i>0.008</i>	<i>0.072</i>	<i>0.005</i>	<i>0.001</i>	<i>0.047</i>	<i>0.002</i>	<i>0.001</i>	<i>0.365</i>	<i>0.000</i>	<i>0.097</i>	<i>0.000</i>	<i>0.000</i>	
<b>Maturity</b>																
Early harvest	1509.0 ± 288.5 a	1.2 ± 0.1 a	0.4 ± 0.1 a	408.0 ± 35.6 a	1924.0 ± 184.9 a	318.4 ± 23.5 a	21.4 ± 6.8 a	34.0 ± 4.8 a	41.5 ± 19.5 a	50.0 ± 12.4 a	17.8 ± 1.1 a	4.4 ± 2.3 c	9.0 ± 0.6 a	0.4 ± 0.0 a	92.9 ± 44.2 b	
Commercial harvest	1559.6 ± 139.9 a	1.9 ± 0.1 b	0.5 ± 0.0 a	408.5 ± 29.6 a	2076.9 ± 19.9 a	377.0 ± 35.1 b	24.9 ± 6.5 b	28.5 ± 9.3 a	39.4 ± 14.6 a	48.3 ± 10.6 a	15.6 ± 1.8 a	3.2 ± 1.5 b	9.0 ± 1.8 a	0.6 ± 0.1 b	84.5 ± 30.8 ab	
Late harvest	1985.7 ± 292.4 b	2.6 ± 0.1 c	0.5 ± 0.1 a	501.9 ± 60.3 b	2195.6 ± 256.2 a	377.9 ± 11.9 b	28.9 ± 9.2 c	35.3 ± 3.5 a	36.3 ± 13.9 a	46.8 ± 10.8 a	16.8 ± 1.2 a	2.1 ± 1.0 a	11.6 ± 1.1 b	0.8 ± 0.1 c	80.4 ± 31.9 a	
<i>p</i>	<i>0.005</i>	<i>0.000</i>	<i>0.104</i>	<i>0.006</i>	<i>0.086</i>	<i>0.003</i>	<i>0.003</i>	<i>0.081</i>	<i>0.205</i>	<i>0.156</i>	<i>0.220</i>	<i>0.001</i>	<i>0.038</i>	<i>&lt;0.0001</i>	<i>0.032</i>	
<b>Maceration</b>																
7 d	1481.6 a	1.9 ± 0.6 a	0.4 ± 0.1 a	404.6 ± 40.7 a	1942.7 ± 170.8 a	373.6 ± 32.1 b	18.6 ± 2.5 a	36.8 ± 2.7 b	25.4 ± 1.7 a	38.6 ± 1.3 a	16.9 ± 0.9 a	1.9 ± 0.5 a	10.5 ± 1.3 a	0.7 ± 0.2 b	55.0 ± 2.6 a	
14 d	1888.0 b	1.9 ± 0.7 a	0.5 ± 0.1 b	474.4 ± 64.2 b	2188.4 ± 174.3 b	342.0 ± 40.5 a	31.6 ± 5.0 b	28.4 ± 5.9 a	52.7 ± 6.1 b	58.1 ± 2.8 b	16.5 ± 2.0 a	4.6 ± 1.7 b	9.2 ± 1.9 a	0.5 ± 0.2 a	116.8 ± 12.8 b	
<i>p</i>	<i>0.003</i>	<i>0.351</i>	<i>0.044</i>	<i>0.007</i>	<i>0.035</i>	<i>0.012</i>	<i>&lt;0.0001</i>	<i>0.008</i>	<i>0.000</i>	<i>&lt;0.0001</i>	<i>0.652</i>	<i>&lt;0.0001</i>	<i>0.097</i>	<i>0.000</i>	<i>&lt;0.0001</i>	
<i>Maturity x Maceration</i>	<i>0.397</i>	<i>0.793</i>	<i>0.353</i>	<i>0.410</i>	<i>0.368</i>	<i>0.058</i>	<i>0.144</i>	<i>0.090</i>	<i>0.191</i>	<i>0.497</i>	<i>0.222</i>	<i>0.009</i>	<i>0.342</i>	<i>0.008</i>	<i>0.037</i>	

<sup>a</sup> Within a column, values followed by the same letter are not significantly different according to Fisher's LSD test at  $p \leq 0.05$ . Significant  $p$  values ( $p \leq 0.05$ ) are shown in italic letters. The data consisted of two experimental replicates.

## 7 Supplementary information on the presented vintages

Temperature data from an on-site weather station at the Weincampus Neustadt and DLR Rheinpfalz, located in Neustadt an der Weinstraße, Germany (lat. 49°24' N; long. 8°11' E), has been documented since the 1990s. Using the temperature data, growing degree days (GDD) per year were calculated according to Method 1 by McMaster and Wilhelm (1997) and the Huglin Index (HI) was calculated according to Huglin (1978). Figure 8 shows the GDD per year for the vintages 1998 – 2021 and the HI for the vintages 2008 – 2021. Neustadt an der Weinstraße is located in the European winegrowing zone A (cool climate), however, only two vintages since 2008 are considered cool climate (H-2, HI range 1500-1800) according to the Huglin Index categories. Nine vintages can be considered temperate (H-1, HI range 1800-2100), two vintages temperate warm (H+1, HI range 2100-2400), and 2018 vintage warm climate (H+2, HI range 2400-2700) (Gaitán and Pino-Otín, 2023; Huglin, 1978).

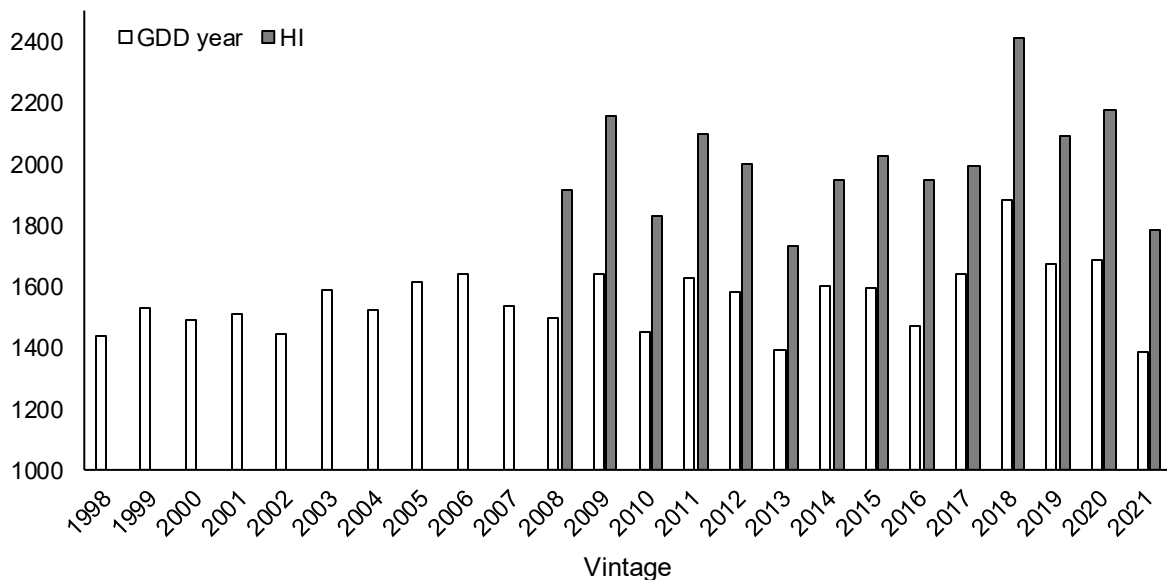


Figure 8 Growing degree days (GDD) per year and Huglin Index (HI) for Neustadt an der Weinstraße. Data was used from an on-site weather station at the Weincampus Neustadt/DLR Rheinpfalz (lat. 49°24' N; long. 8°11' E).

Growing degree days can also be used to categorize a region's climate. Figure 8 shows that the vintages presented in chapters 4, 5 and 0 (2018 - 2020) had the highest GDD in the last 20 years. In 2018, the 10-year average GDD in Neustadt an der Weinstraße was 1548 (20-year average 1540) which is within the Winkler region II (cool). The year 2018 on the other hand was an extremely hot vintage with 1884 GDD (Winkler region III, temperate) and it may be considered as an example of what we can expect from progressing climate change (Jones *et al.*, 2010; Winkler *et al.*, 1974). The data presented in Figure 8 suggests that the actual weather conditions in a winegrowing area might not necessarily coincide with the geographical winegrowing zones regulated by the European Union (European Union, 2013). With

progressing global warming, the European Union might have to reconsider their categories and adjust them accordingly.

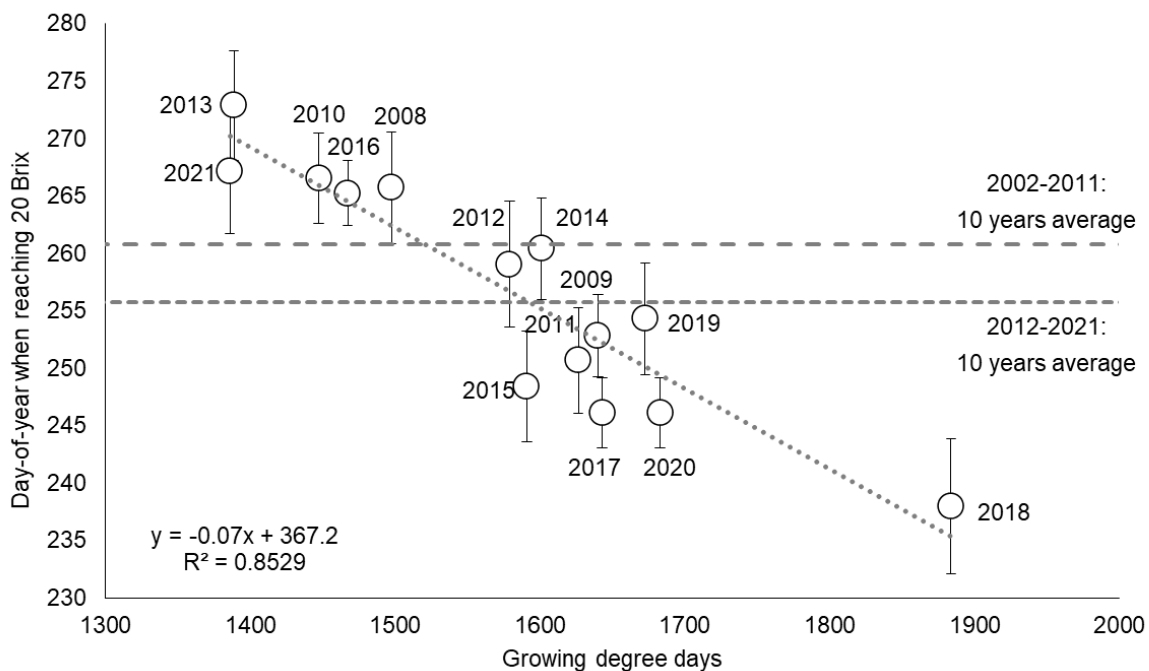


Figure 9 GDD in relation to day-of-year when reaching 20 Brix in Pinot noir ( $n = 10$  vineyards in the Pfalz region; mean  $\pm$  SD) for the vintages 2008-2021 (Feifel *et al.*, 2023).

Figure 9 shows that higher GDD correlated negatively with the day-of-year when 20 Brix were reached. In the decade 2012-2021, 20 Brix were reached approximately 5 days earlier than in 2002-2011 (Feifel *et al.*, 2023). These findings suggest that the vintages 2018 – 2020 had a compressed ripening period which is in agreement with the observations by Jones and Davis (2000) that due to climate change, phenological events tend to occur earlier with a shortening of the intervals. Further information on the impact of climate change on grape cluster structure, grape constituents, and processability is provided in Appendix A.

## 8 Concluding remarks

Viticulture, winemaking and wine consumption are anchored in many different cultures and traditions. Historically, wine was often reserved for kings and religious leaders, therefore winemakers have always aimed to produce a high-quality product. Vines are traditionally cultivated between the 30° and 50° latitude of the Northern and Southern hemisphere due to the climatic conditions (Spellman, 1999). Grapevines are very sensitive to weather conditions and climate, hence their cultivation is affected by changes in weather and climate patterns related to global warming (Jones *et al.*, 2005; White *et al.*, 2006; Winkler *et al.*, 1974). The vineyards used for the cultivation of Pinot noir and Cabernet Sauvignon presented in chapters

4,5, and 6 are located in Neustadt an der Weinstraße, Germany (lat. 49°24' N; long. 8°11' E) which is close to the 50° latitude. The region (Pfalz) is therefore categorized into the European winegrowing zone A (cool climate) (European Union, 2013). Weather data from 1998-2021, specifically temperature data from an on-site weather station located at the Weincampus Neustadt/DLR Rheinpfalz, has shown that most vintages would no longer be considered “cool climate” according to the categories introduced by Huglin (1978) and Winkler *et al.* (1974) but rather temperate climate or warmer. The data presented in chapter 7 shows that the vintages 2018 (Chapter 6), 2019 (Chapter 4), and 2020 (Chapters 4 and 5) were unusually hot for the region. The Pfalz is well known for the cultivation of white (68.3 % of the total acreage) and red grape varieties such as Dornfelder (10.3 % of the total acreage) or Pinot noir (7.3 % of the total acreage) who prefer cooler growing conditions. However, the acreage of red varieties that are commonly grown in warmer climates such as Cabernet Sauvignon has continuously expanded since the 1990s. Cabernet Sauvignon acreage in the Pfalz region increased from 24 ha in 1999 to 256 ha in 2023 (1.1 % of the total acreage) (Statistisches Landesamt Rheinland-Pfalz, 2024). This indicates that the climatic growing conditions in the Pfalz region are getting increasingly more suitable for warm climate-associated grape varieties. Therefore, this thesis aimed to investigate the grape ripening of two red grape varieties – Pinot noir and Cabernet Sauvignon – of international importance in the European winegrowing zone A (cool climate) and to show how progressing grape maturity, viticultural strategies and winemaking techniques influence the phenolic composition and sensory characteristics of these red wines. The ripening progress of Pinot noir grapes from 10 vineyards in the Pfalz region has been documented by weekly measurements of total soluble solids and titratable acidity for the vintages 2008-2021. The data revealed that higher GDD per year correlated negatively with the day-of-year when 20 Brix were reached. Therefore, in vintages with higher GDD, 20 Brix were reached earlier than in vintages with lower GDD. Linear regressions were performed on the ratio of total soluble solids to titratable acidity in the same Pinot noir grapes with day-of-year. The data suggests that vintages with higher GDD cause earlier and accelerated grape ripening. However, earlier and accelerated ripening do not necessarily have to be connected with each other. These findings also suggest that the vintages presented in this thesis (2018 – 2020) had a compressed ripening period (Appendix A: Feifel *et al.* (2023)). Considering the observed effect of higher GDD, related to climate change, on the ripening kinetics of grape constituents, previous studies have also suggested that sugar accumulation might occur ahead of other quality-associated compounds such as polyphenols in red varieties (Sadras and Moran, 2012). This suggested decoupling of ripening kinetics of different grape constituents presents a new challenge for winemakers to determine optimal grape maturity, because traditional grape maturity indicators such as TSS may no longer provide accurate information about the grape maturity perceived in wine. In red varieties the grape maturity

related content and composition of polyphenols is of great importance, because they provide the typical red color and sensory characteristics in wine like astringency. Phenolic maturity is a different grape maturity concept that focuses on the content and extractability of phenolics (Glories, 1986; Saint-Cricq de Gaulejac *et al.*, 1998), whereas aroma maturity relates to the content and extractability of aroma compounds and aroma precursors (van Leeuwen *et al.*, 2022). After decades of research, the definition of phenolic maturity is still very vague. Therefore, this thesis aims to improve the understanding of the concept of phenolic maturity as well as to identify indicators for phenolic maturity perceived in wine which are independent from potential alcohol. The study presented in chapter 4 also raises the question whether potential alcohol is a suitable indicator for the phenolic composition in grapes.

Most studies on grape maturity either anchor their “maturity levels” to total soluble solids, potential alcohol or refer to harvest dates by early and late or day-of-year. In some of these studies the potential alcohol of each “maturity level” is adjusted to achieve similar alcohol concentrations in the wine (Trujillo *et al.*, 2022). It is thought that this measure improves the comparison of grape maturity reflected in wine for sensory studies, because alcohol contributes significantly to the sensory characteristics (Frost *et al.*, 2021; Heymann *et al.*, 2013; Merrell *et al.*, 2018; Sherman *et al.*, 2017). Wines produced from early harvested grapes might lack a full body or taste less sweet due to lower alcohol concentrations. By enhancing the potential alcohol panelists might focus more on maturity related differences in sensory characteristics other than alcohol. Alcohol, however, also acts as a solvent in the extraction of grape constituents such as polyphenols. The extractability of these phenolics depends on the solvent as well as maturity related cell wall permeability (Hanlin *et al.*, 2010). Enhancing the potential alcohol for better comparison might adulterate the natural state of grape maturity by manipulating the extraction of phenolics as well as the sensory characteristics of the wine. It is therefore crucial to understand the extent to which phenolic and sensory parameters are influenced by potential alcohol, obtained by grape maturity or enhanced by chaptalization. In the study presented in chapter 4, Pinot noir and Cabernet Sauvignon grapes of two vintages were harvested at three different harvest time points, representing low, medium and high grape maturity. The potential alcohol of premature grapes was enhanced by sugar addition to simulate low, medium and high phenolic maturity, but yield the same alcohol content irrespective of the harvest time point. An experimental model was developed to determine the effect and contribution of potential alcohol to the sensory characteristics and phenolic composition of red wine. To differentiate between the effect of naturally obtained potential alcohol and enhanced potential alcohol, phenolic data and descriptive sensory data of wines produced at three harvest time points were plotted against natural potential alcohol over two vintages. Data from low phenolic maturity wines with enhanced potential alcohol were then plotted in the same graph to show if a parameter is dependent on potential alcohol and whether

extractability or the content of substance(s) in the grape, that are responsible for the parameter's intensity, is the limiting factor.

Sensory evaluation of 2019 Pinot noir and Cabernet Sauvignon wine revealed the influence of natural grape maturity and enhancement of potential alcohol on sensory characteristics. Wines produced from grapes of higher natural grape maturity received higher ratings in color intensity, purple hue, dark fruit aroma, sweetness and a full body, irrespective of the variety. In contrast, early harvest Cabernet Sauvignon wines were rated higher in sourness, green astringency, and green bell pepper aroma, whereas early harvest Pinot noir wines received higher ratings in harsh mouthfeel, dry astringency, rough astringency, and green astringency. In Pinot noir, ratings for astringency related sensory descriptors got lower with progressing maturity. However, in Cabernet Sauvignon wines only green astringency got less pronounced and dry astringency ratings increased with progressing maturity. Comparison of the sensory profiles of wines with and without enhanced potential alcohol showed that the effect of potential alcohol adjustment on sensory characteristics was marginal, non-existent or even enhancing unripe impressions. Since most significant differences related to grape maturity were still present after alcohol adjustment, it can be concluded that low phenolic maturity grapes with enhanced potential alcohol cannot achieve the sensory characteristics of a late harvest (high phenolic maturity) wine. Therefore, grape maturity reflected in wine sensory is not solely dependent on potential alcohol.

Analysis of phenolic composition showed that early harvest wines of both varieties were associated with higher contents of seed-associated phenolics such as catechin, epicatechin, and procyanidins, whereas late harvest wines had higher contents of anthocyanins, caffeic acid, and np-PP. Pinot noir wines with enhanced potential alcohol had higher contents of gallic acid and lower contents of B-prodelphinidins and Cabernet Sauvignon wines had higher tannin and iron reactive phenolics concentrations. The extent to which potential alcohol adjustment influenced the phenolic composition became smaller with progressing grape maturity which can be explained by the decreasing extent of adjustment required. The phenolic data revealed that the effect of potential alcohol is different from the effect of grape maturity. Therefore, potential alcohol enhancement of low phenolic maturity grapes cannot be driven towards a phenolic composition associated with higher grape maturity. Hence, this effect was observed for both, phenolic composition and sensory characteristics of red wine. It was still evident that grape maturity and potential alcohol adjustment both significantly influenced the sensory characteristics and phenolic composition of Pinot noir and Cabernet Sauvignon wine. An experimental model was developed revealing the contribution of potential alcohol to individual sensory and phenolic parameters. Anthocyanins were found to provide the most accurate information about grape maturity perception in Pinot noir. Their content increased significantly with progressing grape maturity but was not affected by potential alcohol adjustment. This

suggests that anthocyanins are suitable indicators for phenolic maturity perceived in Pinot noir wine. For Cabernet Sauvignon, np-PP were found to be suitable indicators for phenolic maturity reflected in wine. Further studies could build on the hypothesis that grape varieties with low phenolic content show a trend similar to Pinot noir and varieties with a high phenolic content show a trend similar to Cabernet Sauvignon. Overall, the findings presented in chapter 4 highlight that with a potential decoupling of the ripening kinetics of sugar and phenolic compounds related to climate change, the sole use of potential alcohol as grape maturity indicator could be misleading for the intended phenolic composition and sensory characteristics of red wine. Based on the findings of this study, the potential alcohol of wines presented in chapters 5 and 6 was not adjusted to reveal the unadulterated grape maturity perceived in wine.

Grapes can be harvested manually or by using different machines, depending on the size of the vineyard and the intended quality of the wine. Winemakers often remove grape clusters of low quality due to *Botrytis cinerea* infection, sunburn or other reasons for damaged berries before harvesting. However, within the vineyard and within the healthy grape clusters, there are berries of varying size and maturity. Heterogeneous berry sizes provide a challenge for the processing machines used in wineries as well as for winemakers to predict the intended wine style. Berry size heterogeneity was found to either improve or lower wine quality. Since results from previous studies are also inconclusive regarding the question whether small berries are more mature and/or higher in quality than large berries, the effect of berry size on the phenolic composition of Pinot noir wine was investigated and presented in chapter 5. Cluster exposure and timing of harvest were examined as potentially suitable strategies to modulate the berry size heterogeneity in the vineyard. This study also aimed to determine whether these measures lead to a higher phenolic maturity reflected in the wine composition. Leaf removal performed at BBCH stage 75 (pea-sized berries) was compared to untreated Pinot noir vines. At two harvest dates of the 2020 vintage, grape clusters were harvested manually to avoid damaged berries. Healthy and fully intact berries were then sorted into three sizes categories for determination of berry size distribution and winemaking. The effect of cluster exposure and timing of harvest on the berry size distribution was shown to be marginal, which is different than what we expected. A scale of 900 g berries was used for microvinifications. Due to this small experimental scale only chemical analysis of polyphenols was performed and there was no sensory evaluation. Chemical analysis revealed that cluster exposure and harvest date as well as berry size significantly affected the phenolic composition of Pinot noir wine. Leaf removal enhanced the content of seed-associated phenolics in wine, such as catechin, epicatechin, B-procyanidins, B-procyanidin gallates, C-procyanidins, and tannins. Late harvest on the other hand increased the content of skin-associated phenolics, such as epigallocatechin and np-PP. Small berries produced wines that were richer in phenolics than large berries. This

was especially reflected in polymeric pigments and anthocyanins which are considered quality indicators of red wine. Regarding anthocyanins as the indicator for phenolic maturity in Pinot noir wine proposed in chapter 4, cluster exposure and later harvesting did not lead to higher phenolic maturity. Small berries were associated with higher anthocyanin contents; however, this could be either due to higher phenolic maturity or higher proportions of skins compared to large grapes. Further studies could increase the scale of the vinification and perform a sensory evaluation to assess the influence of berry sizes on wine quality. It could also be investigated to which extent phenolic maturity and skin proportions during winemaking related to berry sizes contribute to the phenolic composition of red wine. Studies should further focus on different leaf removal intensities, timing of leaf removal, additional harvest dates to extend the investigation period, and other viticultural measures to modulate berry size or berry size heterogeneity and enhance differences in phenolic maturity.

The proposed indicators for phenolic maturity in chapter 4 were required to be independent from potential alcohol. The extractability of certain polyphenols, however, is not only influenced by grape maturity and potential alcohol content, but also the exposure time to the solvent. Extending maceration time can increase the proportion of phenolics that are better extracted in the presence of alcohol. During maceration oxygen exposure can be modulated by the intensity of mash movement using manual punch downs, air push or other techniques. Once alcoholic fermentation is completed, the protective layer of grape solids and carbon dioxide on the surface gets smaller and grape solids eventually sink to the bottom of the fermenter, because carbon dioxide is no longer being produced. Extending the maceration time after this stage can therefore significantly enhance the oxygen exposure of young wine. The extent of oxygen exposure can potentially favor the formation of polymeric pigments but also increase oxidized aromas in wine. The study in chapter 6 determined the extent to which different maceration times can modulate the phenolic composition and sensory characteristics of Pinot noir and Cabernet Sauvignon wine produced from grapes of three different harvest dates. It was also assessed whether extending maceration time can improve the sensory characteristics of wine produced from immature grapes and if sensory effects can be explained by polyphenol analysis. Pinot noir and Cabernet Sauvignon grapes of the 2018 vintage were harvested manually at three different harvest time points. Maceration lasted either 7 days, just after completion of alcoholic fermentation, or 14 days. Wines were analyzed three months after bottling for phenolic composition and sensory characteristics. Sensory evaluation and phenolic data revealed that the influence of maceration time was different from the influence of grape maturity. Extending maceration time neither compensated for grape immaturity nor enhanced the characteristics associated with immaturity. Similar to the wines presented in chapters 4 and 5, the content of skin-associated phenolics such as anthocyanins and polymeric pigments increased with progressing maturity. Extending maceration time on the other hand increased

the content of seed-associated phenolics such as monomeric catechins and procyanidins. For both grape varieties, anthocyanins and np-PP were suggested as suitable indicators for phenolic maturity perceived in wine which is in agreement with the findings presented in chapter 4. Sensory evaluation revealed higher ratings for color intensity, fruity aroma attributes, sweetness, dry astringency, bitterness, and a full body for late harvest wines which is in agreement with previous studies (Cadot *et al.*, 2012) and the findings presented in chapter 4. Higher bitterness perception in late harvest wine might be explained by the higher alcohol content. Early harvest Cabernet Sauvignon wines received significantly higher ratings for green bell pepper aroma than late harvest wines, which was explained by the content of IBMP that is known to decrease with progressing maturity (Bindon *et al.*, 2013; Roujou de Boubée *et al.*, 2000). Extending maceration time resulted in wines with higher ratings in astringency, as well as tendencies to a lighter color and yellow hue. Astringency perception can be explained by the increased extraction of seed-associated phenolics during the extended maceration time and color characteristics can be attributed to oxidative processes due to higher exposure. These findings are in agreement with previous studies (Cadot *et al.*, 2012; Gil *et al.*, 2012). Overall, changes in the phenolic composition with progressing grape maturity and extending maceration time were reflected in the wine color as well as in taste and tactile ratings in the sensory evaluation of the wines. Further studies should investigate the long-term effects of grape maturity and maceration time on the phenolic composition and sensory characteristics of Pinot noir and Cabernet Sauvignon wine. Red wine is usually not consumed within one year after alcoholic fermentation. Therefore, sensory evaluations performed in wine research are often not a suitable representation of the actual market situation. However, funding for research projects is often limited to a period of approximately three years, therefore studies on winemaking procedures commonly do not include data from aged wines. Wine ageing for three or more years would allow the continuous formation of polymeric pigments as well as changes in tannin structure and show whether grape maturity or maceration actually improve color stability and sensory characteristics. Findings by Hauser (2023) suggest that after three years of bottle ageing, differences in wine sensory characteristics and phenolic composition due to grape maturity and maceration time were less pronounced. Polymeric pigments and tannins increased in Pinot noir wines, although p-PP were only detected in wines produced from grapes of higher maturity. In Cabernet Sauvignon wines on the other hand, tannin content and np-PP decreased significantly whereas p-PP increased in all treatments. Differences in polymeric pigments and tannin content were still better related to grape maturity at harvest than maceration time after three years of wine ageing. Sensory and phenolic data for aged wines would still be interesting for the parameters influencing wine composition presented in chapters 4 and 5.

In conclusion, the studies presented in this thesis highlight why an improved understanding of grape ripening and its influence on sensory and phenolic characteristics is necessary in times of climate change. It was shown that higher GDD related to global warming trigger earlier and/or accelerated ripening of grapes. This thesis also sheds light on how phenolic maturity is reflected in wine and indicators for phenolic maturity perceived in wine were identified. Anthocyanins and non-precipitable polymeric pigments were positively correlated with progressing grape maturity and were independent of potential alcohol enhancement and winemaking procedures, making them suitable indicators for phenolic maturity if sugar accumulation is decoupled from the ripening kinetics of other grape constituents. An experimental model was developed to determine the extent to which the potential alcohol acts as a solvent modulating the extraction of phenolic compounds during winemaking. The studies showed that neither potential alcohol enhancement nor extended maceration time can manipulate the sensory characteristics of wine produced from immature grapes towards the characteristics of late harvest wines. This also holds true for the phenolic composition of the wines. Viticultural strategies such as cluster exposure by leaf removal and timing of harvest were shown to influence the phenolic composition of Pinot noir wine, however these strategies only had a marginal effect on the berry size distribution in the vineyard. Small berries were shown to produce more concentrated wines than large berries, however, it remains unclear whether small berries were of higher phenolic maturity.

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## Appendix

### A. Proceedings of the 43<sup>rd</sup> World Congress of Vine and Wine

#### Impact of climate change on grape cluster structure, grape constituents, and processability

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Disclaimer:

The presented work was performed by Weincampus Neustadt/DLR Rheinpfalz (Sandra Feifel) and University of Bonn (Jan-Peter Hensen and Ingrid Weilack). For more information, refer to the author contribution section.

Author Contributions:

**Sandra Feifel:** Conceptualization, Methodology, Investigation, Winemaking, Chemical analyses, Writing – Original draft, Review and Editing. **Jan-Peter Hensen:** Methodology, Chemical analyses. **Ingrid Weilack:** Chemical analyses. **Fabian Weber:** Writing - Review and Editing, Funding acquisition. **Pascal Wegmann-Herr:** Conceptualization, Winemaking, Writing - Original draft. **Dominik Durner:** Conceptualization, Supervision, Writing - Review and Editing, Funding acquisition.

All authors have read and agreed to the published version of the manuscript.

Citation:

Feifel, S., Hensen, J.-P., Weilack, I., Weber, F., Wegmann-Herr, P., and Durner, D. (2023), "Impact of climate change on grape cluster structure, grape constituents, and processability", *BIO Web of Conferences*, Vol. 56, p. 1016. <https://doi.org/10.1051/bioconf/20235601016>.

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# Impact of climate change on grape cluster structure, grape constituents, and processability

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**Abstract.** Over the last 20 years in the Pfalz region of Germany, GDD were highest in the vintages 2018-2020. To visualize the effect of climate change for the Pfalz region of Germany, the development of TSS and TA were documented during Pinot noir grape ripening for the vintages 1998-2021. It was observed that climate change causes sooner and accelerated ripening, however, sooner or accelerated ripening were not connected with each other. The presented study investigated the impact of vintages on berry physiology development, ripening heterogeneity in grape clusters, changes in grape constituent concentrations and their extractability with progressing grape maturity. Pinot noir and Cabernet Sauvignon, both cultivated in the Pfalz region, were compared during three consecutive vintages. Different maturities were considered in the range of 17-25 Brix and berry size distribution was documented. The changes in grape constituent concentrations and their extractability with progressing grape maturity were analyzed using HPLC-DAD/FD, LC-QToF-MS and protein precipitation assay. It was revealed that tannin extractability differs for grape varieties and vintages and progressing maturity seemed to have only a small influence on the extractability of anthocyanins.

## 1 Introduction

Understanding the potential impacts of climate change on viticulture has become increasingly important for the wine industry. A study by Duchêne and Schneider (2005) has shown a significant increase in temperatures for the vintages 1972-2002 and that the period between budburst and harvest has become both earlier and shorter [1]. In a long-term (1952-1997) climatology study in Bordeaux, Jones and Davis (2000) observed that phenological events such as budburst, floraison, véraison tended to occur earlier with a shortening of those intervals. Cabernet Sauvignon and Merlot tended to produce higher sugar to total acid ratios which was shown to have an influence on Bordeaux vintage quality [2]. Several studies have shown that musts with higher sugar levels due to higher temperatures in the growing interval caused a stress reaction in the yeast metabolism and that higher pH had an influence on the microbial ecology, enhancing the chances for off-flavors and related loss in wine quality [3]. To extend on those findings, our study aims to visualize the effect of climate change in the Pfalz region of Germany for the vintages 1998-2021, using the traditional grape maturity parameter total soluble solids and titratable acidity. It was investigated whether climate change has an impact on the kinetics of berry ripening and the ripening heterogeneity within grape clusters. The tannin and anthocyanin contents in Pinot noir and Cabernet Sauvignon grapes at different maturities were investigated for the vintages 2018-2020 and compared to the tannin and anthocyanin contents in wine. Besides the extractability of phenolics, the phenolic composition of wines was examined to discuss future process options in times of climate change.

## 2 Materials and methods

### 2.1 Growing degree days

To visualize climate change, the growing degree days (GDD) were calculated with data from an on-site weather station at the Weincampus Neustadt (lat. 49°24' N; long. 8°11' E) for the vintages 2008-2021 according to Method 1 by McMaster and Wilhelm (1997) [4]. The GDD were used as explanatory variable for plotting the day-of-year when reaching 20 Brix.

### 2.2 Basic grape juice parameters

Pinot noir berries were sampled weekly, starting at 7 Brix, from 10 vineyards located in the Pfalz region of Germany for the vintages 1998-2021. Total soluble solids (TSS) and titratable acidity (TA) were measured by Fourier transform infrared spectroscopy (FT-IR) (Wine Scan™ FT120 Basic, FOSS GmbH, Hamburg, Germany) using a calibration method provided by the manufacturer.

### 2.3 Berry physiology, constituents, and processability

#### 2.3.1 Vineyard site

The investigation of berry physiology, constituents, and processability was conducted during the 2018-2020 vintage at the Weincampus Neustadt experimental vineyard located in the Pfalz region of Germany (lat. 49°24' N; long. 8°11' E). *Vitisvinifera* L. cv. Pinot noir (PN; clone Mariafeld on SO4 rootstock) was planted in 1987 with a vine by row spacing of 1.88 m × 1.20 m and *Vitisvinifera* L. cv. Cabernet Sauvignon (CS; clone 1Gm

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on Binova 1Opp rootstock) was planted in 2008 with a vine by row spacing of 2.00 m × 1.20 m.

### 2.3.2 Berry analysis

Pinot noir and Cabernet Sauvignon berries were collected at three different time points in the range from 17 to 25 Brix. Lots of 500 berries per time point and grape variety were cut from the rachis using scissors to obtain intact berries ( $n = 3$  experimental replicates). The berries were sorted into three size categories of different diameters (PN: < 13 mm, 13-15 mm, > 15 mm; CS: < 11 mm, 11-13 mm, > 13 mm). The grape constituent concentrations were measured using the protein precipitation assay by Harbertson et al. (2002, 2003, 2015) [5-7].

### 2.3.3 Wine production

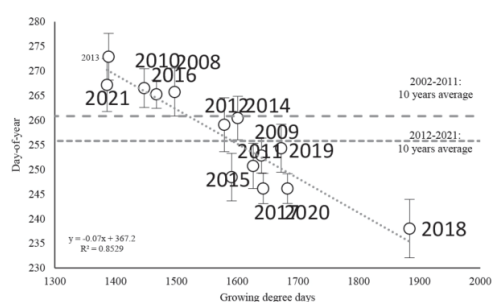
Pinot noir and Cabernet Sauvignon grape bunches were harvested manually in the aforementioned TSS range. After destemming, grapes were crushed, transferred into 100 L experimental stainless-steel fermenters (70 kg each) and inoculated with 200 mg/L ZYMAFLORE® RB2 yeast. At day 5 coinoculation with 10 mg/L *Oenococcus oeni* VP41 (ZEFÜG GmbH & Co. KG, Bingen, Germany) was done. After maceration, wines were <1 g/L residual sugar. Fermentations were carried out in triplicate.

### 2.3.4 Wine analysis

Phenolic contents of the wines were analysed using HPLC-DAD/FD, LC-QToF-MS and protein precipitation assay by Harbertson et al. (2002, 2003, 2015)[5-7].

## 3 Results and Discussion

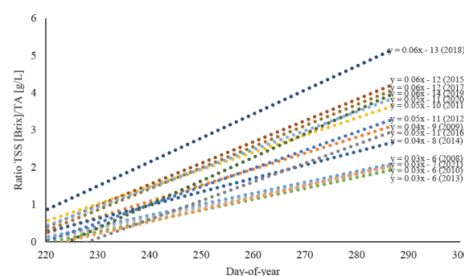
Over the last 20 years, GDD in the Pfalz region were highest in the vintages 2018-2020. The day-of-year when reaching 20 Brix in PN was calculated for the decades 2002-2011 and 2012-2021, as well as the individual vintages. As shown by the correlation in Figure 1, the increase in GDD causes grapes to reach 20 Brix sooner. This observation indicates climate change in the region.



**Figure 1.** GDD in relation to day-of-year when reaching 20 Brix in Pinot noir ( $n = 10$  vineyards in the Pfalz region; mean  $\pm$  SD) for the vintages 2008-2021.

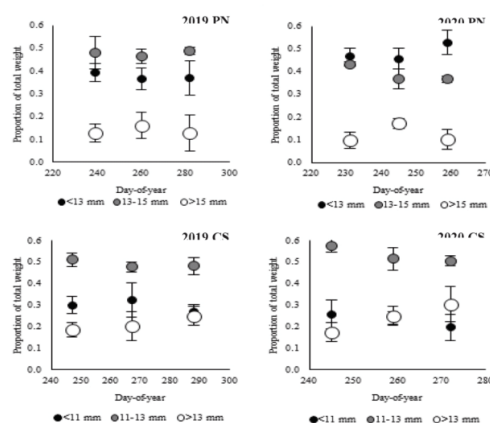
Besides TSS, winemakers consider the decrease in TA when determining their harvest time points. To

evaluate whether climate change only causes a sooner maturity or accelerates the pace of ripening, the decrease in TA was related to the increase in TSS. This ratio is shown for the vintages 2008-2021 in Figure 2. As shown by the linear regressions of the ratio of TSS/TA, climate change causes sooner and accelerated ripening. However, sooner or accelerated ripening do not necessarily have to be connected with each other.



**Figure 2.** Linear regressions on the ratio of TSS/TA with day-of-year for PN of the vintages 2008-2021 ( $n = 10$  vineyards in the Pfalz region).

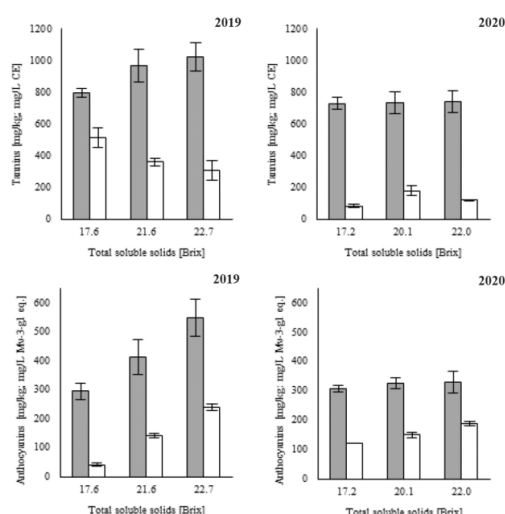
To examine whether the observed differences in grape constituent contents are an effect of dilution or concentration, changes in berry size distribution need to be considered. The evolution of berry size distribution in PN and CS as a possible indicator of ripening heterogeneity was investigated for the vintages 2019 and 2020. In both grape varieties and both vintages, berry size distribution did not significantly change with progressing maturity (Fig. 3). The proportion of <13 mm PN berries was higher in 2020 than in 2019, indicating a higher heterogeneity, which is most likely due to rainfall near flowering of PN, as observed by Zhu et al. (2020) [8]. In contrast, CS berries tended to be larger in size in 2020 than in 2019. The contrasting observations indicate that changed weather conditions manifest differently in different grape varieties.



**Figure 3.** Berry size distribution of Pinot noir (PN) and Cabernet Sauvignon (CS) at different time points in the vintages 2019 and 2020. ( $n = 3$  experimental replicates; mean  $\pm$  SD).

Since wine quality cannot be defined solely by TSS and TA, the concept of phenolic maturity was introduced [9]. Recent research suggests that phenolic composition may be used to monitor grape maturity and predict wine composition and style [10].

The phenolic composition of PN and CS berries and resulting wines at three different maturities in two consecutive vintages was analysed in order to investigate the influence of grape maturity and vintages on the processability of the grapes. Tannin and anthocyanin contents in berries and resulting wines are shown in Figs. 4 (PN) and 5 (CS).

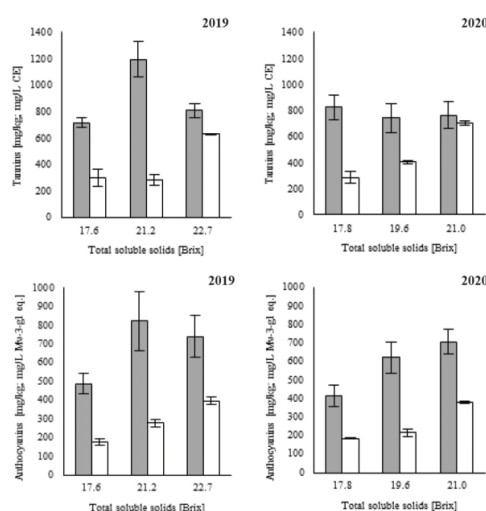


**Figure 4.** Tannin and anthocyanin content in PN berries (grey) and wine (white) at different time points in the vintages 2019 and 2020. ( $n = 3$  experimental replicates; mean  $\pm$  SD).

Irrespective of the vintage and grape variety, tannin and anthocyanin content was higher in berries than in wine. This can be explained by incomplete extraction and multiple reactions of phenolic compounds in the winemaking and ageing process. Tannin content in 2019 PN berries (Fig. 4) increased with progressing grape maturity, the tannin content in the corresponding wine decreased, indicating that tannins are more readily extracted in unripe PN berries. This trend could not be observed in the 2020 vintage. Tannin content in both, berries and wine, did not change with progressing grape maturity.

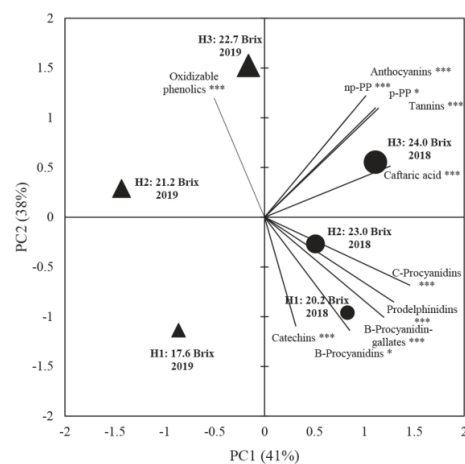
In CS on the other hand, tannin content in berries did not show a trend with progressing grape maturity (Fig. 5). In wine, however, tannin content increased with progressing grape maturity in both vintages, indicating that tannins have a higher extractability with progressing grape maturity. Therefore, the extractability of tannins is influenced by grape variety and grape maturity.

Irrespective of the vintage and grape variety, anthocyanin content in berries and wine (Figs. 4 and 5) increased with progressing grape maturity, indicating that the extractability of anthocyanins was not influenced by grape maturity.



**Figure 5.** Tannin and anthocyanin content in CS berries (grey) and wine (white) at different time points in the vintages 2019 and 2020. ( $n = 3$  experimental replicates; mean  $\pm$  SD).

Many other phenolic compounds than tannins and anthocyanins contribute to the berry and wine composition, such as phenolic acids and proanthocyanidins. Also newly formed phenolic compounds, such as polymeric pigments, which result from reactions during winemaking and ageing, can be found in wine.



**Figure 6.** PCA for Cabernet Sauvignon wines produced from grapes at different harvest time points in two consecutive vintages (2018 circles, 2019 triangles). PCA space was calculated on the thirteen displayed chemical attributes. ( $n = 3$  experimental replicates; \* significant at  $p < 0.05$ , \*\* significant at 0.01, \*\*\* significant at 0.001).

A PCA was performed for the phenolic composition of CS wine in two vintages at different maturities (Fig. 6). The first PC segregated the wines by vintages. 2018 vintage wines were positively loaded on PC1 which

is explained by higher contents of caftaric acid, C-procyanidins and prodelfinidins. PC2 segregated the wines by maturity. H1 wines were negatively loaded on PC2 which is explained by higher contents of catechins and B-procyanidins. H3 wines were positively loaded on PC2 which is explained by higher contents of oxidizable phenolics, polymeric pigments (np-PP and p-PP), anthocyanins and tannins.

Progressing maturity has a great influence on the phenolic composition of wine. However, the difference between vintages was neither enhanced nor reduced by progressing maturity. Accordingly, earlier or later harvest could not compensate for the inter-annual ripening differences as indicated by TSS and TA. Changing weather conditions due to climate change require adaptations in viticulture and enological processing.

#### 4 Conclusion

The effect of climate change on viticulture in the Pfalz region could be shown by relating GDD to TSS. Higher GDD resulted in an earlier and faster increase in TSS in Pinot noir, indicating that climate change causes sooner and accelerated ripening (TSS/TA). The study also revealed that sooner and accelerated ripening are not necessarily connected to each other. Although not significantly changing from 17 to 23 Brix, changing weather conditions over the years affect the grape cluster structure. The extractability of tannins was strongly influenced by progressing maturity and also by different vintage conditions. For anthocyanins, extractability differed for grape varieties and vintages, but progressing maturity seemed to have only a small influence. The

processability of PN and CS grapes seems to be strongly affected by changing conditions due to climate change.

This research project was supported by the German federal ministry of economic affairs and climate action (via AiF) and the FEI (Forschungskreis der Ernährungsindustrie.V., Bonn) project AiF 20024N.

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## **B. Publications in technical journals**

### **Immer noch eine offene Frage: Phenolische Reife im Fokus**

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Citation:

Feifel, S. (2022). Immer noch eine offene Frage: Phenolische Reife im Fokus. *DAS DEUTSCHE WEINMAGAZIN*, 16-17, 36 ff.

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# Immer noch eine offene Frage

## Phenolische Reife im Fokus

Reifeparameter, die sich nicht am Mostgewicht, sondern an anderen qualitätsbestimmenden Traubeninhaltsstoffen orientieren, gewinnen zunehmend an Bedeutung. Doktorandin Sandra Feifel zeigt Ergebnisse aus dem Versuchsprojekt zur phenolischen Reife am DLR Rheinpfalz/Weincampus Neustadt.

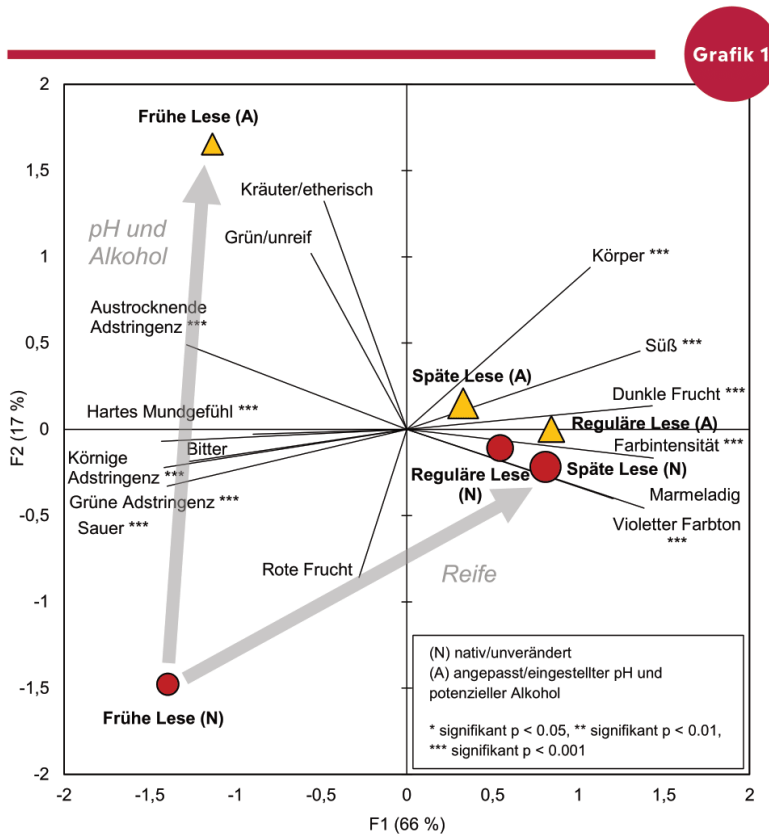


Reifeheterogenität an einem Spätburgunder Rebstock: Zu sehen sind bereits durchgefärbte Cluster/Trauben, Misch-Cluster und unreife Cluster.

Foto: Sandra Feifel

**B**ei den Reifeparametern spielt das Mostgewicht und damit der potenzielle Alkohol zwar eine wichtige, aber bei Weitem nicht die alleinige Rolle. Das Konzept der phenolischen Reife

kommt aus Bordeaux und zielt darauf ab, den optimalen Lesezeitpunkt für Rotweintrauben zu ermitteln. Saint-Cricq et al. (1998) definieren die phenolische Reife als den Zeitpunkt, an dem die Anthocyan-



Spätburgunder Jahrgang 2019: Folgen der Anpassung von pH-Wert und potenziellem Alkohol auf die sensorische Wahrnehmung der Weine in Kombination mit dem Lesezeitpunkt.

und Tanninkonzentrationen in den Traubenschalen ihr Maximum und Kernphenolgehalte ihr Minimum erreichen. Studien gehen davon aus, dass diese beiden Prämissen nicht zwangsläufig zeitgleich vorliegen (Bisson 2001) und der Fokus vor allem auf die Anthocyane gelegt werden sollte (Vivas de Gaulejac et al. 2001).

Im Unterschied dazu empfehlen Ristic und Iland (2005) die Traubenkerne in den Mittelpunkt zu rücken und schlagen vor, den Lignifizierungsgrad anhand der Verfärbung der Kerne mittels einer Farbskala zu bestimmen und somit die phenolische Reife von Trauben sensorisch zu beurteilen.

## Projekt zur phenolischen Reife

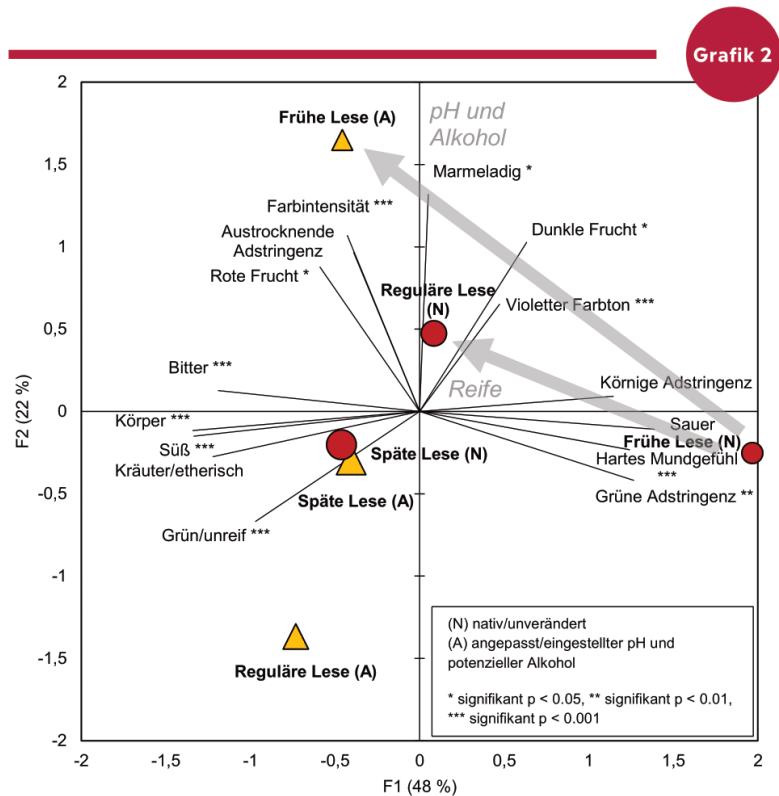
Das Projekt am DLR Rheinpfalz/Weincampus Neustadt hatte zum Ziel, den Begriff der phenolischen Traubenreife zu definieren und analytische Methoden zu etablieren, anhand derer Winzer die Extrahierbarkeit wertgebender phenolischer Inhaltsstoffe bestimmen können, damit die Weinbereitung gegebenenfalls angepasst werden kann. Um die qualitative Spannbreite von Spätburgunder und Cabernet Sauvignon Rotwein zu erfassen, wurden insgesamt über 170 kommerzielle Weine analytisch auf einen Zusammenhang zwischen dem Polyphenolprofil, der sensorischen Qualität und dem Verkaufspreis untersucht.

Zur Ermittlung des Einflusses der Traubenreife auf die Gehalte phenolischer Verbindungen der Beere und Veränderung ihrer Extrahierbarkeit wurden Beeren an jeweils drei Leseterminen in mehreren Jahrgängen analysiert. Die phenolische Reife wurde durch Konzentration und Extrahierbarkeit der Polyphenole definiert und durch Polysaccharid-Interaktionen maßgeblich beeinflusst. Die Messungen ergaben, dass im Verlauf der Reife die Anthocyangehalte und die der nicht-präzipitierbaren (ausfällbaren) polymeren Pigmente in den Weinen signifikant zunahm. Die Tannin- und Gesamtphenolgehalte der Weine zeigten wenige Änderungen. Die Ergebnisse deuten darauf hin, dass die beiden Rebsorten eine unterschiedliche Entwicklung der phenolischen Traubenreife hinsichtlich der Tannin- und Gesamtphenolgehalte aufweisen, mit einer frühen Reife des Spätburgunderweines und einer späteren Reife des Cabernet Sauvignons.

Der Einfluss der Rotweintechnologie auf die Gehalte phenolischer Verbindungen und sensorischer Eigenschaften von Rotweinen aus einem früheren und einem späteren Reifestadium wurde sensorisch und analytisch untersucht. Dabei wurden die Weine beider Rebsorten des ersten Lesezeitpunkts nach Einstellung von pH-Wert und Mostgewicht sensorisch mit einem verstärkten unreifen Eindruck beschrieben. Mit zunehmender physiologischer Reife nahmen die Unterschiede zwischen den unveränderten und eingestellten Weinen ab. Durch die Einstellung konnten jedoch nicht die sensorischen Eigenschaften reiferen Leseguts nachgestellt werden. Zudem konnte analytisch keine signifikant verbesserte Extraktion der Kern- und Schalenphenole bei den eingestellten Weinen beobachtet werden.

**Entwicklung des Entscheidungshilfesystems**

Das Entscheidungshilfesystem (EHS) basiert auf der Extrahierbarkeit und den Gehalten von Polyphenolen. Das entwickelte Modell trifft präzise Vorhersagen des Tannin- und Anthocyangehalts im späteren Wein. Weiterhin können konkrete Handlungsempfehlungen abgeleitet werden, die Winzer nach eigener Zielvorgabe interpretieren können. Für die Entwicklung einer einfachen Analyseverfahren zur Bestimmung der phenolischen Reife und zur Nutzung des EHS wurde die



Spätburgunder Jahrgang 2020: Folgen der Anpassung von pH-Wert und potenziellem Alkohol auf die sensorische Wahrnehmung der Weine in Kombination mit dem Lesezeitpunkt.

Probenaufbereitung mittels verschiedener Extraktionsverfahren optimiert und an die Analytik mittels Protein-Präzipitationsassay angepasst. Zusätzlich wurde eine Bilderkennungssoftware programmiert, die Anwender unabhängig von Weinlaboren einsetzen können. Mit den drei Extraktionsmethoden, die unterschiedliche mechanische Einflüsse simulieren, kann die Extrahierbarkeit abgebildet werden.

**Ergebnisse belegen sensorische Unterschiede beim Lesezeitpunkt**

Bei den Weinen des Jahrgangs 2019 sind die sensorischen Unterschiede der Lesezeitpunkte erkennbar. Die Weine einer frühen Lese wurden unabhängig der Anpassung klassischer Parameter wie Mostgewicht und pH-Wert immer noch mit Attributen, die mit Unreife assoziiert werden, beschrieben. Ein höherer Alkoholgehalt und niedriger Säuregrad führen zu einer intensiveren unreifen, ätherischen und alkoholischen Wirkung anstelle einer

Verstärkung des süßeren Eindrucks. Im Jahrgang 2020 führte die Anpassung von pH-Wert und potenziellem Alkohol bei einer frühen Lese zu farbintensiveren Weinen, die marmeladiger und austrocknender wahrgenommen wurden. Für den Gesamteindruck mit einem vollen Körper und Süße, der bei einer späten Lese erreicht wird, reichte die Anpassung bei der frühen Lese jedoch nicht aus.

**Fazit**

Die am Rebstock selbst erreichte Reife von Spätburgunder-Trauben kann durch Maßnahmen, wie die Erhöhung des potenziellen Alkohols und des pH-Werts, nicht nachempfunden werden. Im Gegenteil können eine Entsäuerung und Chaptalisation von unreifem Lesegut sogar zu einer Verstärkung der unreifen Charakteristik im Wein führen. Die Ergebnisse zeigen, eindrucksvoll, dass die phenolische Reife vom potenziellen Alkohol und von der Säure in Spätburgunder-Trauben entkoppelt ist.