Dorothea Leis, Dipl.-Ing. BSc

Analytical Methods for the Characterization of Aroma Compounds in Wine and Grape Must

DOCTORAL THESIS

to achieve the university degree of
Doktorin der Naturwissenschaften

submitted to

Graz University of Technology

Supervisor

Univ.-Prof. Dipl.-Ing. Dr.techn. Erich Leitner
Institute of Analytical Chemistry and Food Chemistry

Graz, May 2021

“Wine makes every meal an occasion, every table more elegant, every day more civilized.”
Andre Simon

AFFIDAVIT

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Acknowledgment

My first thanks go to my supervisor, Professor Erich Leitner for the possibility to research such an interesting topic and all the amazing opportunities to develop and expand my knowledge and for the possibility to work with many different instruments. I want to thank him for his guidance, patience and all the insights into food chemistry and analysis and the joys good wine and food. I also want to thank all the technicians and all-round lovely people at the Institute of Analytical Chemistry and Food Chemistry of the Graz Technical University. Their hard work allowed my experiments to run smoothly. So, a big thank you to Nina, Sigi, Claudia and Evi for keeping the lights on and the instruments running. In addition, I want to thank Elisabeth. It was great to share an office with you during my time at the institute. Another big thank you goes to Barbara, who would always answer my questions and also for the lovely, mostly food related, talks over coffee. My next thank you is for the other PhD students: Lisa, Andrea, Milica and Iris, who I want to thank for the interesting and stimulating talks about our work, but also for all the great times we had outside of the office, be it at conferences, at summer schools or just over a drink after work. Another thank you goes to Franz, Monica and Tanja, and all the other people who made teaching lab courses fun and entertaining. And last, but certainly not least, to all the other ACFC members for welcoming me and letting me be a part of the team for the past five years.

Another big thank you goes to all our project and cooperation partners, without whom this work would never have been possible. I want to especially thank Wolfgang Renner and the team of the Versuchsstation Obst- und Weinbau Haidegg, for all the interesting experiments and samples they provided. I also want to mention our partners at the University of Zagreb and the University of Arkansas and thank them for the fascinating projects about different aspects of wine we worked on together.

A group of people who I could not have done without while working on this thesis are my friends, especially Miguel, Filo, Fiona, Vio, Noemi, Lisa, Sebi and Seyf. Thank you for all the good times and for being there for me when I needed encouragement. Thank you for making this crazy last year bearable and sometimes even fun. I cannot thank you enough!

And finally, my family: I want to thank my parents, Albrecht and Dagmar and my siblings, Alex and Katha for always being there for me and for all the support, without which this would not have been possible.

Abstract

Wine and wine production have been part of human culture for a long time. One of the most important quality indicators in wines is their aroma. Many scientific studies deal with the understanding how choices made by the winemaker in the vineyard, the microorganisms carrying out the fermentation or even the maturation in different cooperage can influence the final product. As a wine producing region, Styria is known for its excellent white wines, which do particularly well in this environment. However, winemakers will be facing challenges in the upcoming years due to changing climatic conditions, like hotter temperatures and more extreme weather phenomena. Finding new ways to deal with these challenges is crucial. A better understanding of how certain conditions impact the aroma of established cultivars, like Sauvignon Blanc, as well as evaluating the potential of new varieties in the context of Styrian wine production, are needed to find possible solutions.

This is what this work set out to do. Using a mixture of qualitative and quantitative gas chromatography based methods in combination with several statistical tools, the impact of certain viti- and vinicultural techniques on wines and must was examined. Several new methods for the quantification of character impact odorants were developed and others optimized. In addition, instrumental analytical techniques were used to describe volatile compounds in wines produced from fungus resistant grape varieties grown in Styria over several vintages. The data was compared to results of sensory evaluations of the same wines.

Using mostly gas chromatography based techniques, the characterization of wines from different cultivars, including Riesling and Sauvignon Blanc was carried out. Several impact odorants of various cultivars were successfully identified using newly developed and improved analytical methods. In addition, a chemical characterization of wines produced from selected fungus resistant grape varieties (PIWI) grown in Styria was performed. The resulting dataset allowed the grouping of wines from several PIWI cultivars. The grouping was based aroma compounds that are also found in wines from conventional, non-hybrid cultivars, including the Muscat and Sauvignon families, which are well established in Styria. The results correlated with those from the sensory evaluations.

It is essential to understand how changes at any step of wine production will influence the final product. The use of instrumental analysis of wines offered a unique insight into the composition of different facets of wine aroma. These insights can help winemakers to better understand the consequences of their choices and help them to find solutions to challenges they might face in the future.

Kurzfassung

Wein und Weinherstellung sind seit langer Zeit Teil der menschlichen Kultur. Einer der wichtigsten Qualitätsindikatoren bei Weinen ist ihr Aroma. Zu verstehen, wie die Entscheidungen des Winzers im Weingarten, die an der Fermentation beteiligen Mikroorganismen, oder auch der Ausbau in verschiedenen Materialien das Endprodukt beeinflussen können, war daher Gegenstand vieler wissenschaftlicher Studien. Die Steiermark ist als Weinbauregion für ihre hervorragenden Weißweine bekannt, die in dieser Umgebung besonders gut gedeihen. Allerdings werden die Winzer in den kommenden Jahren durch die sich ändernden klimatischen Bedingungen, wie heißere Temperaturen und extremere Wetterphänomene, vor Herausforderungen gestellt. Neue Wege zu finden, um mit diesen Herausforderungen umzugehen, ist daher entscheidend. Ein besseres Verständnis darüber, wie sich bestimmte Bedingungen auf das Aroma etablierter Sorten, wie Sauvignon Blanc, auswirken, sowie die Evaluierung des Potenzials neuer Sorten im Kontext der steirischen Weinkultur, sind notwendig, um mögliche Lösungen zu finden.

Das war das Ziel dieser Arbeit. Mit einer Mischung aus qualitativen und quantitativen gaschromatographischen Methoden in Kombination mit verschiedenen statistischen Techniken wurde der Einfluss bestimmter Wein- und Weinbautechniken auf Weine und Most untersucht. Mehrere neue Methoden zur Quantifizierung bestimmter charakteristischer Geruchsstoffe wurden entwickelt und andere optimiert. Darüber hinaus wurden instrumentelle Analysetechniken eingesetzt, um flüchtige Verbindungen in Weinen aus pilzresistenten Rebsorten, die in der Steiermark angebaut wurden, zu beschreiben. Die Daten wurden mit Ergebnissen der sensorischen Bewertung der gleichen Weine verglichen.

Die Charakterisierung von Weinen aus verschiedenen Rebsorten, einschließlich Riesling und Sauvignon Blanc, wurde hauptsächlich mit Hilfe der Gaschromatographie durchgeführt. Mit Hilfe neu entwickelter und verbesserter Analysemethoden wurden mehrere typische Aromaverbindungen verschiedener Sorten erfolgreich identifiziert. Zusätzlich wurde eine chemische Charakterisierung von Weinen aus ausgewählten pilzwiderstandsfähigen Rebsorten, die in der Steiermark angebaut werden, durchgeführt. Der resultierende Datensatz erlaubte die Gruppierung von Weinen mehrerer PIWI-Sorten. Diese basierte auf Aromastoffen, die auch in Weinen aus konventionellen Sorten gefunden werden, einschließlich der in der Steiermark gut etablierten Muscat und Sauvignon Familien. Die Ergebnisse korrelierten mit jenen aus den sensorischen Auswertungen.

Es ist wichtig zu verstehen, wie Veränderungen in jedem Schritt der Weinproduktion das Endprodukt beeinflussen. Der Einsatz der instrumentellen Analyse von Weinen bot einen einzigartigen Einblick in die Zusammensetzung der verschiedenen Facetten des Weinaromas. Diese Einblicke können Winzern helfen, die Konsequenzen ihrer Entscheidungen besser zu verstehen und ihnen helfen, Lösungen für Herausforderungen zu finden, mit denen sie in Zukunft konfrontiert sein werden.

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Introduction

Practical Part

List of Commonly Used Abbreviations

°C	degree Celsius
µg	microgram
µL	microliter
3SH	3-sulfanylhexan-1-ol
3SHA	3-sulfanylhexyl acetate
4MSP	4-methyl-4-sulfanylpentan-2-one
AA	acetic acid
AAB	acetic acid bacteria
AAP	2-aminoacetopheone
AD	Anno Domini
ADP	adenosine diphosphate
APY	2-acetylpyrroline
ATHP	2-acetyl-tetrahydropyridine
ATP	adenosine triphosphate
BC	Before Christ
bp	boiling point
CAS	Chemical Abstracts Service
CE	collision energy
CO_2	carbon dioxide
CWR	carbon wide range
DAC	District Austriae Controllatus
DAP	diammonium phosphate
DBU	1,8-Diazabicyclo(5.4.0)undec-7-ene
DEG	diethylene glycol
DMS	dimethyl sulfide
DVB	polydivinylbenzene
EA	ethyl acetate
EEC	European Economic Community
Et3N	triethylamine
ETHP	2-ethyltetrahydropyridine
EtOH	ethanol
ETP	ethyl propiolate
FDA	Food and Drug Administration
FID	flame ionization detectors
g	gram
GC	gas chromatography
GCxGC	Comprehensive GC
H2O	water
H2S	hydrogen sulfide

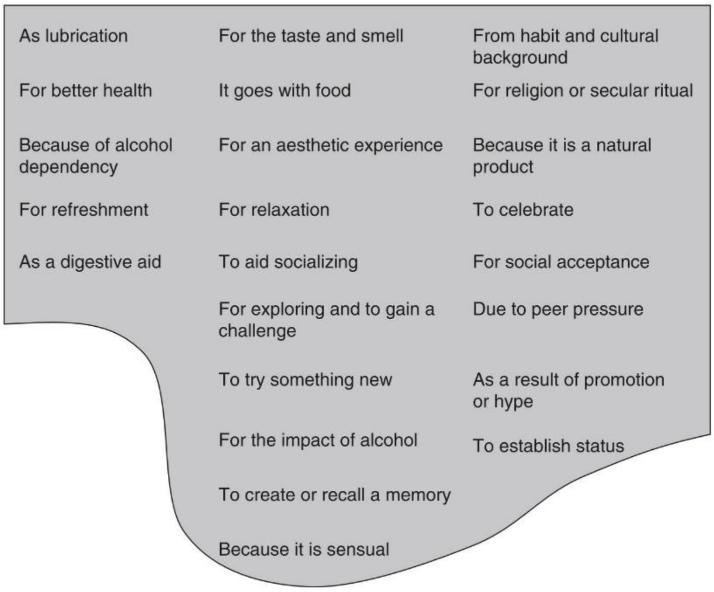
ha	hectare
HPLC	high performance liquid chromatography
HS	headspace
HSD	Honest Significant Difference
IAA	indole-3-acetic acid
IBMP	2-isobutyl-3-methoxypyrazine
ICP	inductively coupled plasma
IPMP	2-isopropyl-3-methoxypyrazine
IS	internal standard
kg	kilogram
KOH	potassium hydroxide
kPa	kilopascal
kV	kilovolt
L	liter
LD_{50}	median lethal dose
m	meter
m/z	mass-to-charge ratio
MABL	Multicolored Asian Lady Beetle
MDGC	Multidimensional GC Techniques
MeOH	methanol
mg	milligram
mhl	million hectoliters
min	minute
mL	milliliter
MLF	malolactic fermentation
MO	microorganism
MOG	material other than grapes
MRM	multiple reaction monitoring
MS	mass spectrometry
MS/MS	tandem mass spectrometry
Na_2SO_4	sodium sulfate
NaOH	sodium hydroxide
ND	not determined
ng	nanogram
NIST	National Institute of Standards and Technology
OAV	odor activity value
OES	Optical Emissions Spectrometry
OIV	Organisation Internationale de la Vigne et du Vin
ÖWM	Österreich Wein Marketing GmbH
PA	polyacrylate

PCA	principal component analysis
PDMS	polydimethylsiloxane
pg	picogram
PIS	product ion scan
PIWI	fungus resistant grape variety
PP	poly propylene
PPA	plant protection agent
RDA	Recommended Dietary Allowance
RI	retention index
rt	retention time
SIDA	stable isotope dilution analysis
SIM	selected ion monitoring
SO_2	sulfur dioxide
SPE	solid phase extraction
SPME	solid phase microextraction
SSB	sub-standard berries
TBA	2,4,6-tribromoanisole
TCA	2,4,6-trichloroanisole
TCP	2,4,6-trichlorophenol
TDN	1,1,6,-trimethyl-1,2-dihydronapthalene
TIC	total ion current
Tr	traces
UTA	Untypical Aging Off-Flavor
V. vinifera	*Vitis vinifera*
v/v	volume per volume
VA	volatile acidity
VSC	volatile sulfur compounds
w/w	weight per weight
WCOT	wall-coated open tubular
WHO	World Health Organization
YAN	yeast assimilable nitrogen

Introduction

Wine has been part of human culture for a long time. Romans, Greeks and other cultures had gods for it, it is part of ceremonies in different world religions, and some people value it, especially red wine, for its medical benefits and others enjoy the status that comes with owing a particularly rare and expensive bottle.

At an auction at Sotheby's in New York in 2018 one bottle of 1945 Romanee-Conti sold for $ 558,000 (Frank 2018). According to a Consumer Market Outlook (Statista 2019) the world wide turnover of the wine industry in 2018 was at 354,695 million $, which is more than the Gross domestic product of Denmark (351,299 million $) of the same year (The World Bank Group 2018). With an average price of about 12 € per bottle consumers expect to get good quality. To understand why people are willing to spend sometimes enormous amounts of money on a product like wine scientists have looked into the reasons people give.



As lubrication
For better health
Because of alcohol dependency
For refreshment
As a digestive aid

For the taste and smell
It goes with food
For an aesthetic experience
For relaxation
To aid socializing
For exploring and to gain a challenge
To try something new
For the impact of alcohol
To create or recall a memory
Because it is sensual

From habit and cultural background
For religion or secular ritual
Because it is a natural product
To celebrate
For social acceptance
Due to peer pressure
As a result of promotion or hype
To establish status

Figure 1: Why do people drink wine (Charters 2006, p. 133)

Charters in his book 'Wine and Society' (2006) distinguishes between three different main reasons that people name when asked why they drink wine. Figure 1 shows three columns, the first one focuses on physical or utilitarian reasons, the second one on experiential or hedonic and the third one on symbolic ones. The first group focuses on the physical results people gain or expect from wine. Experiential reasons focus more on the enjoyment of the act, the sensory experience and pleasure connected with consuming wine, while symbolic reasons focus on the way wine consumption is used to convey a certain image to others that also includes the cultural background, like religion or heritage. In most cases, the reasons for the consumption of wine fall into different categories that can change each time.

To try to understand this one has to look into the product, which on the most basic level is mainly a mixture of water and ethanol. These two compounds make up about 97%. The remaining 3% make wine the interesting product it undoubtedly is. In their book ‘Understanding Wine Chemistry’ Waterhouse, Sacks and Jeffery (Waterhouse et al. 2016) show the composition of a dry red wine:

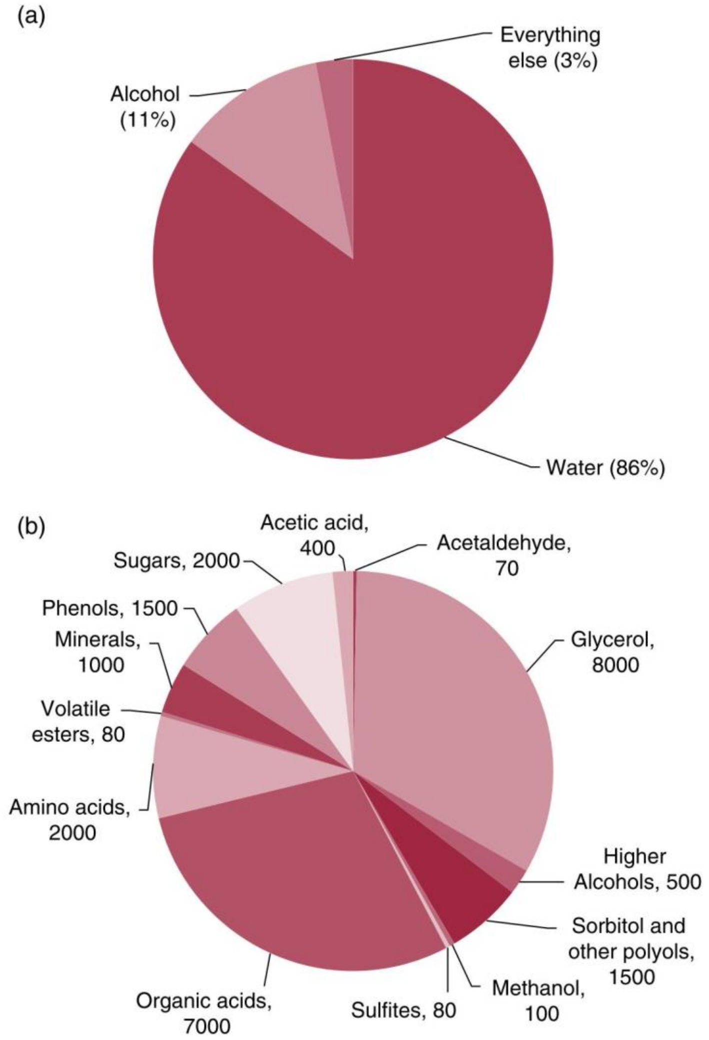


Figure 2: Composition of a representative dry red table wine (a) on a % w/w basis and (b) typical concentrations (mg/L) of major wine components excluding water and ethanol, that is, the main contributors to “Everything Else.” Key trace components (0.1 ng/L–10 mg) would not be visible and are therefore not included (Waterhouse et al. 2016)

The composition of a wine depends on a variety of factors that include the grape variety, the climate and weather conditions of the vineyard, the microorganisms used in the fermentation and the way of aging and storing the finished wine. A variation in any of these factors can lead to variations in the product. Unlike other industries working with agricultural products, these differences are, at least in most cases, wanted and contribute to the unique experience of wine drinking. To archive this, it is important for wine growers and makers to know what influences the taste of their product and how.

Volatile compounds, which are mainly responsible for the aroma of a wine, can have a big influence on the overall perception of a wine. For some volatiles, concentrations of few ng/L are enough for them to have an impact on the aroma. This is due to their low sensory thresholds (Poláskoyá et al. 2008). Just knowing the concentration of a compound will therefore only provide a limited insight into its effects on the aroma of the product. A useful tool to describe the impact one single compound can have is the use of odor activity values (OAV) (Rothe and Thomas 1963).

Equation 1: Calculation of the odor activity value (OAV)

$$\text{OAV}=\frac{\text{Concentration of compound x}}{\text{Sensory threshold of compound x in the same matrix}}$$

Sensory thresholds of important compounds range from mg/L for some higher alcohols (1-hexanol 8 mg/L (Guth 1997b)) to pg/L for some halogenated anisoles (Callejón et al. 2016).

In order to understand how certain factors of wine growing practices and winemaking procedures influence different analytical tools can be applied. The aim of this work is therefore to examine different facets of wine aroma using gas chromatography-based analysis techniques, which are a standard tool in aroma analysis. For this, several case studies were conducted to examine the impact of certain wine growing factors, including harvest time and location. Furthermore, wines from certain cultivars were looked at that are traditionally grown in Styria, like Sauvignon Blanc and Riesling, using various instrumental analytical techniques. In addition, wines from fungus resistant cultivars, which require less plant protection agents compared to conventional vines, were characterized and examined for their similarities to wines from conventional varieties. This is the first time this was done in detail for such wines from Styria spanning several vintages. The results should show how the choices made by wine-growers and makers impact the final product and how those can improve the final product.

1. Wine worldwide

The International Organization of Vine and Wine (Organisation Internationale de la Vigne et du Vin, OIV) estimates the area under vines destined for the production of wine grapes, table grapes or dried grapes, in production or awaiting production to be 7,429 million of hectares (Organisation Internationale de la Vigne et du Vin 2019). Out of this, 57% are dedicated to vines for the production of wine. In 2018, the world wine production is estimated to be around 292 million hectoliters (mhl). With a world population of around 7.7 billion, that equals five standard bottles of wine for every person living on the planet per year. Most vines are grown in the area between the 40th and 50th degrees northern latitude and the 30th and 40th degrees southern latitude (Figure 3) in the 'vine-belt'.

The countries with the highest wine production are Italy (54.8 mhl), France (48.6 mhl), Spain (44.4 mhl) and the United States of America (23.9 mhl). Austria's production with 2.8 mhl makes up for about one percent of the total world production.

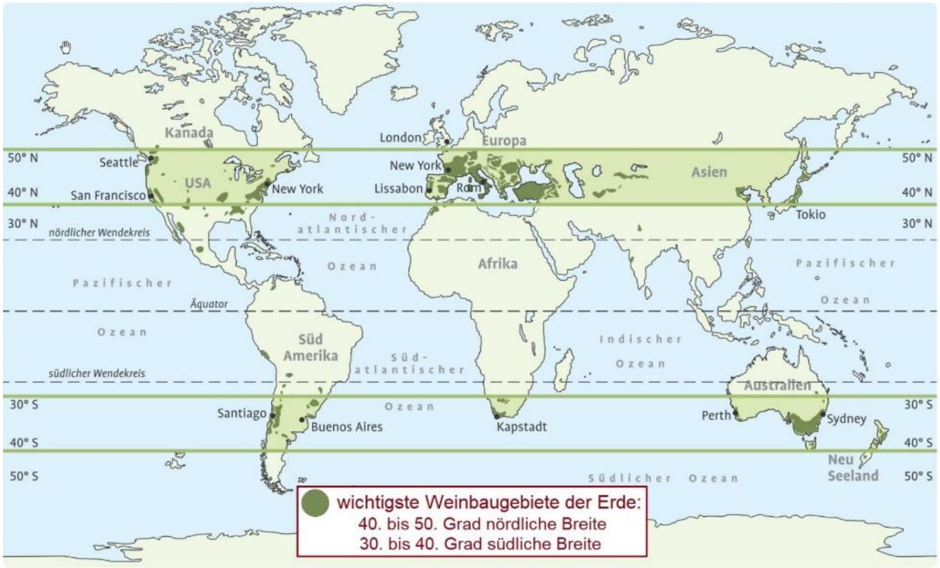


Figure 3: Rebengürtel (Kadisch and Müller 2008)

The main varieties of grapevine grown worldwide for wine production are Cabernet Sauvignon (341,000 ha in 2015, 4% of the total area under vines), Merlot (266,000 ha) and Tempranillo (231,000 ha) according to the OIV (Organisation Internationale de la Vigne et du Vin 2017). These are followed by the first white variety, Airen (218,000 ha), which is mainly grown in Spain, where it accounts for 22% of the vineyard area.

According to the OIV the consumption of wine in 2018 was 246 mhl, the main wine consuming countries being the USA (33 mhl), France (26.8 mhl) and Italy (22.4 mhl) (Organisation Internationale de la Vigne et du Vin 2019). Looking at the consumption per person, this distribution shifts. In first place is Andorra with 56.9 liters per capita, followed by the Vatican (56.2 liters) and Croatia (46.9 liters) (Smith 2017). In Austria, people consumed 2.4 mhl of wine, or 26.8 liter per person. The next part will examine the Austrian wine industry a bit closer.

1.1 Austria

In 2017, the total vineyard area accounted for 46,515 ha. Out of this area, 67% were dedicated to white varieties. Most of the wine growing and wine making focus on the four federal states: Lower Austria (28,145 ha, 60.5%), Burgenland (13,100 ha, 28.2%) Styria (4,633 ha, 10%) and Vienna (637 ha, 1.4%). The remaining states have a total vineyard area of 237 ha. In 2018 2.75 mhl of wine were produced.

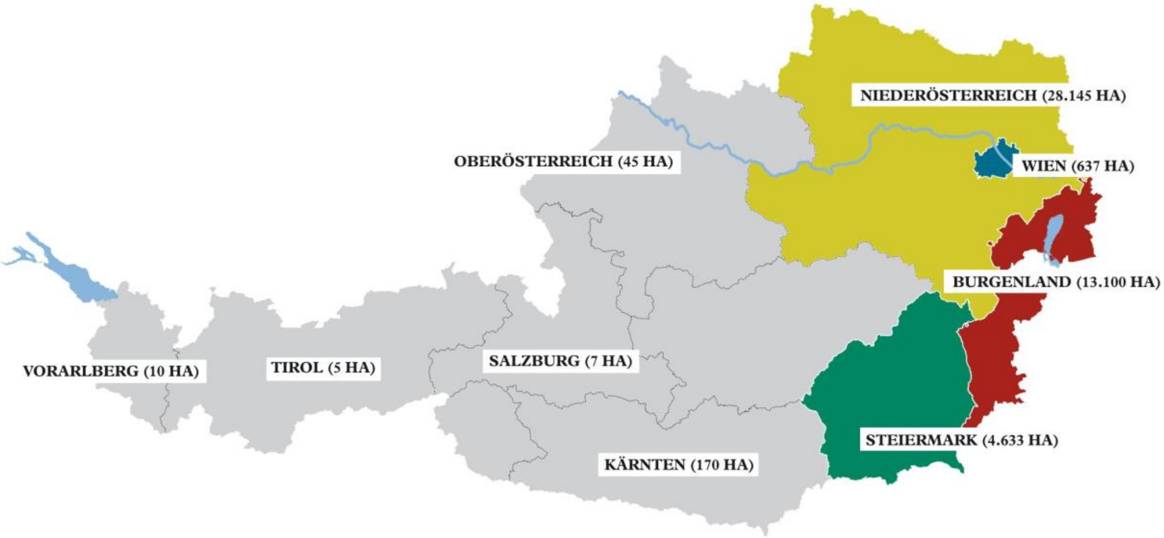


Figure 4: Austria's wine regions with vineyard area (Österreich Wein Marketing GmbH (ÖWM) July 2020)

The statistics and numbers in this part are taken from Dokumentation Österreich Wein (Österreich Wein Marketing GmbH 2019; Heidinger 2021).

The main cultivars are Grüner Veltliner, making up 31% of the vineyard area, and Zweigelt, with 13.8%. Other important varieties are Welschriesling (7.2%), Blaufränkischer (6.5%), Riesling (4.3%), Müller-Thurgau (3.8%), Chardonnay or Morillon (3.5%) and Blauer Portugieser and Sauvignon Blanc (each 2.7%). All of these varieties and others are Qualitätswein grape varieties. According to Austrian wine law, only wines made from those varieties can be used to produce wine that carries a geographical indication on the label.

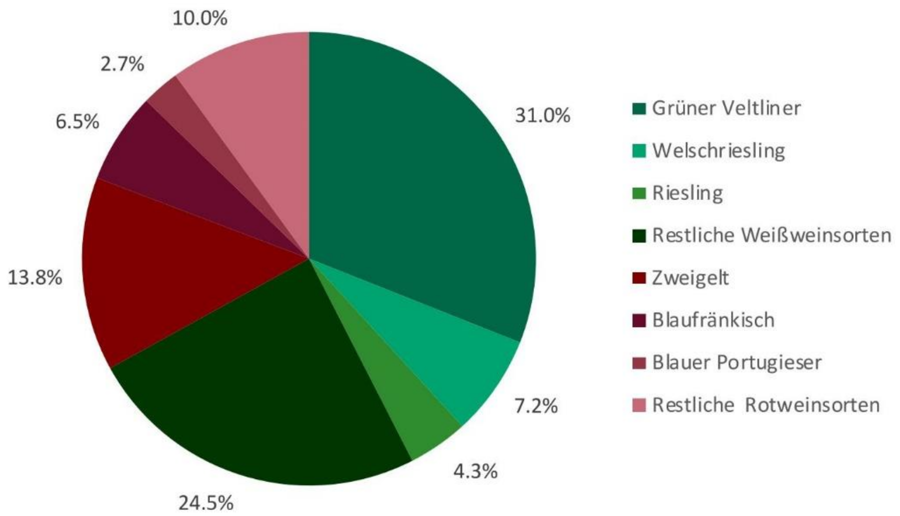


Figure 5: Distribution of grape varieties in Austria (Österreich Wein Marketing GmbH 2019)

Austrian wine law differentiates between several wine categories based on the origin of the grapes. The least specific is Tafelwein (table wine). Wines is this category do not carry a geographical indication, other than Österreich or österreichischer Wein. The indication of the cultivar on the label is not permitted. Any wine with this designation must be free of defects in appearance, smell and flavor. Wines with geographical indications are also separated further, in Landwein and Qualitätswein. For Landwein, grapes used for the winemaking have to originate from the same winegrowing area, Weinland (Lower Austria, Wien and Burgenland), Steirerland (Styria) or Bergland (other federal states). Wines of this category have to exhibit a minimum must weight of 14° KMW. Only juice from Qualitätswein varieties can be used and the wine has to exhibit characteristics that are typical for to the designation. All wines must be free from defects. Wines of this category are comparable to ‘Wines with Protected Geographical Indication’ (PGI). The third category are wines with protected designation of origin (PDO). Grapes for these wines have to be harvested in a single winegrowing region and the wines have to be produced in either the same area or in a region bordering it. Qualitätswein has to have a minimum of 9% v/v of alcohol, a minimum must weight of 15° KMW and pass a sensorial testing by a government tasting authority in order to be awarded a Prüfnummer (Federal Inspection Number). In the tasting, the wines have to fulfill requirements of typicity and regional characteristics. This category also contains Kabinett- and Prädikatswein (Bundesministerin für Nachhaltigkeit und Tourismus 2009).

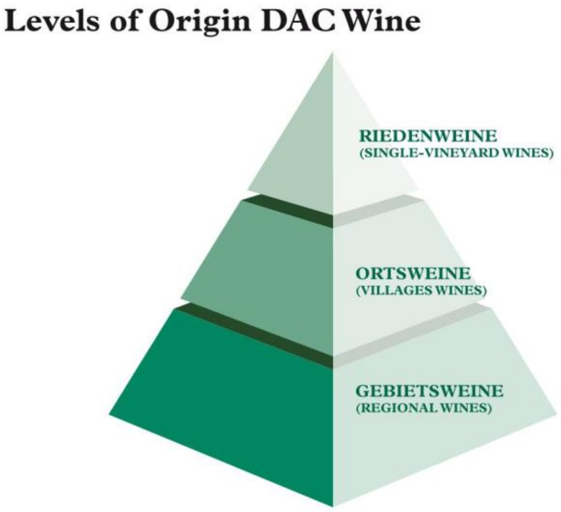


Figure 6: Levels of DAC wine (Österreich Wein Marketing GmbH (ÖWM) July 2020)

In recent years, the District Austriae Controllatus (DAC) was introduced. Until today, there are sixteen DAC regions, six in Lower Austria and Burgenland respectively, three in Styria and the Wiener Gemischter Satz. Winegrowers and winemakers in these regions aim for products that exhibit flavor profiles that are characteristic for their region. Only certain of the Qualitätswein cultivars are eligible for the DAC label, which ones depends on the region.

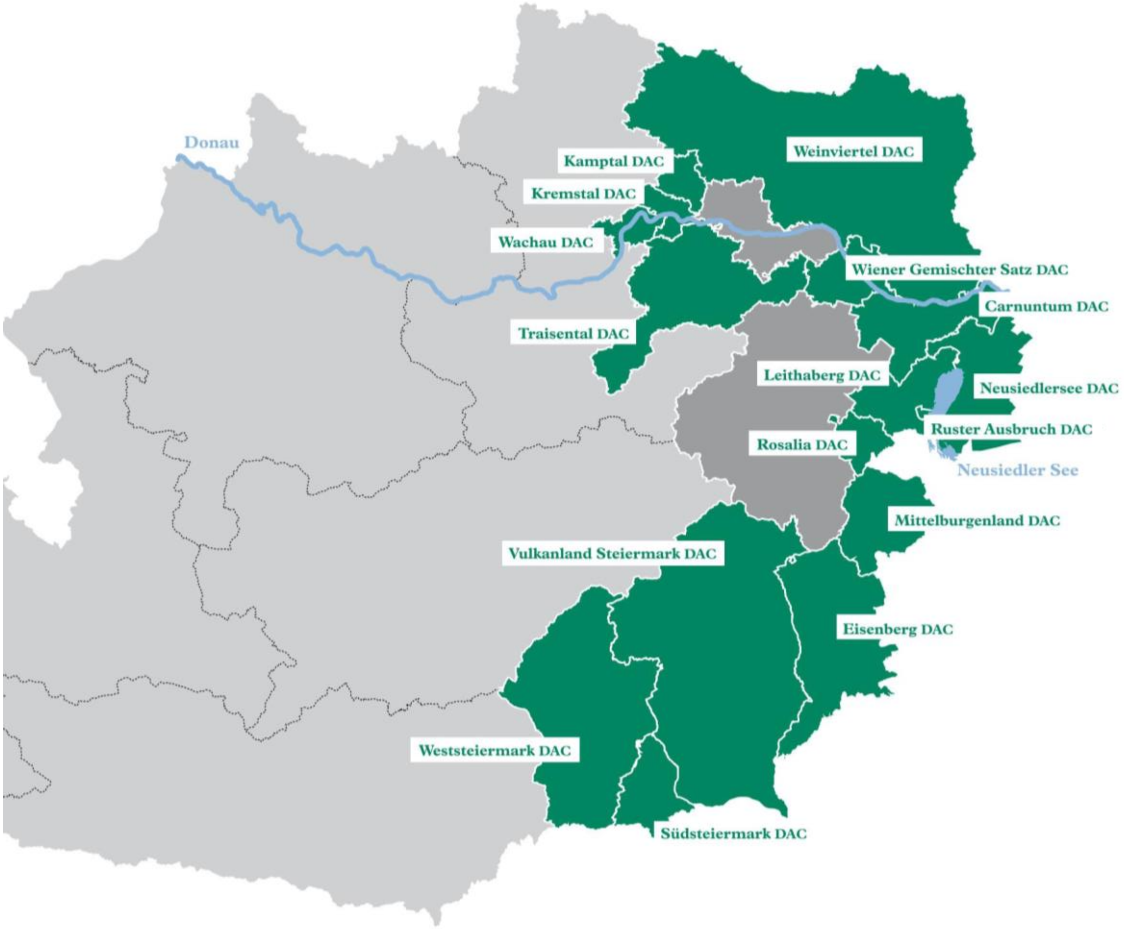


Figure 7: DAC regions (Österreich Wein Marketing GmbH (ÖWM) July 2020)

DAC wines are separated into three levels: Gebietswein, Ortswein or Großlage and Riedenwein. Gebietswein, regional wine, has to exhibit a style that is typical for its winegrowing region. Grapes for this level have to be harvested in the particular region. For Ortswein, local wine, the grapes come from a smaller area inside of the DAC region; wines from this level are usually more complex but must still exhibit a typical flavor profile. The highest and most specific level are single vineyard or Riedenweine (Österreich Wein Marketing GmbH 2018a).

1.1.1 Styria

> *Without a doubt – there are winegrowing regions where more powerful wines with more abundant alcohol are vinified. But in the whole world there are to be found no more refreshing and brilliant wines – thus in an elegant way typical of their origins – as in the south of Austria's federal state Steiermark.*
>
> (Österreich Wein Marketing GmbH 2018a)

This is an extract from the Austrian Wine Marketing Board's (ÖWM) description of Styrian wine. With a vineyard area of 4,633 ha and three designated wine growing areas, Styria is the most southern of the four main wine producing federal states in Austria. With 77.6% of its area dedicated to white grape varieties, most of Styria focuses on the production of white wines. The main cultivars are Welschriesling (16.2%), Sauvignon Blanc (14.7%) and Weißburgunder (12.4%) (Österreich Wein Marketing GmbH 2019). Styria has three winegrowing areas with DAC status: Vulkanland Steiermark DAC, Südsteiermark DAC and Weststeiermark DAC.

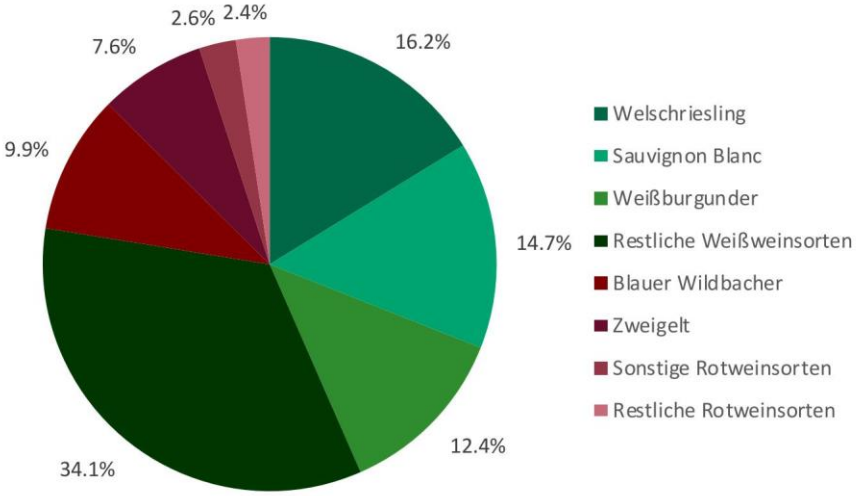
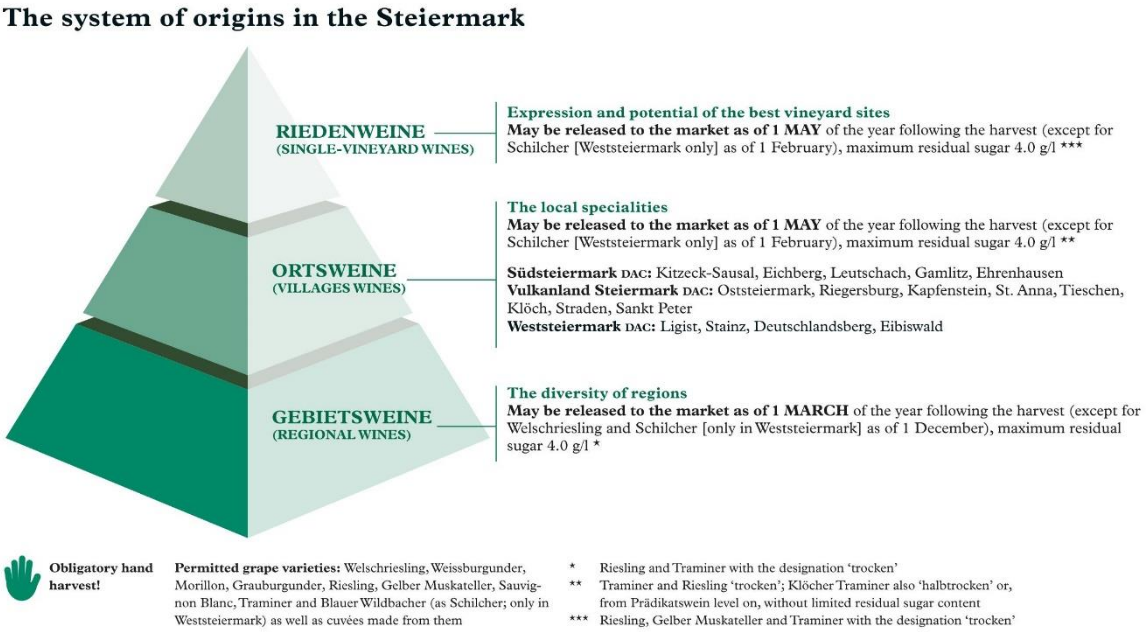


Figure 8: Distribution of grape varieties in Styria (Österreich Wein Marketing GmbH 2019)

The Vulkanland region focuses on Welschriesling, Weißburgunder, Sauvignon Blanc and Traminer. It encompasses 1,524 ha of vineyard area. In the Südsteiermark DAC region, the biggest of the three with its 2,563 ha of vineyard area, the main cultivars are two aromatic varieties Sauvignon Blanc, Muskateller as well as Weißburgunder, Morillon (Chardonnay) and in the area of Sausal particularly of Riesling. The Weststeiermark DAC winegrowing region is the smallest, with 546 ha of vineyard area. Beside Sauvignon Blanc and Weißburgunder, a local autochthonous variety, the Blauer Wildbacher is grown here. With 66.8% of the vineyard area are dedicated to this variety, Weststeiermark is the only Styrian region that focuses on a red grape variety. Wines made from the grapes are sold under the name Schilcher.



The system of origins in the Steiermark

RIEDENWEINE
(SINGLE-VINEYARD WINES)

Expression and potential of the best vineyard sites
May be released to the market as of 1 MAY of the year following the harvest (except for Schilcher [Weststeiermark only] as of 1 February), maximum residual sugar 4.0 g/l ***

ORTSWEINE
(VILLAGES WINES)

The local specialities
May be released to the market as of 1 MAY of the year following the harvest (except for Schilcher [Weststeiermark only] as of 1 February), maximum residual sugar 4.0 g/l **

Südsteiermark DAC: Kitzeck-Sausal, Eichberg, Leutschach, Gamlitz, Ehrenhausen
Vulkanland Steiermark DAC: Oststeiermark, Riegersburg, Kapfenstein, St. Anna, Tieschen, Klöch, Straden, Sankt Peter
Weststeiermark DAC: Ligist, Stainz, Deutschlandsberg, Eibiswald

GEBIETSWEINE
(REGIONAL WINES)

The diversity of regions
May be released to the market as of 1 MARCH of the year following the harvest (except for Welschriesling and Schilcher [only in Weststeiermark] as of 1 December), maximum residual sugar 4.0 g/l *

Obligatory hand harvest!

Permitted grape varieties: Welschriesling, Weissburgunder, Morillon, Grauburgunder, Riesling, Gelber Muskateller, Sauvignon Blanc, Traminer and Blauer Wildbacher (as Schilcher; only in Weststeiermark) as well as cuvées made from them

* Riesling and Traminer with the designation 'trocken'
** Traminer and Riesling 'trocken'; Klöcher Traminer also 'halbtrocken' or, from Prädikatswein level on, without limited residual sugar content
*** Riesling, Gelber Muskateller and Traminer with the designation 'trocken'

Figure 9: DAC System in Styria (Österreich Wein Marketing GmbH 2018a)

According to the most recent survey of areas under vines from 2020, the total vineyard area in Styria has increased to 5,096 ha (+6% since 2016) and Sauvignon Blanc has become the most prevalent variety. It now covers 902 ha or 17.7% of the total area, which is an increase of 27%, compared to the previous report from 2016 (P. 2021).

2. History of wine

> *One can imagine a group of early humans foraging in a river valley or upland forest, dense with vegetation, at some distance from their cave dwelling or other shelter. They are captivated by the brightly colored berries that hang in large clusters from thickets of vines that cover the deciduous or evergreen trees. They pick the grapes and tentatively taste them. They are enticed by the tart, sugary taste of the grapes to pick more. They gather up as many of the berries as possible, perhaps into an animal hide or even a wooden container that has been crudely hollowed out. A hollow or crevice in the rock might also serve the purpose. Depending on the grapes' ripeness, the skins of some rupture and exude their juice, under the accumulated weight of the grape mass. If the grapes are then left in their "container," gradually being eaten over the next day or two, this juice will ferment, owing to the natural yeast "bloom" on the skins, and become a low-*alcoholic wine*. Reaching the bottom of "barrel," our imagined caveman or -woman will dabble a finger in the concoction, lick it, and be pleasantly surprised by the aromatic and mildly intoxicating beverage that has been produced accidentally. More intentional squeezings and tastings might well ensue.*
>
> (McGovern 2019)

Like this or in a similar fashion humans or their ancestors might have first encountered wine. Since then, wine has been part of human culture. McGovern et al. (2017) found evidence that indicates the production of wine and domestication of vines dating back to at least 6000 BC in the region of Georgia in the South Caucasus region. Clay shards with residues of tartaric acid as well as archaebotanical evidence, grapevine wood and agglomerations of pollen, suggest the cultivation of vines and wine production in this area in early Neolithic times in that region. How the first wine was produced is not clear but as a natural product, it was likely discovered by accident.

From there vines and wine spread (Figure 10) to Turkey, Egypt and other parts in Europe and Asia. Egyptians are regarded as the first civilization to grow and make wine. Grapes for wine were pressed by in large canvas bags and the finished product was stored in carefully labeled jars, sometimes for many years. Archeologists found wine, given as a funeral offering, which had been more than two centuries old before being buried.

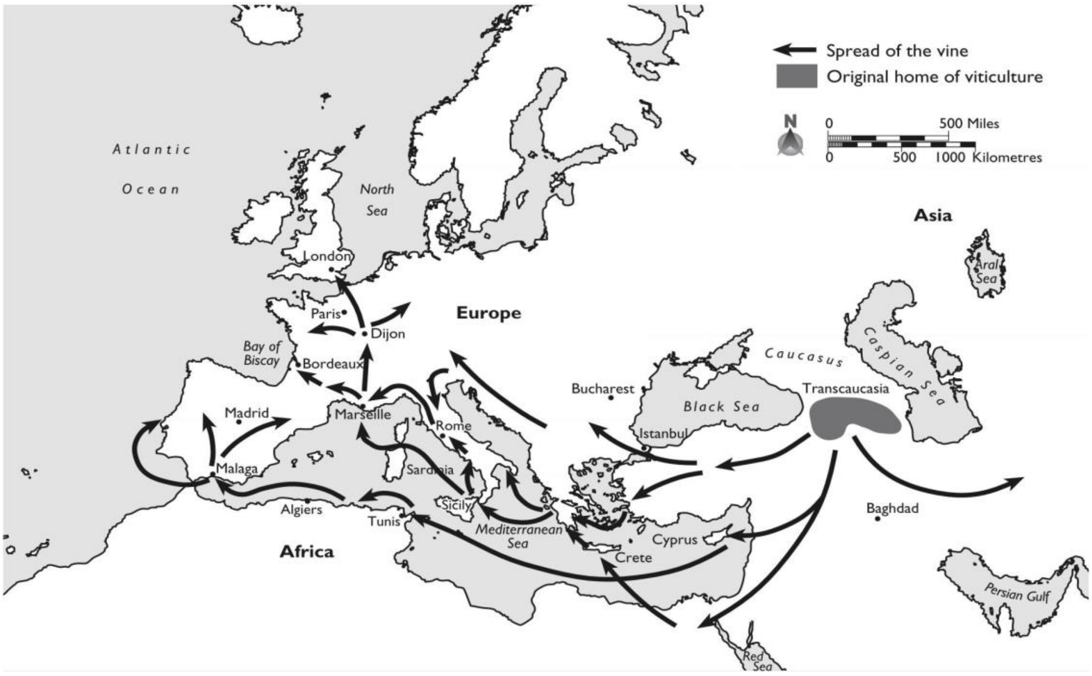


Figure 10: Spreading routes of wine through Europe (Charters 2006, S. 17)

Vines and winemaking technology spread through the Mediterranean. The Greeks, for example, valued wine so much, that they gave it its own deity, Dionysus. Drinking alcohol, particularly wine, was used to create a bond between man and god (Toussaint-Samat 2009). They used to drink it diluted with water, as their version of wine was a rather jam-like strong, sweet and thick product. The fermented juice was cooked multiple times and thickened with honey or cane sugar. The daily consume of wine was reserved for the upper class. For other Greeks it was a drink reserved for special occasions or medicinal use. The Greeks also exported their wine to other nations, like the Roman Empire, where it was highly regarded. Wines were usually blended to make them more palatable, Wine merchants used wines from different locations in Greece to balance out shortcomings of the individual wines.

Greek settlers brought their winemaking practices and vines with them when they settles on Sicily and in southern Italy (Charters and Pettigrew 2008). In central Italy, the Etruscans did the same. Once the Roman Empire started to take over bigger parts of Italy, they also took over these regions and started to assimilate the wine culture. Romans generally preferred white wines, to fulfill the demands ‘black’ (red) wines would be bleached with sulfur fumes. Similar to the Greeks, Romans also cooked their wines, but to a lesser extent. They reduced the wine to about two thirds and flavored it with different aromatics. The Romans also developed and improved viticultural practices. Grafting, for instance, is said to have been developed by the Etruscans. Wines were stored in pitch-coated amphorae after being aged at least two to three years and, unlike Greek wines, they were never blended. Glass bottles started to be more common at the end of the first century BC. This brought also the development of the cork stopper. The Romans brought it with them further inland during their conquests. They needed wine to be able to ensure enough supply for their soldiers. The Romans were the first to name different cultivars. At the end of the first century AD, wine was widely distributed. Remains of amphorae from that time were found in parts of Spain, France, Germany and England (Charters and Pettigrew 2008).

In France, vines was first introduced in the area of Marseille by the Phocaeans about 500 BC. The wine made there was mainly sent back to Greece. At the end of the second century BC, the Romans spread vines through Provence, and again the French did not get to consume the products of their work. The wine was instead sent back to Rome, where they were very popular with rich patricians. The people living in France received only thin piquette and young wine. One invention made in France during that time was the barrel. It was first used for storing and transporting beer, wine was still mainly stored and transported in amphorae but barrels proved to be the better option. Unlike the Romans, the French drank their wine pure and undiluted, pitch was added for table use and sometimes it was flavored or made bitter.

After the decline of The Roman Empire, the Catholic Church took over the distribution of grapevines. Wine had an important symbolical and sacral meaning to the church as it was used in the Holy Communion (Dougherty 2012). The quest of the Catholic Church to bring Christianity to the entire world helped to spread wine and winemaking across the world. For this, the missionaries needed wine. During the Renaissance *V. vinifera* vines reached several New World countries with Catholic missionaries. They were introduced in the Americas, South Africa and Australia and New Zealand. Some of the grape varieties in these areas still carry names like ‘mission’. In Europe, Monks and nuns during that time furthered the development of winemaking practices. In the Loire valley, Monks for instance developed hard pruning while nuns ripened harvested grapes on straw for the production of vin de paille or straw wine. Another important invention of the time is credited to a Benedictine monk by the name of Dom Pérignon, who was the administrator of the monastery of Hautvillers. He did make white wine from red grapes, ‘Blanc the rouges’. Some sources also credit him with the invention of a way to make sparkling wine, something the Champagne region is still known for today. Other sources say that the method originated from England, where sparkling wine first became popular. The British had filled non-refined wines with residual sugar from the Champagne region in bottles and closed them. When the temperature increased after winter the yeast, that had been dormant over winter started working again, turning the sugar in the bottle into carbon dioxide that could not escape and accidentally resulted in sparkling wine (Tardi 2016). Christopher Merret presented “Observations of ordering wines” presented to the Royal Society on December 17th 1662, several years before Dom Pérignon’s appointment to the monastery in 1668. In this presentation, Merrett describes how the addition of sugar or molasses make “*the wines drink brisk and sparkling*”. In addition, by using coal instead of charcoal in the furnace, the British also developed a stronger glass, the “verre anglaise”, needed to contain the added pressure from the carbonization. It took until the 18th century for Champagne to gain popularity in its own country, leading to the founding of companies of which some still operate today (Unwin 1996). Another development in the wine industry during the 17th century was Port, fortified wine from northern Portugal. For this, alcohol is added during the fermentation to stopping it, giving the resulting wines the residual sweetness the must had, when the fermentation was stopped. It was not the first time wine was fortified. Spanish wine makers in the 16th century added cooked wine or brandy to their wines to extend their aging potential and stabilize them for long sea voyages. The wines made in this style became known as sherry (Unwin 1996). In addition to the vinicultural advances, there were other important developments during the 17th century that aided winemakers. Two of the most important ones were probably the introduction of glass bottles closed with fitted corks to store wine. Until then wine was mostly stored in barrels, which could lead to wine spoiling due to acetobacter infections turning the wine into vinegar in the presence of oxygen. Using bigger barrels and topping of the liquid after taking out some of the wine helped avoiding most of the damage for a certain amount of time. With the introduction of cork sealed bottles oxygen damage could be reduced and wines stored for longer periods of time (Charters 2006).

During the 18th century, the industrial revolution led the formation of the middle class. Common people had more money and were able to spend it on food and wine to showcase their newfound wealth. Books on wine and wine auctions started during this time. Winemakers were able to spend more money on improving the quality of their wines. While the upper and middle classes were able to spend more money on better quality wines, the lower class had to make due with cheap wines prone to systematic adulteration. Producing higher quantities of wine, even if it meant a loss in quality, became the goal of an increasing number of winemakers in France and Germany. This lead to the development of two different markets, on the one hand one for expensive, high quality wines and on the other hand one for mass produced wines of lower quality. While in France the demand for cheap, low quality wines grew due to a large increase in the urban population from 2.7 million to about 5 million, with around 0.5 million people in

Paris alone, the German wine market did not exhibit the same behavior. The population of German had been reduced to around 10 million in 1650 as a result of the Thirty Years War. This, along with other factors like the lack of large cities and the existence of many smaller states instead of one centralized government like in France, led to a smaller demand of wine. In addition, wine growing areas in the valleys of Rhine and its tributaries had been struggling with climatic deterioration and a lack of winemakers and workers. While the beer industry expanded, wine and its industry lost demand, leading to a reduction in wine growing areas during the 17th century. Most reporting from that time focuses therefore on France. More growing area was planted with grape varieties that had high yields but only produced wines of a mediocre quality at best, like the Gamay. Better connection to bigger cities and a high demand of cheap wines led many winemakers to restructuring their vineyards and expanding them. Overproduction was either turned into vinegar or distilled. While the common people were content with the low quality wine, the nobility wanted high quality and exclusivity. Different areas in France produced for the two different markets, while Médoc, Burgundy and Champagne focused on the higher quality wines, wines from the Loire valley and the south of France catered to the lower price market. Wines of both categories were prone to fraud and adulteration. While cheaper wines were mostly diluted with water, people tried to sell lower quality wines for higher prices by adding different substances to pass for higher quality. The Portuguese were the first who introduced regulations to ensure quality and origin of their port wines and therefore making sure that the profits remained with the merchants and owners of larger vineyards in the Douro region. They introduced fixed prices for wines used in the production of port wines in 1756 (Unwin 1996).

The French revolution of 1789 affected especially the Burgundy wine regions where most of the vineyards belonged to the church and monasteries. Most of the land was confiscated by the state and redistributed leading to their fragmentation. In Bordeaux, the amount of confiscated estates was much smaller, most of the properties stayed intact. Overall, even though the revolution had an effect on the distribution of the land and the ownership of vineyards, the quality of wines coming from either of the regions did not decrease. In the case of Burgundy, it actually raised the amount of high quality vineyards. After almost a century, the French winemakers followed the Portuguese example. In 1855, a classification system was introduced, dividing higher quality red wines from the Gironde into different five different quality classes and white wines into three groups for white wine. Wine brokers conducted the division mainly based on the price the wines sold for. The most expensive wines from the Médoc could cost up to four times the price of a common wine and up to 20-times more than a lower quality wine (Unwin 1996). In general, most of the 19th century was a good time for the French wine industry. People of the middle class from all over Northern Europe had more money to spend on luxury goods, including wine. In addition, the free trade agreement between France and Great Britain in 1861 led to an increase in exports. This also fueled the wine production in other European countries, like Germany, Spain and Italy. Technological advances in different areas made wine trading faster and safer. Better means of transport developed during that time, like railways and steam ships, made transport of wine across countries and worldwide possible. Preservation techniques, in particular different ways of cooling wine helped as well. Wines could be transported to outposts of different European empires and the United States of America, increasing profits (Charters 2006). The 19th century was also a time of scientific discoveries. In 1815, Joseph-Louis Gay-Lussac found that cooked grape juice would not ferment, if it was sealed in an airtight container while it was still hot. Fermentation only started once he added yeast cells to the cooled juice, proving that yeast played a vital part in alcoholic fermentation. A few years later, it was discovered that yeast cells reproduce during alcoholic fermentation and that yeast is a living organism and not, as previously believed, organic residue. Louis Pasteur was the first one to demonstrate that the sugar in grape juice was converted into alcohol by yeast cells in the absence of oxygen. He also proved that alcohol and carbon dioxide are not the only products of fermentation (Segal 2008). In his book *Études sur le vin: ses maladies, causes qui les provoquent, procédés nouveaux pour le conserver et pour le vieillir*, (Pasteur 1873) first published in 1866, he describes how wine can spoil due to the presence of microorganisms, mainly lactic and acidic bacteria (Ribéreau-Gayon et al. 2006). This, he discovered, happens especially when oxygen is present (Tattersall and DeSalle 2015). Pasteur observed that wines would not spoil if they were heated to 55°C without oxygen present, as most of the harmful organisms are killed in the process (Unwin 1996). His findings lead to a big change in the quality of wines produced by reducing the probability of spoilage making it easier to transport across the world.

The advances in transportation during the 19th century also made the spreading of pests and diseases possible. In 1845, a gardener from England found a powdery substance that covered the leaves of some of his vines. This was the discovery of powdery mildew, oïdium. This fungus had most probably come to Europe with American varieties that were bread in greenhouses. Oïdium spread across Europe, causing a significant reduction of yields. In 1852, the head of the greenhouses of Versailles, Grison, found that spraying the leaves with a mixture of boiled lime and sulfur, called Eau Grison, reduced the effects. As this method was impractical for larger vineyards. In the early 1860s, Henry Mares found that dusting vines with fine sulfur successfully treated infected plants. This method became the European standard (Unwin 1996). Another way to lessen the thread of powdery mildew was the introduction of resistant vines from the Americas, mainly the United States. Unknowingly the vintners invited another thread to Europe. Starting in 1863, vines in France started dying with no obvious cause. First leaves would turn red and fall from the vine, full bunches of grapes would dry up and root tips started to rot. Finally, the affected plants died. Starting from Arles these symptoms spread through France. Five years later, researchers, namely Jules- Émile Planchon and his team, found the reason: a small, barely visible aphid. It sucked sap from the roots of the infested plants and poisoning them. First named *Rhizaphis*, it later became known as *Phylloxera vastatrix* (Riley 1874).

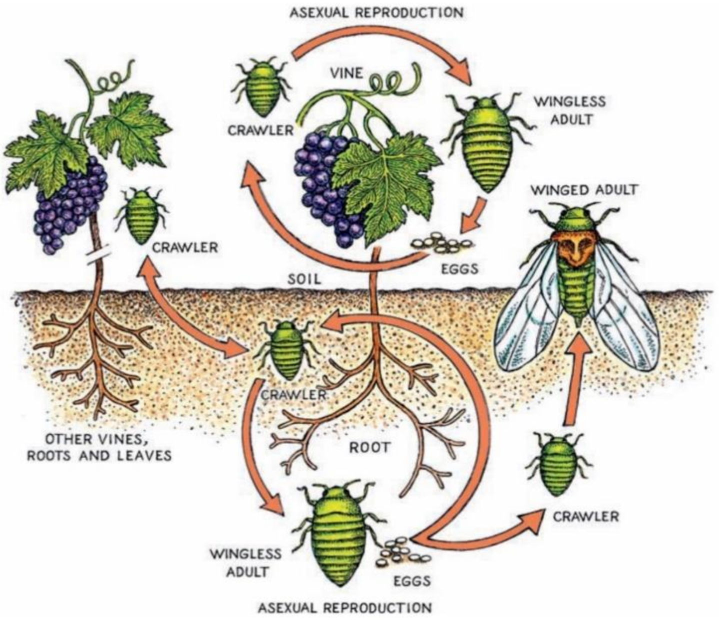


Figure 11: Life cycle of the Phylloxera bug (Tattersall and DeSalle 2015, p. 121)

From France phylloxera expanded to rest of Europe, devastating most of the vineyards. In the 1870s it reached Portugal, Turkey, Austria-Hungary, Switzerland, Spain and Italy and in 1881 Germany. 1875 phylloxera was first recorded in a vineyard in Lower Austria. It was most probably introduced with American cultivars which were supposed to fight powdery mildew (Lantschbauer and Barwirsch 1989). In Styria, the first infestations were reported in 1880 (Postmann 2003). In France alone 6.2 million acers of vineyards fall prey to phylloxera, leading to a drop in production from 2.2 billion gallons to only 600 million from 1875 to 1889 (Estreicher 2006). In 1869, a commission to investigate a solution for the problem was installed.

The government even offered a price of 20,000 francs for a sustainable solution. Finding a solution to this thread took longer due to several external forces. In 1870, the Franco Prussian war broke out and the third republic was declared. The government only started to focus on the phylloxera once the war was over. In 1874, they raised the price money to 300,000 francs. The commission tested more than 300 different proposed remedies. Out of these, only two showed some sort of improvement, the application of potassium sulfide or sulfide with colza cakes. In addition, flooding effected vineyards worked where it was practical. The vineyards needed to be flooded for at least 40 days during autumn or winter. In addition, the application of the highly toxic carbon disulfide proved effective. The protection these remedies brought only lasted for one season and had to be repeated every year. Finally, the solution came from the same source as the problem. At an international phylloxera congress held in Bordeaux in 1881, it became widely accepted, that the only effective protection is archived by grafting French vines onto American rootstocks that had proven resistant. Experiments were carried out in order to find suitable rootstocks that were able to grow in European vineyards and provide a good base for the European cultivars. By 1885, 110,000 acers of French vineyards had been replanted with vines grafted on American roots (Estreicher 2006). This development continued into the 20th century. By the 1920s, most vineyards had vines grafted onto American rootstocks. The replanting and grafting of whole vineyards came with considerable capital investments that mostly only bigger wineries could afford. Small peasant producers on the other hand often were not able to manage the growing costs for replanting and spraying for fungal diseases. Many abandoned their vineyards or planted different crops. This, in combination with other factors, resulted in a decrease of vineyard area in France (Figure 12). Even though the total area under vines kept shrinking, the production volume of wine increased. This development was partially due to the creation of hybrid varieties that had a higher yield compared to their predecessors. Vines exhibiting high production and resistance to at least one of the new diseases or pests were chosen and combined with others for their wine quality. The American species provided the resistance while the European cultivar contributed the quality. These hybrids enabled an easier wine production as they required less care compared to their grafted counterparts. With higher yields and easier handling, hybrids were cheap way to produce high quantities of wine, laying the basis for a new, industrialized viticulture (Charters 2006; Unwin 1996; Clarke 2015; Tattersall and DeSalle 2015; Simpson 2011).

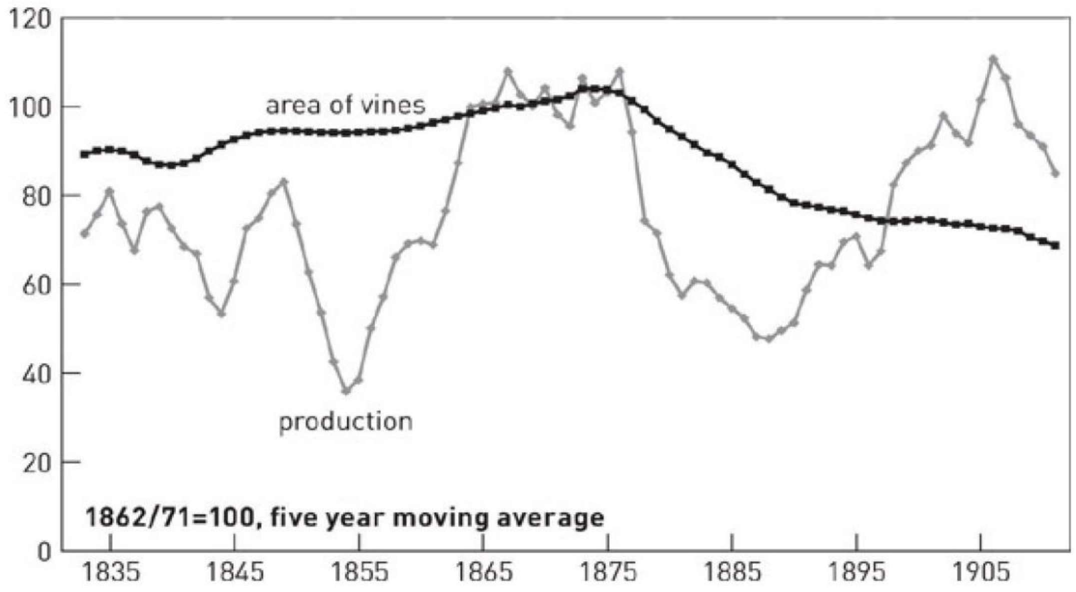


Figure 12: Area of vines and wine production in France, 1833-1911 (Simpson 2011)

During the phylloxera crisis, another new threat was introduced in France, most likely due to American vines introduced for grafting. Downy mildew was first noted in 1878. A few years later, in 1882, it affected the already weakened vines in most of the major wine producing regions and further augmented the decline of wine production. In the case of this fungal parasite, the discovery of a remedy followed a year later. In 1883, Pierre-Marie-Alexis Millardet successfully treated vines with copper sulfate sprays. A year later, the application became common practice (Unwin 1996; Estreicher 2006).

In the years after the outbreaks of first mildew followed by Phylloxera and to a lower degree oidium, the wine production could not fulfill the high demand. France, at this point, had the highest per capita consumption of wine. Wine fraud and adulterated wines became more widespread than ever. The most common practice was the dilution of wine with water. There are records from the tax authorities in France that show about one sixth of the wine sold in Paris in the early 1880s was water. To reach the, at that time average, alcohol content of 10 per cent, the diluted wines were fortified with spirits. Another practice used during this time was the production of 'wine' from raisins and currants. A popular book of the time, '*L'art de faire le vin avec les raisins secs*' by Joseph Audibert, detailed the process. The import of raisins went from 8 million kilograms in the years leading up to 1880 to 65 million in 1885 (Stanziani 2003). To produce 300 liters of raisin wine with a strength of about 8 per cent 100 kg of dried raisins or currants were soaked in warm water and fermented for about two weeks. The resulting pale yellow beverage could be turned into 'red wine' by adding real red wine, elderberries or a chemical dye. Another way of producing ‘wine’ was the deuxième cuvée. For this, the solids from a previous pressing, mainly seeds and skins, would be mixed with warm water and sugar. After fermentation, the color was fixed by adding tartaric acid. There are estimates that in 1890 one tenth of the wines sold were either raisin wine or deuxième cuvee. With limited amounts of raw material even lower quality grapes were used to produce wines. In order to reach a desirable product, sugar had to be added before fermentation. This process is known as chaptalization, named after a chemist from the 18th century and one of Napoleon’s ministers, Jean-Antonine Chaptal. While French vineyards had already fallen prey to phylloxera, vineyards in Spain remained unaffected for another thirty years. During this time, producers from France, especially from Bordeaux, would buy Spanish wines, mix them with their own production and sell the product as pure Bordeaux. The same was true for wines from Portugal, except for Port, and Italy (Estreicher 2006). To prevent fraud and misleading consumers, France introduced various new laws during this time. In the 1889 Griffe law wine was defined as a product produced from fresh grapes only. The production of other ‘wines’ was still permitted, as long as the consumer was made aware of the real content. After the introduction of several other laws regarding wine made from raisins and the addition of gypsum in 1891, the watering down of wines in any form was made illegal in 1894 (Phillips 2018; Simpson 2011; Unwin 1996).

At the same time France was recovering from phylloxera, new grafted vines were planted and wine production recovered and wine prices started to fall (see Figure 13). In the beginning of the 20th century wine prices in the Midi had fallen from 33 francs per hectoliter in 1882 to 9 francs in 1907 (Simpson 2011). A commission looking into the reasons for this development found that overproduction was not the main reason, but rather that the price of genuine wines was adjusted those of artificially manufactured ones. The low wine prices led to large demonstrations in parts of France, mainly in the Midi. As a result, the French government introduced several laws. In 1905, a law giving customers the possibility to redress wines that were not what they claimed to be was passed. In 1907, a law limiting the amount of sugar that could be added to produce wine was introduced. Further regulations were introduced in 1919 and 1927. Form this point on, producers have to declare the sum of their annual production and blending of French wines with wines from other countries, like Spain and Algeria, became illegal. During this time, wine producer associations started to form in some wine regions. One of the main goals of these groups was to guarantee the authenticity of wines. This in combination with the 1905 law were the first steps toward introducing the *Appellations* *d'Origine Contrôlées* system, which was introduced in 1935. This law also restricted the use of high-yielding varieties, mainly hybrid cultivars that resulted from breeding efforts in early post-phylloxera times (Unwin 1996; Simpson 2011; Estreicher 2006).

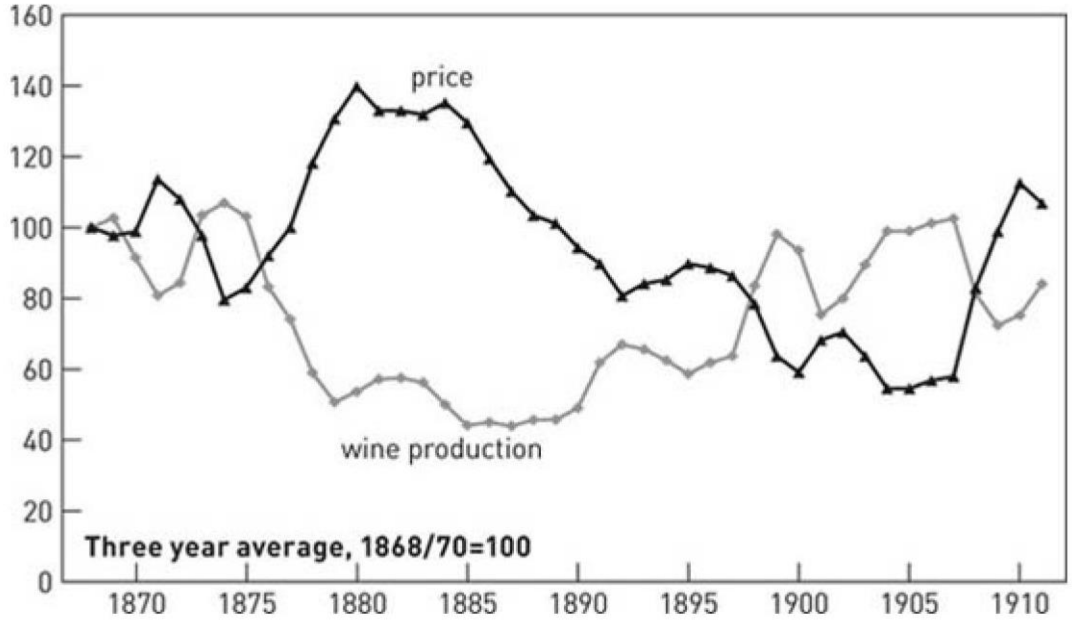


Figure 13: Development of wine production and wine prices in France in the times before and after phylloxera (1851-1912) (Simpson 2011)

Other parts of the world followed France and introduced laws protecting wine makers and consumers from fraud and adulteration. Spain, for example, introduces its denomination of origin in the 1930s, first in the Rioja. Jerez and Malaga follow a few years later. In 1930, Germany introduced a law according to which vineyard names had to be registered and that only European cultivars that had been grafted onto American rootstocks could be planted and cultivated. This resulted in the registration of around 30,000 vineyards but could not prevent adulteration and fraud. Austria introduced the first laws regulating the production and selling of wine in 1880. The production of artificial wine was banned in 1907. Austria, which had been the forth-biggest producer of wine worldwide (5.6% in 1905), lost about 95% of its vineyard area after World War I with a drop from 560,000 ha in the beginning of the 20th century to around 30,000 ha in 1922 (Postmann 2010). From 1940 until 1945, the German wine law also applied in Austria. In the United States, the 18th amendment is passed in January 1920, making the production, transporting, selling and even the possession of intoxicating liquors (containing more than 0.5% of ethanol) illegal. As a result, the wine production in the US fell from more than two mhl to 0.13 mhl, only 6%. While prohibition ended in 1933, it took the American wine industry decades to recover (Estreicher 2006; Unwin 1996).

The national regulations regarding the origin and naming of wines were only applicable inside the country. As a result, people in Britain were able to buy ‘Perelada Spanish Champagne’, produced by the Costa Brava Wine Company. In 1959, several French producers, led by Bollinger, sued the Spanish company in London over the use of the name, as it might lead consumers to believe, what they were drinking was real champagne. The French producers won the court case and set a precedent for the use of the name champagne only for wines produced in the area. This led to most international producers refraining from using it for their sparkling wine, except for some Californian winemakers (Charters 2006; Unwin 1996).

After the Second World War, some European countries, mainly Germany and Italy, used wine exports to earn foreign currency. To optimize this business, they went for quantity, most times at the expense of quality. New vineyards were planted away from traditional wine growing areas in fertile valleys. This in combination with rapid technological advances, both in viti- and viniculture led to an increased yield. In the 1980s, German vineyards reached a high of 11 t/ha (about 88 hl/ha), the highest in Europe at the time (Storchmann 2018). At this time, sweet wines were popular with consumers, both inside of Germany as well as in other countries. Blue Nun, a sweet, white wine in a blue bottle, was popular in Great Britain in the 1970s, selling 3.5 million bottles a year (Veseth 2011). Wines like this, also known as Liebfraumilch, shaped the image of German wine for the upcoming years. The wine producing countries of the European Economic Community, a predecessor of the European Union formed in 1957, adopted a form of the French regulatory system, as France was regarded as the leading wine nation at the time. West Germany, one of the founding members, did not. Their wine law from 1971 divided the country into different region and introduced hierarchy in wine quality. Both, the European and the German regulations were unable to prevent overproduction; therefore, a ban on new plantings was introduced in 1976. This measure proofed unsuccessful as well. In 1979 and 1980, European Economic Community (EEC) members produced 182 and 164 mhl respectively. With further regulations, like making the distillation of surplus wine the standard way to regulate overproduction, the market stabilized. The increase in yield was made possible due to many advances in both wine growing as well as wine making practices. Canopy management changes, like the introduction of the Lenz Moser Hochkultur and others instead of the Stockkultur (Figure 14), made working in the vineyards easier and reduced the chances of losses. The use of small tractors for ground working and spraying of plant protection agents brought additional workload relief. New cultivars were introduced, because some of the old cultivars were not able to withstand the cold during winter. In Austria, cultivars like Riesling, Traminer, Grüner Veltliner, Weißburgunder and for Styria in particular, Sauvignon Blanc and Welschriesling were recommended, as they could withstand the cold winters and were more productive in the new vine training versions.

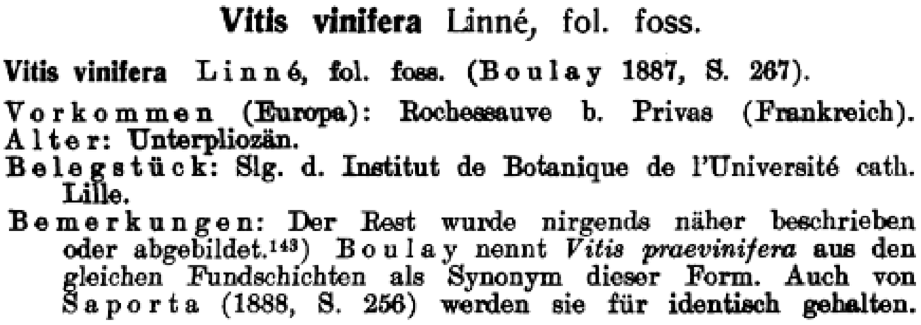
Figure 14: Stockkultur and Lenz Moser Hochkultur (Postmann 2010, p. 32&p. 55)

In the cellar, new techniques made winemaking safer and more reliable. Temperature controlled fermentation, the use of cultivated yeast and new, more effective stabilization methods became commonplace. The increases in yields were significant. In Austria, they rose from 1.14 mhl in the 1950s to 2.81 mhl in the 1980s. The vineyard area also increased by more than 30%, from 41,821 ha to 56,042 ha over the course of 13 years. This was in part due to a study in the early 1970s that had predicted a rise in per capita consumption of wine to more than 52 liters. In reality, the consumption reached its highest in 1989 with 35 liters per person. All of these developments contributed to the biggest wine scandal in Austrian history. After receiving a tip, German authorities found diethylene glycol (DEG), a main ingredient in anti-freeze, in wine samples from Austria in 1985. The addition of DEG to wine makes it appear to be sweeter and fuller bodied, so more desirable, based on the wine standards of the time. This discovery was possible due to the development of more sensitive analytical methods. As a result, all Austrian wines were taken off the shelves of German supermarkets and the news spread all over the world. The following year, Austrian wine exports dropped to 10% of the previous years. Black lists, published by the Austrian ministry of health, listed more than 130 products from 46 producers that had been found to contain some amount of DEG. More than 26 million liters of wine had to be destroyed and 325 people were prosecuted. The highest concentration found was 48 grams of DEG per liter of wine. In reaction to the discovery of the adulteration, the Austrian government introduced stricter wine laws and consumers started to demand higher quality wines. The adulteration was first discovered because one of the wine makers tried to claim the DEG as expense on their tax form (Charters 2006; Unwin 1996; Postmann 2010).

At the same time, institutions in California and Australia gained importance in wine research and wines from the new world, first from California, followed by New Zealand, Australia and Chile, but also South Africa, started to appear on the international market more and more frequently (Charters 2006). Over the last century, the production share of the three biggest producers of wine, France, Italy and Spain, had slowly declined. At the beginning of the 20th century, they were responsible for 80% of the world's wine production. By 1960, their share was reduced to around 60% and in 2018 they were responsible for 50% (Postmann 2010; Organisation Internationale de la Vigne et du Vin 2019; Anderson and Pinilla 2018). This shows the continuing globalization of the wine market, where new world and smaller countries in Europe become more important as the world becomes more interconnected. Since 2011 the United States have been the world's biggest wine consumer, being responsible for 13.4% of the worldwide consumption in 2018 (Organisation Internationale de la Vigne et du Vin 2019). They have also been the biggest importer by value (5.2 billion Euro, 11.5 mhl in 2018) while Germany is still the leading importing nation regarding volume (14.5 mhl in 2018) (Organisation Internationale de la Vigne et du Vin 2019).

3. The grapevine

The main species for cultivation and wine making in the *Vitaceae* family is the *Vitis* genus. There are about 60 interfertile species, out of which the *Vitis vinifera* is the one most important to the wine industry.



Vitis vinifera Linné, fol. foss.

Vitis vinifera Linné, fol. foss. (Boulay 1887, S. 267).

Vorkommen (Europa): Rochessauve b. Privas (Frankreich).
Alter: Unterpliozän.
Belegstück: Slg. d. Institut de Botanique de l'Université cath. Lille.
Bemerkungen: Der Rest wurde nirgends näher beschrieben oder abgebildet.143) Boulay nennt *Vitis praevinifera* aus den gleichen Fundschichten als Synonym dieser Form. Auch von Saporta (1888, S. 256) werden sie für identisch gehalten.

Figure 15: Descriptions of fossils of vitis vinifera (Kirchheimer 1939)

Figure 15 shows an entry in a museum's catalog detailing artifacts related to *Vitis vinifera* specimens, dating back to the Zanclean (Early Pliocene) age (between 5.3 and 3.6 million years ago). These originate most probably of the subspecies *silvestris*, the predecessor of the modern varieties. Modern varieties show important morphological differences compared to their ancestor (Figure 16). Botanists believe that these are most likely due to the domestication by humans and not geographical isolation (This et al. 2006).

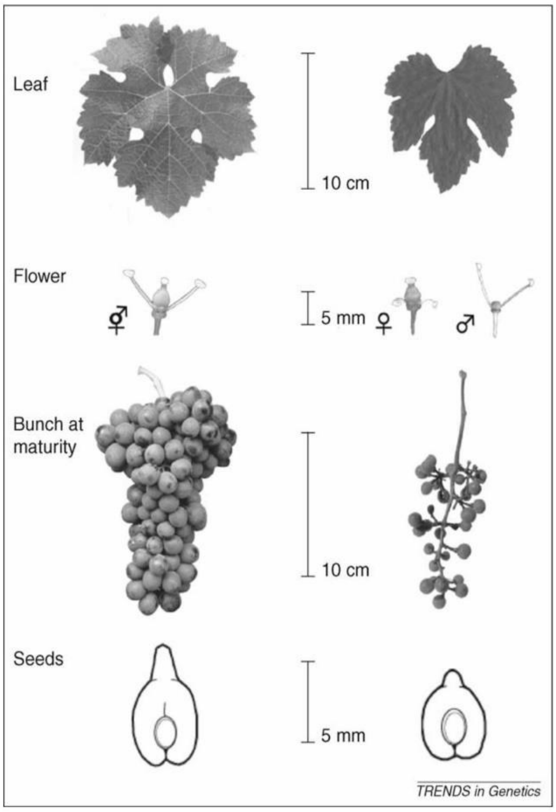


Figure 16: Comparison of Vitis silvestris and Vitis vinifera (This et al. 2006, p. 513)

The main differences are beneficial for the production of wine, like higher sugar content in the ripe fruits to ensure better fermentation, greater yield and plants that carry fruit more regularly. The amount and the size of berries changed as well as the anatomy of the flower. *Vitis silvestris* plants are dioecious, meaning that male and female plants exist separately and fruits are only produced if it comes to a pollination of the female flower. Cultivars used today are monoecious or hermaphrodites making self-pollination possible and ensuring stable yields. Other characteristics that changed are the shapes of the leaves and the seeds. These changes do not influence the wine production directly; they do, however, help with the identification of archeological discoveries of vine and grape residues. It is not entirely clear if these changes happened slowly through sexual crosses and human or natural selection over time or quickly because of mutations. While breeding new varieties is still of importance, most new vines today are a result of vegetative propagation by using cuttings. This ensures that all plants have the same genotype and therefore behave in a predictable way and similar to each other (This et al. 2006).

Currently there are around 10,000 varieties of grapevine. Out of these, only a few are cultivated for human use, for wine, raisins and table grapes. Only 13 cultivars are responsible for covering one third of the worldwide area under vines, with 33 varieties the coverage raises to 50% (Organisation Internationale de la Vigne et du Vin 2017). Even though less than 4% of the known cultivars are widely used for winemaking, winemakers still have a great multitude of varieties to choose from, making sure their cultivars fit with the conditions of the individual vineyards and climatic conditions (Clarke and Rand 2015; Boulton 1996). In the 1980s, winemakers in the US started declaring the grape variety on the label of their bottles. Since then, varietal labelling has gained popularity all over the world. It presents consumers with an easier way to understand what they are buying. At the same time, it gives winemakers the opportunity to explore the potential of the cultivars in their own way. With the exception of some traditional European wine growing areas, like Bordeaux and Burgundy, varietal labelling has brought the consumers' focus to the specific cultivar, reducing the importance of the origin to a certain degree. A knowledge of both factors allows for educated choices and an easier way of understanding what wine you are buying (Robinson et al. 2013). In addition to the varieties used for wine making, there are some that are intended for the production of table grapes and raisins. Requirements for these cultivars are different to those of grapes for winemaking, which is also observed in their genes. Table grapes generally show greater genetic differences from wild grapes than those used in wine making (Jackson 2014). Migicovsky et al. (2017) compared genomic and phenomic differences of table and wine grapes. They found that table grapes are generally larger in length, width and weight when compared to wine grapes. Additionally, wine grapes generally showed higher titratable acidity and higher sugar content. Table grape clusters were on average larger and less dense, as dense clusters are more prone to damage during packing or transport. The berries were also firmer than wine grapes. Of the 77.8 million tons of grapes produced in 2018, 57% were grapes for wine production, 36% table grapes and 7% were for the production of dried raisins (Organisation Internationale de la Vigne et du Vin 2017).

Ampelography is the science concerned with the identification and classification of grapevines
(HarperCollins Publishers)

Ampelography as science relies on the characterization of certain traits of the vine in different stages of growth during the year. In spring, one can best judge the color and shape of the shoots and young leaves. Beginning with the flowering, the mature leaves can be observed. The berries and bunches are inspected starting after veraison. Characteristics interesting to the ampelograph range from the amount and type of hair on the tendrils and leaves, over color and shape of the leaves, their texture and appearance to the characteristics of bunches and berries. These observations vary based on the environmental circumstances and the genetic makeup of the cultivar. It is very important to look at multiple different, healthy vines to minimize these effects. Ampelography is a complex science and will not always give the same results, depending on the region and the observer. A more objective way to differentiate between cultivars is DNA analysis. This can also be used to identify the lineage of a variety (Tassie 2010).

Some varieties of grapevine are not primarily grown for fruit production, but rather to provide a phylloxera resistant rootstock for other cultivars that have to be grafted onto resistant plants in order to be able to survive. Of the 1,902 varieties registered in the European Union, about 4% (77) are used primarily for this purpose (Lacombe et al. 2011).

In the following part, the taxonomy of vines and the significance and use of certain species are explained in more detail.

3.1 The *Vitis* Genus

As of 2013, *The Plant List* (2018), an online collection of scientific plant names, lists 466 names for species in the genus Vitis, out of these 79 are accepted names. These are divided into two subgenera: *Vitis* (formerly *Euvitis*) and *Muscadina*. The latter are separated from the first due to the fact that representatives of this sub-genera have 20 pairs of chromosomes, while *Vitis* species have 19 (Boulton 1996; Jackson 2014). They make up the larger sub-genera and include the main species used for wine making, *Vitis vinifera*. Other representatives of the *Vitis* sub-genera are *Vitis labrusca*, *Vitis riparia* or *Vitis berlandieri*, which are used, either directly or in crosses, for rootstocks onto which *Vitis vinifera* cultivars can be grafted to provide resistance to phylloxera and other pests and diseases (Clarke and Rand 2015). As all of the *Vitis* representatives share the same amount of chromosomes, interspecific hybridization, the crossing of two species in the same sub-genera, is possible. The resulting hybrids are usually fertile, making multiple hybridization possible. Crossing representatives of *Vitis* and *Muscadinia* sub-genera is more complicated and will generally result in infertile progeny due to the unequal number of chromosomes (Jackson 2014). Figure 17 shows an overview of the vine family and its taxonomy.

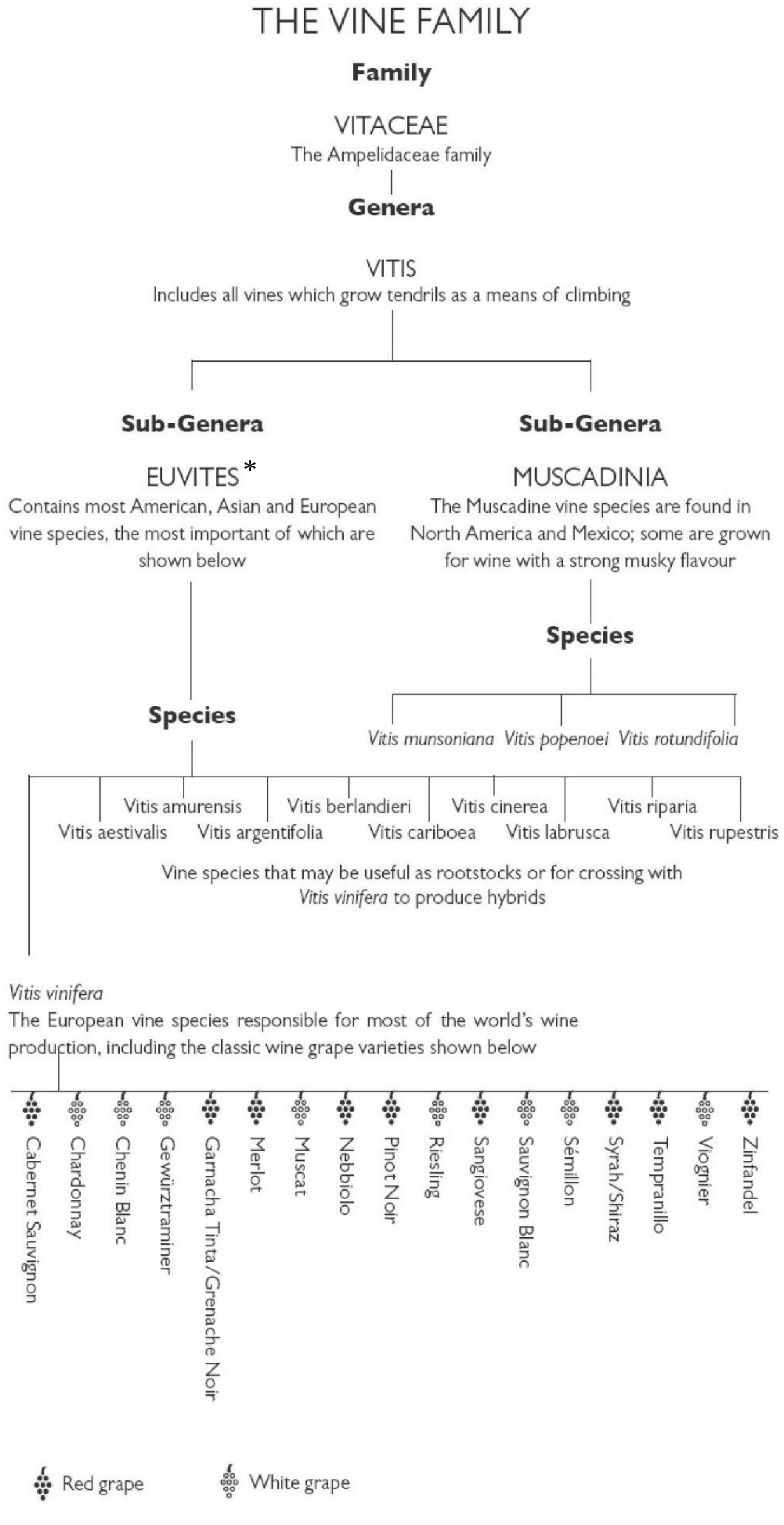


Figure 17: The vine family (Clarke and Rand 2015)

**The prefix Eu- is no longer used in the case that the name of the subgenera is the same as the one of the genera. The name Euvitis for the subgenera is therefore outdated and was replaced by Vitis (Jackson 2014).*

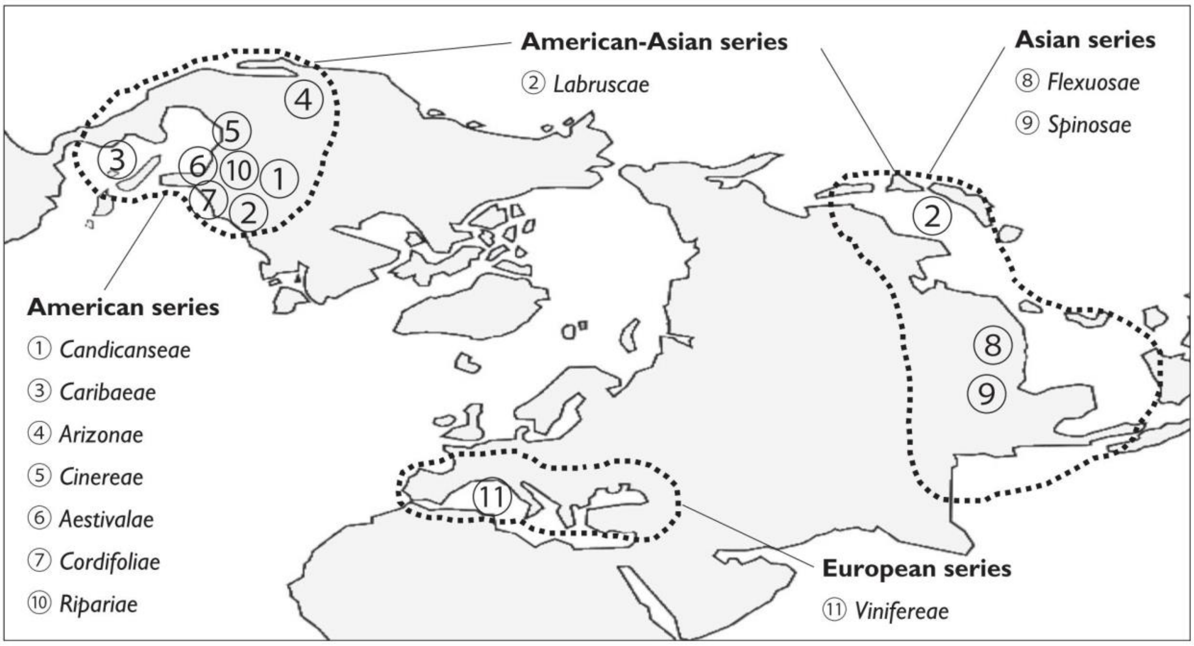


Figure 18: Distribution of Vitis worldwide (Péros et al. 2011, p. 472)

Assigning representatives of the *Vitis* sub-genera to groups can be difficult. This is mainly due to facts that cultivars that show the same ampelography can be genetically different from each other while others that share the same genetics can show different traits based on their growing conditions. (Jackson 2014). Galet (1988) sorted 59 species into 11 categories (Figure 18). This was mainly based on morphological traits but also on where they are grown and their biogeography (Péros et al. 2011). Another, broader, way of grouping is by hybrid origin. This can either be specific, as with most commercial *V. vinifera* cultivars, or interspecific. Among the interspecific hybrids there is a further distinction based on when the hybridization happened and between what species. French-American hybrids are the largest group. They are crosses between *V. vinifera* and one or more partners, like *V. rupestris*, *V. riparia* and *V. aestivalis*. These varieties go back to the time when phylloxera was spreading in Europe and winegrowers needed to find a way to deal with the aphid. The second group are early American cultivars that are either indigenous or crossed with *V. vinifera*. The most recent hybrids are interspecific cultivars where the crossing was closely monitored and the parentage is known. These are usually crosses between *V. vinifera* and *V. riparia*, *V. amurensis*, *V. armata* and *V. rotundifolia*. The last one is a representative of the sub-genera *Muscadinia*, making it more complex, not only due to the different chromosome count but also due to the fact that it is not possible for *V. Vinifera* pollen to penetrate the style of the muscadine cultivars (Jackson 2014).

In the European Union 1,825 varieties are used for grape production. Out of these, 87% are *V. vinifera*, 5% are interspecific hybrids. For the remaining 8% there is no record of the genetic origin (Lacombe et al. 2011).

3.1.1 Non *Vinifera* species

Some American varieties are not primarily used for the production of grapes and wine but as rootstocks. Rootstock varieties have to be chosen based on the soil and climate of the vineyard, as well as the compatibility between the scion (upper part of the graft (Kunkee and Großmann 2003)) and the rootstock. Figure 19 shows two different mechanical grafting methods, the scion is shown with a flower bud.

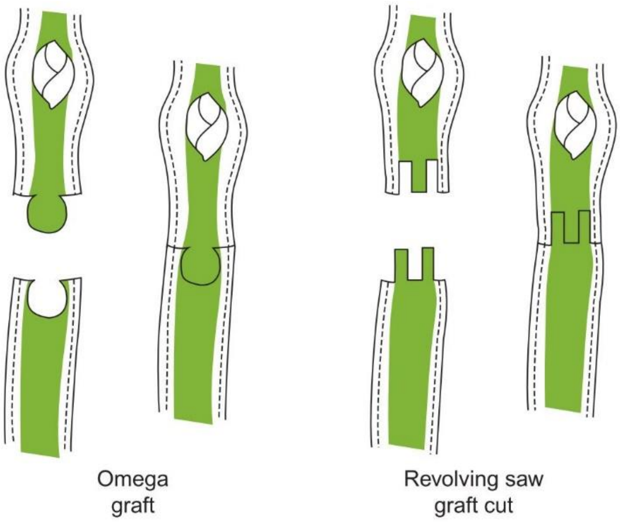


Figure 19: Different grafting techniques (Jackson 2014, p. 187)

In order to choose the most fitting rootstock many factors have to be taken into account. These factors include the adaptability to the growing circumstances, like humidity or dryness or the type of soil, as well as the tolerance towards salt and limestone. The decision has to be made with an awareness that, once the vineyard is planted, there is no possibility to change the rootstock until the replanting of the vineyard (Jackson 2014). Trials to find the most suitable cultivars started in the late 1800s, at the height of the phylloxera outbreak. At that time, most of the chosen rootstocks were either *V. riparia*, *V. rupestris* or a cross between the two, due to their tendency to root easily and the possibility to propagate them from dormant cuttings. Other cultivars used in the search for the best variety included *V. berlandieri*, now known as *V. cinerea* var. *helleri* (Heinitz et al. 2019). Rootstocks with *V. riparia* parentage are used if early fruit maturation is needed, for instance in regions with short growing periods. In addition, they might provide a certain resistance to high climatic variability and strong winters (Jackson 2014) and can be used to reduce the vigor in scions that have been planted in fertile and humid soil to improve fruit quality (Heinitz et al. 2019). One of the main disadvantages is the low drought resistance. *V. rupestris* rootstocks allow the adaption to gravel rich soils that are low in nutrients and provide a salt tolerance by excluding chloride from reaching the scion. When *V. riparia* and *V. rupestris* rootstocks were first used in Europe, the vines in some regions developed chlorosis (a lack of chlorophyll in plant tissue, resulting in lighter colored, yellow leaves (Jackson 2014)). To avoid this, *V. cinerea* var. *helleri* was introduced as a breeding partner in the search for rootstocks better suited for the limestone rich soils of some European wine regions. The variety originated from the Edwards Plateau in Texas where the soil is shallow and quite rich in limestone. In addition, this cultivar provides some drought resistance as it can be found growing in dry areas where other plants cannot survive (Heinitz et al. 2019).

In recent years, rootstock breeding has focused on including cultivars that provide a better resistance to drought, heat and nutrient deprived or salt-rich soils. Nematode resistance is of interest as well. Varieties like *V. vulpina* and *V. rotundifolia* can provide the latter while *V. dirdiana* and *V.* X *doaniana* showed potential for increasing salt tolerance and provided high growth rates, even at high temperatures. *V. moticola* might be able to provide high drought resistance as it is often found growing in areas without obvious access to water sources, though propagation is difficult and cuttings exhibit very slow growth (Jackson 2014; Heinitz et al. 2019). In order to choose the right rootstock winegrowers usually take seven factors in consideration: first the resistance to phylloxera, followed by the resistance to nematodes, the adaptability to alkaline soil, adaptability to salt rich soil, tolerance of soil with a lower pH, adaptability to poorly drained or wet soil and drought tolerance. The importance of the factors is adjusted to the vineyard specific requirements (Reynolds and Wardle 2001). Most of the rootstock varieties currently used in Europe are crosses between *V. riparia* and *V. berlandieri* (20%) (Lacombe et al. 2011). In Austria, the rootstock most commonly used is Kober 5BB, a *V. berlandieri* x *V. riparia* cross, followed by SO4, with the same linage (Höhere Bundeslehranstalt und Bundesamt für Wein- und Obstbau Klosterneuburg 2019). In Styria, the situation is similar; the two most common rootstocks are SO4 and Kober 5BB. Until 2007 5BB was the main choice for new vines, since then SO4 has become more popular, despite its susceptibility to dryness. One the explanation for this trend is that most Sauvignon vines use SO4 as rootstock. In addition, 1103P and Ferca are used in some cases (Renner 2018a).

Some American cultivars and their hybrids are used for the production of wine and table grapes. The most important ones are related to *V. labrusca*. Wines produced from these cultivars can usually be recognized by their characteristic “foxiness”. High concentrations of methyl anthranilate and 2-aminoacetophenone cause a sweet smell of wild strawberries that is considered a fault by many (Rapp and Versini 1996; Jackson 2014). *V. labrusca* is native to the northwestern United States where, in addition to wine, the grapes of related cultivars are used to produce juices and preserves. Vines with this parentage are usually resistant to both, powdery and downy mildew. This is one of the reasons why it was commonly used as a breeding partner in early hybrid varieties (Heinitz et al. 2019).

Some representatives of the second sub-genera, Muscadinia, are used to make wine and other products. Muscadine grapes have been used for these purposes in the southern United States for a long time as they are indigenous to the region (Heinitz et al. 2019). Figure 20 shows a bunch of *V. rotundifolia* grapes. They are larger than *V. vinifera* ones and have a thicker skin. Due to their number of chromosomes, *Muscadinia* grapes have 20 pairs compared to the 18 of *Vitis* cultivars, hybridization among the two sub-genera is difficult, as mentioned before. The flavor of muscadine grapes and their products is very distinct. It contains notes of roses and orange blossoms (Jackson 2014).

Figure 20: Muscadine grapes at the fruit breeding station of the University of Arkansas

American cultivars are interesting and important for the wine industry worldwide, not only as rootstocks for *V. vinifera*, but also in crossings where they can add resistance and tolerance to certain challenges winegrowers face. These challenges include climate change, cultivars in the future will need to be able to deal with longer, hotter summers, but also more intense weather phenomena, like storms, extended heavy rainfall and sudden cold spells. Pests and diseases pose additional challenges (Heinitz et al. 2019; Jackson 2014). Later in this chapter, the importance of some hybrid varieties will be discussed further.

The Caucasus region in central Asia is regarded as the cradle of wine (McGovern et al. 2017). It stands to reason that there is a diversity of native cultivars. Péros et al. (2011) note two Asian Vitis series: *V. flexuosa* and *V. spinosa*, as well as one American-Asian: *V. labrusca* (based on Galet (1988)). Compared to species of (North) American and European origin, there was however less, easily-accessible research available. In recent years, this has somewhat changed, as more research has become available. This is especially true for research into possible benefits these grapevines can provide, like resistance to fungal diseases and low temperatures (Marrano et al. 2015; Riaz et al. 2013; Venuti et al. 2013; Liu and Li 2013; Riaz et al. 2018). One of the best researched subspecies in this group is *V. amurensis*. Its origins are in China and it can be found there as well as in Russia and Korea (Liu and Li 2013). It is used for the production of wines that different to those produced from *V. vinifera* (Zhao et al. 2010, 2011). *V. amurensis* vines can withstand temperature of down to -50°C and are resistant to downy mildew, both of which make them interesting potential partners for hybridization (Liu and Li 2013; Schwander et al. 2012; Jackson 2014). Other species like *V. coignetiae* and *Vitis ficifolia* var. *ganebu* have been investigated as potential source of different nutrients (Jeong et al. 2007; Shiozaki and Murakami 2016).

3.1.2 Vinifera species

Most wines sold today are made from grapes of one of the many varieties of *Vitis vinifera*, the European grapevine. Figure 21 shows a drawing of *Vitis vinifera* from the 19th century that appeared in a book about medical plants.

Figure 21: Ampelographic drawing of Vitis vinifera (Brandt et al. 1883)

Cultivars are grown all over the world and even though there are more than 5,000 named, the most of the wines sold are produced from a few well-known varieties (Boulton 1996; Lacombe 2012). This large number has several reasons; one of the main ones is the fact that wine is among the crops that have been cultivated by humans for the longest time, more than 6,000 years in this case. Winegrowers would select useful or beneficial traits in vines and cross them with others. Some varieties probably also resulted from natural intraspecific crosses or mutations (Jackson 2014). In order to keep the same characteristics among one cultivar, vines are propagated by cuttings and are effectively clones of the same vine. In some cases, somatic mutations can occur during this procedure resulting in slight differences among clones (Boulton 1996). Should they be beneficial, this clone might be used as the basis for future cuttings. One cultivar can therefore have many different clones. Grapes produced by different clones of the same cultivar might be slightly different in their characteristics, like ripening time, final sugar and acid concentration or in their appearance. Some clones are better suited for certain situation than others are. A careful selection of the right clone can sometimes make a difference in the final wine (Renner 2017).

The varieties cultivated for wine production with the largest vineyard area worldwide are Cabernet Sauvignon, Merlot, and Tempranillo, as already mentioned in chapter 1. All of these produce black grapes. The white variety with the largest vineyard area is Airen, followed by Chardonnay (210,000 ha) (Organisation Internationale de la Vigne et du Vin 2017).

In Austria, 67% of the vineyard area is planted with white cultivars, mainly Grüner Veltliner and Welschriesling. The main red variety is Blauer Zweigelt. In Styria 78% of the grown varieties produce white grapes, with the main varieties being Welschriesling, Sauvignon Blanc and Weißburgunder. The main red variety in Styria is the autochthonous Blauer Wildbacher.

Of the varieties grown in Styria Sauvignon Blanc is of particular interest. Wines produced from this variety have been among the best worldwide, wining prices and medals for their characteristic taste and aroma. At the Concours Mondial du Sauvignon, an international contest of Sauvignon Blanc wines, Austrian wines won 45 medals in 2019 and 78 in 2018, making it the country with the second most after France (Phillips 2019). In recent years, more and more people from all over the world discovered Styrian Sauvignon, frequently awarding them top scores in rankings (Kavanagh 2019; Berry 2019; Schiessl 2017; Zecevic 2019). The variety was introduced to Styria by Archduke Johann in the 19th century under the name Muskat Sylvaner and has been grown ever since (Österreich Wein Marketing GmbH 2018a). The parentage is uncertain, but the variety most likely resulted from a cross of Chenin blanc and Traminer (Höhere Bundeslehranstalt und Bundesamt für Wein- und Obstbau Klosterneuburg 2019). Wines produced from this variety can exhibit different characteristics depending on their origin (Green et al. 2011). Styria is part of the cool climate region for Sauvignon Blanc. Wines from these regions usually have green and vegetal aromas that are mainly related to the presence of methoxypyrazines (Marais 1994; Allen et al. 1991; Lacey et al. 1991; Jackson 2014). These are primary aroma compounds that are already present in the grapes and remain unchanged during fermentation (Waterhouse et al. 2016). Other important aroma compounds in Sauvignon Blanc wines are thiols (Tominaga et al. 1998). These sulfur containing compounds are present as non-volatile precursors and are released during fermentation (Waterhouse et al. 2016).

Another variety that was investigated as part of this thesis is Riesling. From its origins in Germany, it spread to France and Austria (before 1700) and was brought to the Americas by European settlers. These days, it is grown on six continents, covering a total vineyard area larger than 50,000 ha (Anderson and Aryal 2013; Sechrist 2012). Riesling is still the most widely planted variety in Germany, covering around 23% of the vineyard area (Organisation Internationale de la Vigne et du Vin 2017).

3.1.3 Hybrids

Hybrids are interspecific crossing of different species of the Vitis genus as briefly discussed in 3.1 and 3.1.1. The aim of this process is the introduction of resistance to one or more diseases by crossing a *V. vinifera* cultivar with another *Viti*s or *Muscadinia* variety that possesses the desired trait. Crossings are usually carried out without the use of genetic engineering, but by using the pollen of one breeding partner to pollinate the other (Basler and Scherz 2011). In addition to this resistance, newer breeding efforts also try to include tolerance to certain climatic conditions, like drought (Heinitz et al. 2019). A study in 2000, found that about 70% of all fungicides in Europe were used for the production of grapes (EUROSTAT 2000). With the use of cultivars resistant to one or more fungal diseases the amount of treatments needed can be reduced, depending on the climatic conditions, to two or three (four in some cases) from six to nine treatments with fungicides with conventional cultivars. This carries the additional advantage of less work in the vineyard, which can be dangerous, particularly in areas with steep slopes. The use of less plant protection agents can be interesting for producers of ecological wine, as consumers associate organic wine with no or little treatment (Basler and Scherz 2011). This part will focus of the history and the use of different hybrids, as well as the current situation in Austria and Styria, in particular.

History

The first purposeful performed hybridizations were performed in the beginning of the 19th century in the United States of America. Settlers had been unable to grow *V. vinifera* cultivars due to different pests, like phylloxera, fungal diseases and hard climatic conditions. In order to be able to produce wines with pleasant aromas, researchers at Harvard proposed crossing resistant American varieties with European ones in 1822. The resulting first generation of cultivars are known as American hybrids (Eibach and Töpfer 2015).

When phylloxera first arrived in Europe at the end of the 19th century, winemakers were keen on finding a fast and permanent solution for the problem. One approach was the crossing of American cultivars with European ones to combine the resistance of the first with the flavor of the latter. While a full phylloxera resistance could not be achieved, the newly bred cultivars showed resistance to some of the other diseases that reached Europe around the same time, mainly downy and powdery mildew. This was the birth of the French-American hybrids. They had names like Clinton, Noah, Othello, Herbemont, Chambourcin, Concord or Isabella (Basler and Scherz 2011; Heinitz et al. 2019). The new cultivars had *Vitis vinifera* as well as other members of the *Vitis* sub-genera in their pedigree. *Vitis aestivalis* or *Vitis labrusca* were some of the most common crossing partners for the first generation hybrids. During the first half of the 19th century, there had already been cultivations of crossings of American cultivars, some natural other intentional. One of the most famous examples is the variety Concord, which is still used to produce more than 360,000 tons of grape juice and preserves in the United States today (Heinitz et al. 2019). The main problem with early hybrids that had and have *V. labrusca* in their pedigree is their tendency to exhibit “foxy” flavors. As mentioned before this is considered a fault by many wine drinkers. To avoid this, *V. labrusca* became less popular as breeding partner. Instead, crosses would include *V. rupestris*, *V. riparia*, or, mentioned before, *V. aestivalis* (Jackson 2014). In France, producers started to cultivate hybrid varieties starting at the end of the 19th century. In the following years, the cultivation area for hybrids increased, fueled by enormous losses due to multiple years of downy mildew outbreaks. Other factors that helped the spread of hybrids in France were the lack of workers after the First World War, as hybrids needed less attention in the vineyard. In addition, breeders produced new cultivars and promoted them in special publications dedicated to the cultivation of hybrids like “La Vigne américaine” “La Viticulture modern” and others. During the time of the Second World War, a shortage of plant protection agents fueled the further spread of hybrids. Most of the wines produced from hybrid cultivars were used to produce wines that were consumed locally or sold to wine merchants at very low prices. In 1953, the situation for growers of hybrid cultivars changed. Varieties were put in three different categories, recommended, allowed and tolerated. Most hybrids were in the last one except for about 20 cultivars including Chambourcin. Despite this, the largest vineyard area under hybrids was recorded in 1958, at around 400,000 ha, about 31% of the total vineyard area in France at the time. Ten years later the area had been reduced to less than 300,000 ha and in 1979, all of the tolerated cultivars had to be cleared. From that point on, the vineyards grew smaller. Today, not much remains of the first hybrid cultivars in France, except for some small areas, where the wines produced from hybrids are a local specialty (Basler and Scherz 2011).

The situation in Austria was similar. Starting in 1929, wine from direct producer vines had to be declared on the label. With the wine law reform in 1936 the vineyard area for hybrids had to be reduced to a maximum of 25% over the following 10 years and no new cultivars were allowed to be planted. One year later, a law was introduced prohibiting the mixing of wine produced from hybrids and that from *V. vinifera* grapes. Due to the war, some of the uprooting did not take place and the laws from 1936 were not valid anymore. A new wine law came into effect in 1967 outlawing all distribution of wines produced from direct producer hybrids. This affected winemakers from southern Burgenland in particular who had been selling “Uhudler”, a wine produced only from early American hybrid varieties like Isabella, Noah, Concord and others. Wines like these could only be produced for “personal consumption” (Haustrunk). From 1971 on, only 400 L per person working in the wine industry were allowed, all the remaining wine had to be made into vinegar, distillate or used of technical purposes. With the Austrian wine scandal in 1985 the term Haustrunk was removed from wine law and all wines produced from American vines were declared “non-wines”, which put them on the same level as spoiled wine (Postmann 2010). While the law prohibited the production of wine with hybrid gapes, the breeding program for new resistant cultivars in Klosterneuburg continued in 1959 after a 15 year break (Kaserer et al. 1996). When the public interest in untreated table grapes started to rise in the 1980s, more breeding programs in Austria started to focus on introducing resistances by crossing European and American varieties. At the Versuchsstation Obst- und Weinbau Haidegg of the Styrian government, breeding efforts first focused on table grapes, but later widened to include grapes for winemaking (Renner 3/13/2020). On the 28th of September 1995, the EEC (European Economic Community) added three new varieties to the list of authorized vine varieties as an addendum to Commission Regulation No 3800/81 (European Economic Community 12/16/1981). The varieties Roesler, Rathay and Seifert are all fungus resistant crossings that created by Dr. Gertrude Mayer of the breeding program in Klosterneuburg. All three new varieties were based on the French-American Hybrid Seyve Villard 18-402 and established Austrian varieties, including Blauburger, Blaufränkisch and Zweigelt (Kaserer et al. 1996). In 2000, Rathay and Roesler were elevated to Qualitätsweinsorten (varieties that can claim a geographic designation on the bottle (explained in 1.1)) (Österreich Wein Marketing GmbH 2018b).

Breeding efforts also took place in Germany, mainly Freiburg, Geisenheim and Siebeldingen, but also in Bordeaux and Montpellier in France and multiple places in the United States of America. Starting in the 1980s, private breeders experimented with the creation of resistant cultivars in Switzerland and since 1996 the national research institute Agroscope in Changins does the same (Basler and Scherz 2011; House of Switzerland 2019). The association PIWI international was founded in December two years later in Einsiedeln in Switzerland. Their goals are the further dissemination of fungus resistant grape varieties (PIWI) as well as the facilitation of exchange and collection of information about the new varieties (PIWI International; Basler and Scherz 2011). A few years later, in 2005, the Austrian chapter was founded to promote the use of PIWIs in Austria (Renner 3/13/2020). PIWIs have a lower need for plant protection agents (PPAs), especially fungicides, and are, therefore, of interest for wine growers that want to reduce their use of PPAs. 13% of the Austrian vineyards are under ecological management and the interest in PIWIs is growing, especially in Styria where some fungal diseases caused by rain are abundant in some years. In 2018, four new varieties were elevated to Qualitätsweinsorten, among them three new PIWI varieties: Blütenmuskateller, Muscaris and Souvignier Gris (Österreich Wein Marketing GmbH 2018b; BGBl. II Nr. 184/2018 7/24/2018). In addition, eight other PIWI cultivars can be used to produce wine without a designation of origin: Bronner, Cabernet blanc, Donauriesling, Donauveltliner and Johanniter (white varieties) and Cabenet Jura, Pinot Nova and Regent (red varieties) (Renner 2020).

Current situation

Due to the breeding efforts in Germany, there are a lot of registered new cultivars that can be used for wine production. These include: Cabernet Blanc, Bronner, Helios, Hibernal, Johanniter, Merzling, Muscaris, Orion, Phoenix, Prinzipal, Saphira, Sirius, Solaris, Souvignier Gris, Staufer, Villaris (white cultivars); Accent, Allegro, Baron, Bolero, Cabernet Carbon, Cabernet Carol, Cabernet Cortis, Calandro, Monarch, Pinotin, Piroso, Prior, Reberger, Regent and Rondo (red cultivars). The most important among the listed varieties is Regent. With a total area of 1,784 ha it covers about 1.7% of the German vineyard area. Solaris and Johanniter are the main white PIWI cultivars in Germany, both with more than 100 ha (Bundessortenamt 2015; Deutsches Weininstitut GmbH 2019).

In South Tyrol, which is part of Italy, the interest in PIWIs is large, especially compared to the rest of the country. In Switzerland, as mentioned before, resistant cultivars represent an important part of the vine landscape, with about 10% in some cantons (Basel-Landschaft, Basel-Stadt, Solothurn and Thurgau) and about 1.5% of the entire vineyard area of Switzerland in 2016. Most of the PIWI vineyards are located in the German- and Italian-speaking part with about 5.5% of the vineyard area, compared to 0.3% in western Switzerland, where the main language is French. The most important cultivar is Regent, as in Germany, with about 1.1% of the whole and about 74% of the PIWI vineyard area. Other important varieties are Cabernet Jura (30 ha), Johanniter (24 ha) and Divico and Solaris (both 22 ha). The winegrowers listed ecological concerns as the main reason for planting PIWIs (Holzwarth and Häseli 2018).

In the United States, hybrid cultivars are relatively wide spread due to their intrinsic properties, such as their cold hardiness. This is especially true for the states of New York, Pennsylvania and Ohio, where hybrid cultivars cover a significant part of the vineyard area. Most of the cultivars have *V. labrusca* in their pedigree. While hybrid grapes are used for wine production, they are mostly used for the production of juice and confections like jams and jellies (Sabbatini and Howell 2014).

As of the writing of this thesis, there are no exact areas available for the extent of the PIWI areas in Austria, but there are estimations that PIWIs could make up about 1% of the total vineyard area (Österreich Wein Marketing GmbH 2018b; Renner 2020). Only for the varieties Roesler and Rathay, which have been used for the production of Qualitätswein since 2000, areas are available. In 2017, they covered 273 ha (0.6%) of the Austrian vineyard area with Roesler being the more abundant variety (238 ha) (Österreich Wein Marketing GmbH 2019).

Styria takes a special place with regard to the amount of PIWI vineyards. Winemakers have been working with fungus resistant cultivars for a long time due to the high amount of precipitation (Österreich Wein Marketing GmbH 2018b). Over the last years an upwards trend regarding the amount of newly grafted PIWIs can be seen. In 2019, about every tenth vine grafted was a fungus resistant cultivar with more than 160,000 new plants (Renner 2020). Figure 22 shows the distribution of the grafted PIWI vines from 2017. The main varieties are Muscaris and Souvignier gris, two of the varieties from which Qualitätswein can be produced (Renner 2018b). In Styria, the Landesversuchsanstalt für Obst und Weinbau grows different

PIWI varieties and produces wine from them. As part of this thesis, these wines were analyzed in order to obtain a more complete profile and gain more information about the potential of these varieties. In addition, the data was compared to sensory data obtained at the sensory testing laboratory in Haidegg, Graz.

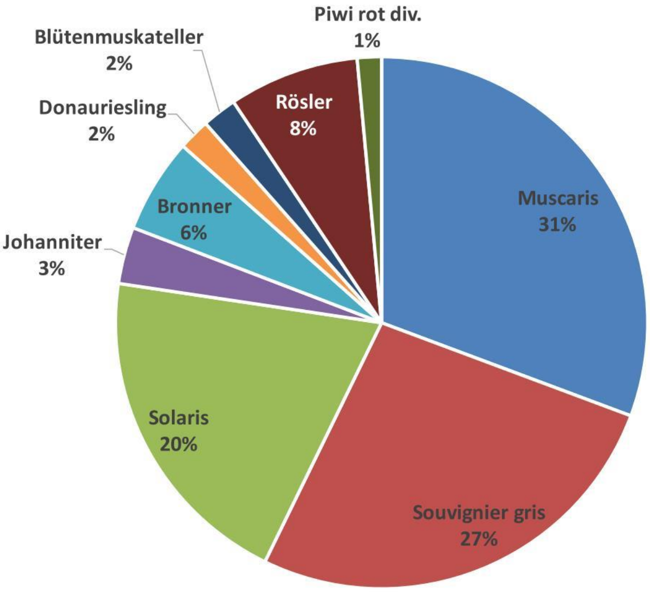


Figure 22: Distribution of PIWI vines in 2017 by variety (Renner 2018b, p. 4)

A majority, especially of casual, of wine consumers can be hesitant when it comes to buying and consuming wines produced from new, relatively unknown varieties (Jackson 2014). When purchasing PIWI wines directly from the producer, consumers usually require extensive explanation of the wine. And while consumers are willing to try new things, they are likely to fall back into their previous habits of buying wines produced from traditional varieties. Wines from resistant cultivars will not replace wines from conventional varieties, but extend the spectrum of possibilities for the consumers (Renner 2020).

How to create a hybrid

Most wild representatives of the *Vitis* genus are dioecious, they produce either female or male flower parts. *V. vinifera* cultivars are monoecious, or hermaphroditic, meaning that they contain both male and female flower parts. This results in self-pollination, which in a regular setting is advantageous as the system does not have to rely on outside factors for a successful pollination. This has to be avoided when crossing different species or varieties. The hermaphroditic flowers have to be emasculated in order to be able to use them as female flowers. This is a process that requires high accuracy as all male parts have to be removed by hand at the right time. This is usually done using tweezers. The emasculated flower is then pollinated using the pollen of the selected crossing partner. To protect the crossing experiment, the pollinated flower has to be protected from further cross-pollination. This can be done by placing a paper bag over the flower as shown in Figure 23 (Eibach and Töpfer 2015).

Figure 23: Pollinated flowers covered with a paper bag to exclude the possibility of cross-pollination with other pollen

Mayer (1988) describes a five step breeding program. The first step is the crossing of French-American hybrids with European cultivars. In the second step, the resulting offspring are raised and from them future breeding elites are selected. These are crossed with European cultivars in the third step. In the fourth step, the progeny of these crosses are raised and tested for their fruit and general properties. In the fifth and final step, the best cultivars are chosen and grafted onto phylloxera resistant rootstock and tested for their performance in the vineyard and wine quality of the produced grapes. As this process is quite lengthy, it can take years until a hybrid is ready for the last stage. In the case of Donauriesling, for example, the first vines were planted in 1978 in a place that was not easy to manage. In 1994, it was selected and evaluated annually. In the following years, it was planted by winegrowers in different parts of Austria to evaluate its properties. While it is allowed to sell the wine in bottles labeled with the name of the variety, it is yet to be accepted for the production of Qualitätswein (Regner 2015).

Using multiple, at least two, backcrossings with *V. vinifera* cultivars ensured the production of high quality hybrids (Kaserer et al. 1996). One example of the pedigree of a PIWI variety is shown in Figure 24. In order to be accepted as a Qualitätswein-variety certain standards have to be met. This includes the absence of “hybrid aroma” caused by methyl anthranilate and 2-aminoacetophenone and of malvidin 3,5-diglucoside in red wines (Regner 2015).

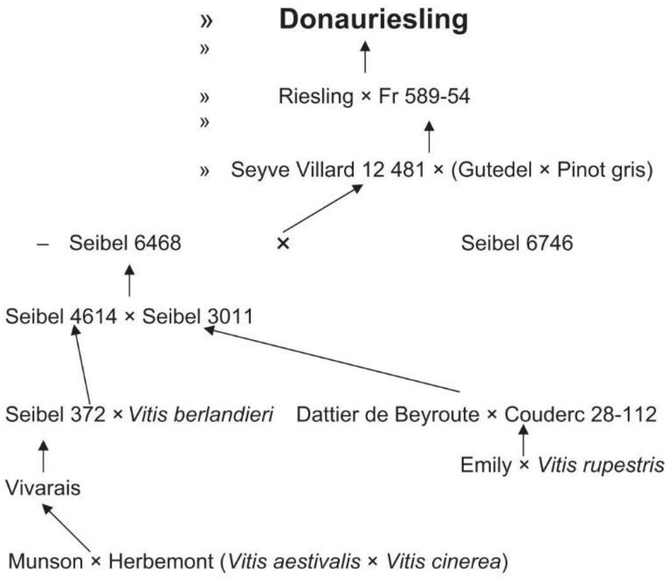


Figure 24: Pedigree of the PIWI variety Donauriesling (Regner 2015)

4. Wine making

Winemaking, also called vinification, has come a long way since wine was first discovered in 6,000 BC. Better understanding of the fermentation process and possibilities to control it, made vinification more secure and less prone to faulty results. Advances in analytical techniques for monitoring of intermediate steps during the production and of the final product made adulteration easier to detect as well as increased the reliability of the entire process. The advancement in innovations, both in winemaking techniques and analytics, has happened at a more and more increasing rate due to the efforts of winemakers and scientists alike (Jackson 2014).

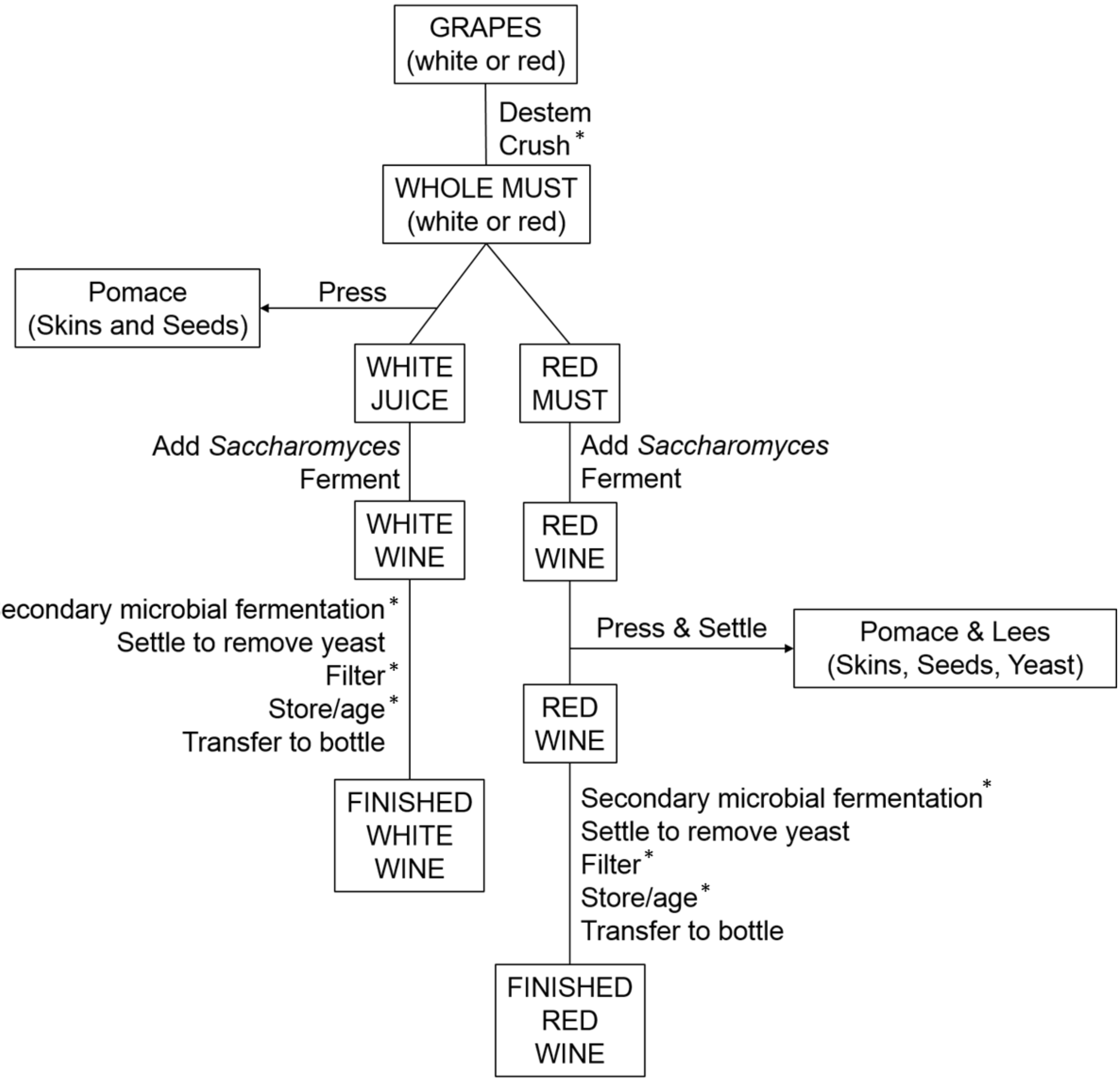


*Figure 25: Process steps for the production of red and white wine, steps marked with * are optional (Polásková et al. 2008)*

When producing wine, every step of the process can influence the final product. Figure 25 shows a flow diagram of the different stages of making red and white wine. While for some vinification formally only starts once the grapes or the grape juice reach the winery (Jackson 2014), other people argue that the wine is made in the vineyard and, therefore, consider grape maturity and harvesting as part of the process (Ribéreau-Gayon et al. 2005). Others say that the main influence on the final product stems from the vinification techniques, the handling in the cellar and the choices of the winemaker. "A wine is made in the vineyard" is a common saying among winemakers (Reynolds 2010). This sentiment implies that one needs good quality material to archive a good quality product. Therefore, *good* grapes are needed to produce *good* wine and while it is possible to produce *bad* wine from *good* grapes, it is almost impossible to produce *good* wine from *bad* grapes. It is important to note that good and bad are largely subjective in this context. Good vineyard practice, choosing the right variety and a good clone for the specific circumstances are important to produce grapes that exhibit the characteristics desired in a raw material for the production of wine. This includes the right levels of sugar content and acidity as well as other qualities including the color of the grapes and their aroma (Young and Vivier 2010; Polásková et al. 2008).

The previous chapter provided an insight into the basic component for each wine, the grapes. This one will focus on how the steps in wine production can influence the final product and will discuss some of the tools winemakers can use to create wines that can capture their unique style of vinification.

4.1 Harvest, destemming and pressing

The timing of the harvest is one of the most important decisions of the entire winemaking process (Grainger and Tattersall 2016). Grapes should be ripe but not overripe at the time of picking. For the determination of the ripeness of the grapes, several factors are taken into account. Zoecklein et al. (2010) list factors that are taken into account, including the grape's aroma and its intensity, the tannins of the skin, the number of seeds per berry, sugar content (in °Brix), the acidity as well as the appearance of the grape and the bunch based on several factors. Grapes should be harvested before desirable fruit characteristics are lost. Therefore, testing for maturity markers like pH and °Brix happens regularly towards the time of harvest. Testing the sugar concentration can be done directly in the vineyard by using a refractometer. Once the decision to harvest has been made there are two main possibilities: a manual or mechanical harvest (Grainger and Tattersall 2016).

Before the beginning of the 1970s, almost all harvesting was done manually as mechanical harvesters were still relatively rare. Manual harvesting provides several advantages for the production of high quality wine. One of the reasons for this is the possibility to reject grape bunches that are not ripe or show other defects like diseased grapes (Jackson 2014). Unlike mechanical harvest, this method can be used in any terrain and for any vine training system. In manual harvesting it is important to ensure the integrity of the grapes as broken grapes can lead to problems later on in the wine making process. In this process, the whole bunch is cut off the vine and placed into a container. All of this is very labor and time demanding work and, therefore, relatively cost intensive (Grainger and Tattersall 2016). Some quality classification systems, including the grapes intended for the production of Champagne and the DAC wines in Styria, require manual harvesting (Österreich Wein Marketing GmbH 2018a; Fink 2017). In some production methods, for example harvesting noble-rot, also known as "botrytis bunch rot“, or late-harvest grapes, require manual harvesting, as well as producers that want to reduce the amount of sulfur needed for stabilization require intact grapes (Fink 2017).

The main disadvantage of manual harvesting listed in multiple publications are the labor costs and the required time to harvest all grapes (Dalton 2017; Fink 2017; Boulton 1996; Grainger and Tattersall 2016; Jackson 2014).

Mechanical harvesters are very efficient in terms of time needed for harvesting and costs, if the initial costs are disregarded. This initial cost includes the machine as well as the costs for preparing the vineyard (Grainger and Tattersall 2016). When only looking at the costs of the harvest itself, mechanical harvesting can reduce harvest costs by up to two-thirds and the time needed per hectare from 160-300 hours to 0.6-1.2 (Jackson 2014). The faster harvest can be an advantage in some cases, for instance if bad weather is forecast or in hotter climates where night harvests are needed to make sure the grapes reach the winery in the best possible condition (Grainger and Tattersall 2016). Mechanical harvesters use similar techniques to harvest the grapes. In most cases, a force is applied to the vines and the grapes are separated from the rest of the plant. This means that most of the stems remain on the vines and are, therefore, not included in the harvest. There are two main categories of mechanical harvesters: the ones that apply force to the trunk of the vine (shakers) and those that apply force to the fruit bearing shoots (strikers). The choice of harvester depends on the age of the vines and the training system. Both systems have drawbacks; a combination of both systems is also possible to reduce the damage done to the vines and the efficiency of the harvest (Jackson 2014). Since their introduction in the 1960s, the technology has improved significantly. As a result, many of the original limitations, like broken grapes and a high amount of material other than grapes (MOG, including stones, insects and plant material), have been reduced (Fink 2017; Grainger and

Tattersall 2016). Some grape varieties with thin-skinned berries that are particularly prone to breaking might not be suited for mechanical harvesting, unless a pressing step is included in the process in case skin contact is not desired. One remaining limitation of most mechanical harvesters is their inability to operate on steep slopes, leaving them unsuited for some wine growing regions (Jackson 2014).

Both, mechanical and manual harvesting, have advantages and drawbacks. Some winemakers might choose a combination of both systems, for example cutting off damaged bunches before using a mechanical harvester. A sorting of the harvested grapes can additionally reduce the amount of MOGs and damaged grape material. Parenti et al. (2015) compared different harvesting and sorting methods regarding the quality of the resulting must during two consecutive years. As parameters they used MOG as well as the amount of sub-standard berries (SSB) between two different harvesting methods (manual and mechanical) and three different sorting techniques (no sorting, manual sorting and densimetric sorting). The results depended greatly on the vintage and the overall ripeness of the grapes. While manual harvest was beneficiary for riper grapes, there was no difference in the second year when the grapes exhibited less overall ripeness. The results for the sorting technique gave more conclusive results with densimetric sorting reducing the amount of MOG and SSB most efficiently for both vintages, while manual sorting did not provide a significant advantage over no sorting at all. The choice of harvest technology can also influence the concentration of certain metals in the final product, the zinc concentration for example can be increased due to damaged wires caused by the mechanical harvester (Ribéreau-Gayon et al. 2006).

Overall the main goal is to maintain the highest possible grape quality for further processing. The way to archive this will depend on the preferences and possibilities of the winemakers as well as the final vinification style (Christmann and Freund 2010; Dalton 2017). To examine the influence of different harvest parameters (time of day, type of harvest) several experiments were conducted in cooperation with Haidegg and Silberberg. The results of these experiments will be discussed in the experimental part of this thesis.

After the grapes reach the winery the further processing depends on the final wine style. While grapes intended for the production of white wine are usually destemmed and pressed, this might not be the true for red wines (see Figure 26). Destemming is the process of separating berries and stems and other plant material from each other. The stems, or stalks, are discarded while the grapes are further processed to produce wine. This step can be combined with the crushing of the grapes. In some winemaking practices, like when using whole bunch pressing for white wines or for carbonic maceration for black grapes, the destemming step is omitted (Robinson and Harding 2015). In some winemaking regions in France, namely Burgundy and parts of the Rhône, a portion of the stems are retained to achieve higher tannin concentrations and more structure in the finished wine. Pinot noir is the grape variety most closely associated with whole bunch fermentation as this variety lacks a certain type of anthocyanins and the addition of the stem material can compensate for this shortcoming (Goode 2012). After destemming the grapes are crushed. During this process the berries are broken to release the juice but not damage the seeds as they contain phenolic compounds that can add bitterness and astringency to the final wine. Most modern processing equipment consecutively performs the destemming and crushing steps to keep the amount of extracted compounds as low as possible (Bakker and Clarke 2012; Jackson 2014). When using mechanical harvesting, crushing can take place directly in the vineyard as most of the stems are already removed. Some mechanical harvesters offer the possibility to crush the grapes directly. Field crushing offers the possibility to cool the must on the way to the winery and can be beneficial for long transports. There are some production methods that do not require crushing. These include wine styles with carbonic maceration, the production of late harvest or botrytised wine and the production of some sparkling wines from black grapes, these grapes are pressed directly to avoid coloring of the juice (Boulton 1996; Jackson 2014).

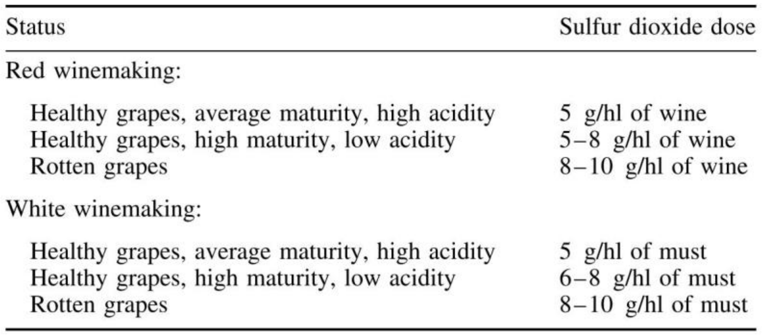
For white wine, especially when it is made from black grapes, the skin contact time (maceration) is kept relatively short. For some white cultivars, a certain skin contact time is beneficial for the final aroma of the wine as some varietal aroma compounds are located in the skin. Maceration times depend on the winemaker and can last a few hours or a few days. Winemakers have to choose a duration where the balance between beneficial aromas and skin and seed derived phenolics are in balance. The extracted amount of phenolics does not only depend on the duration, but also on the circumstances during maceration especially the temperature. For the production of red wines, fermentation usually takes place without removing the skins and seeds. Some exceptions are the production of rosé wines and early maturing red wines. These are pressed after maceration times of up to 24 hours and 3-5 days respectively. For other red wines, particularly ones intended for long aging periods, maceration can last several weeks (Jackson 2014). For the production of orange wine, white grapes are treated like red grapes. After destemming and crushing, the grapes are fermented with skins and seeds, in some cases without the addition of yeast, using only the indigenous yeasts occurring on the grape skins. This results in amber colored, tannin rich white wines (Robinson and Harding 2015).

While destemming and crushing happens before fermentation in most cases, the timing of pressing depends on the style of wine. The earliest pressing techniques for wine production were developed at least 5,000 years ago with some illustrations of the process being depicted in ancient Egyptian tombs. Since then, the equipment and its efficiency have been improved greatly. From the beam press in 1600 BC to modern pneumatic, membrane or continuous presses the main purpose has remained the same: separating juice and pomace (skins and seeds). Modern machines are gentler, this avoids crushing the seeds and releasing additional solids into the juice. With continuous presses the throughput can be increased as time-consuming steps like filling and emptying can be avoided (Jackson 2014).

4.2 Sulfurization

Using sulfur dioxide (SO_2) to preserve food has a long tradition and was most likely first done by either the Romans or the Egyptians. It took until the 18th century before it was widely used in wine making (Jacobson 2006). Today, SO_2 is an essential part of the tools available to winemakers and has many uses. However, its main purpose is to act as a disinfectant and antioxidant (Grainger and Tattersall 2016).

In industrial settings, SO_2 is added as gas or liquefied gas to large volumes of must or juice. For smaller amounts, an addition of liquefied SO_2 gas diluted in juice or must can be used for better distribution. One of the disadvantages with these methods is the strong SO_2 smell. Other, less odorous options are sodium or potassium metabisulfite ($Na_2S_2O_5$ and $K_2S_2O_5$), with the potassium salt being the more common option. Sulfuring of barrels and other wooden storage containers is done by burning a certain amount of sulfur inside of the receptacle. This is probably the oldest form of adding SO_2 in winemaking and has a double sterilization effect, once for the wine and additionally for the surface of the container (Dalton 2017; Ribéreau-Gayon et al. 2005). SO_2 can be added during many different stages of the wine making process. The needed quantity depends considerably on the health and condition of the grapes. Healthy, good quality grapes need less SO_2, while grapes that are of a lower quality require more. They might break open and release juice during the harvest or transport to the winery. In this case wild yeasts that naturally occur on the grapes can cause an early onset of fermentation. With the addition of an appropriate amount of SO_2, this can be delayed or avoided completely, preventing off-flavors in the final wine (Grainger and Tattersall 2016). Figure 26 shows common concentrations of SO_2 depending on the health and type of grapes. The addition of SO_2 impacts *Saccharomyces cerevisiae* to a lesser degree allowing for alcoholic fermentation to take place (Dalton 2017). Other factors that have to be taken into account are pH, temperature and the general contamination risk (Ribéreau-Gayon et al. 2005). During fermentation, SO_2 inhibits enzyme activity. A concentration of 50 mg/L is sufficient to reduce the activity by more than 90% (Boulton et al. 1996d).



Status	Sulfur dioxide dose
Red winemaking:	
Healthy grapes, average maturity, high acidity	5 g/hl of wine
Healthy grapes, high maturity, low acidity	5–8 g/hl of wine
Rotten grapes	8–10 g/hl of wine
White winemaking:	
Healthy grapes, average maturity, high acidity	5 g/hl of must
Healthy grapes, high maturity, low acidity	6–8 g/hl of must
Rotten grapes	8–10 g/hl of must

Figure 26: Concentration of SO_2 used in winemaking in temperate climate zones (Ribéreau-Gayon et al. 2005)

While the addition of SO_2 during the first stages of the winemaking process reduced the chances of malfermentation and lowers the enzymatic activity, in the later stages, for example during racking, SO_2 functions as a stabilizing agent. This makes it possible to store and age wines for longer periods. Its presence prevents a second fermentation due to its anti-microbial effects. As an antioxidant, it binds free oxygen, therefore preventing browning and other oxidative reactions. At suitable storage conditions, concentrations of free SO_2 concentrations should exceed 5-10 mg/L for red wine and 20 mg/L for white wine (30 mg/L for white wine produced from low quality grapes) (Boulton et al. 1996d; Ribéreau-Gayon et al. 2005).

SO_2 is also produced by yeast during the fermentation process. The amounts produced rarely exceed 10 mg/L but can be up to 30 mg/L in some cases. This has to be taken into account before the addition of SO_2 as the concentration of free SO_2 is regulated by many countries (Ribéreau-Gayon et al. 2005). In the European Union the maximum amount depends on the wine style. For red wines the concentrations are lower than the ones for white and rosé wines. The limits for the total content of sulfur dioxide are 150 mg/L and 200 mg/L, respectively. Additional 50 mg/L can be added in some wine regions whenever necessary due to weather conditions up to a maximum concentration of 300 mg/L. For sweet wines with residual sugar concentrations higher than 5 g/L, the permitted concentrations are higher (200 and 250 mg/L). Other sweet wines can have concentrations of up to 400 mg/L, including Beerenauslese, Trockenbeerenauslese and Eiswein. For sparkling wines, the limits are 185 mg/L for quality sparkling wine and 235 mg/L for all other sparkling wines (European Commission 2018). In the International Code of Oenological Practices, published by the OIV, the limits are 150 mg/L for red and 200 mg/L white and rosé wines until a limit of 4 g/L of reducing substances, 300 mg/L for wines with higher concentrations of reducing substances and up to 400 mg/L for certain sweet wines (Moio and Organisation Internationale de la Vigne et du Vin 2015). In other countries, including the USA, the limit of total SO_2 is 350 mg/L for all types of wine (Ribéreau-Gayon et al. 2005). These limits have decreased over time. In France for example, the maximum amount was 450 mg/L SO_2 for all wines in 1926, which is three-times the concentration permitted today by the EU for red wines. In reality, the maximum concentration is rarely reached. In France, the average concentration of SO_2 in white wines was found to be 105 mg/L and 75 mg/L for red wines (Ribéreau-Gayon et al. 2005).

While a certain amount of sulfur dioxide is generally needed for the stabilization of wines, too much of it can have different negative effects. A high concentration of SO_2 can neutralize favorable aromas or, in even higher concentration, cause a characteristic burned match off-flavor and cause a burning aftertaste. High concentrations of SO_2 can also slow or even prevent malolactic fermentation. The most important reasons to avoid excessive SO_2 concentrations are related to health concerns. In animal studies, an acute toxicity was determined with an LD_{50} of 0.7-2.5 g per kg bodyweight, depending on the species. Chronic toxicity studies by Til et al. (1972) showed three consequences: a deficiency in thiamine, histopathological changes of the stomach and slowed growth. Based on this, the WHO set a recommended dietary allowance (RDA) of 0.7 mg/SO_2 per kg body weight. In humans, acute SO_2 poisoning can cause nausea, vomiting and gastritic irritation at an ingestion of a single dose of 4 g of sodium sulfite. In the 1980s, several studies found that SO_2 can cause adverse reactions in certain people suffering from asthma. This was particularly true for steroid depended patients (Bush et al. 1986; Dahl et al. 1986; Gunnison and Jacobsen 1987; Lester 1995). Therefore, a declaration of SO_2 on bottle labels is required by the Food and Drug Administration (FDA) and in the European Union at levels higher than 10 mg/L (Ribéreau-Gayon et al. 2005; European Parliament; European Council 10/25/2011).

SO_2 has been an important additive in winemaking for many years, making it easier to avoid fermentation by undesirable microorganisms and store wine for longer periods of time. It is important to add the right amount at the right times in order to archive the desired antimicrobial and antioxidant effects and avoid stuck fermentation and off-flavors due to over-sulfurization.

4.3 Fermentation

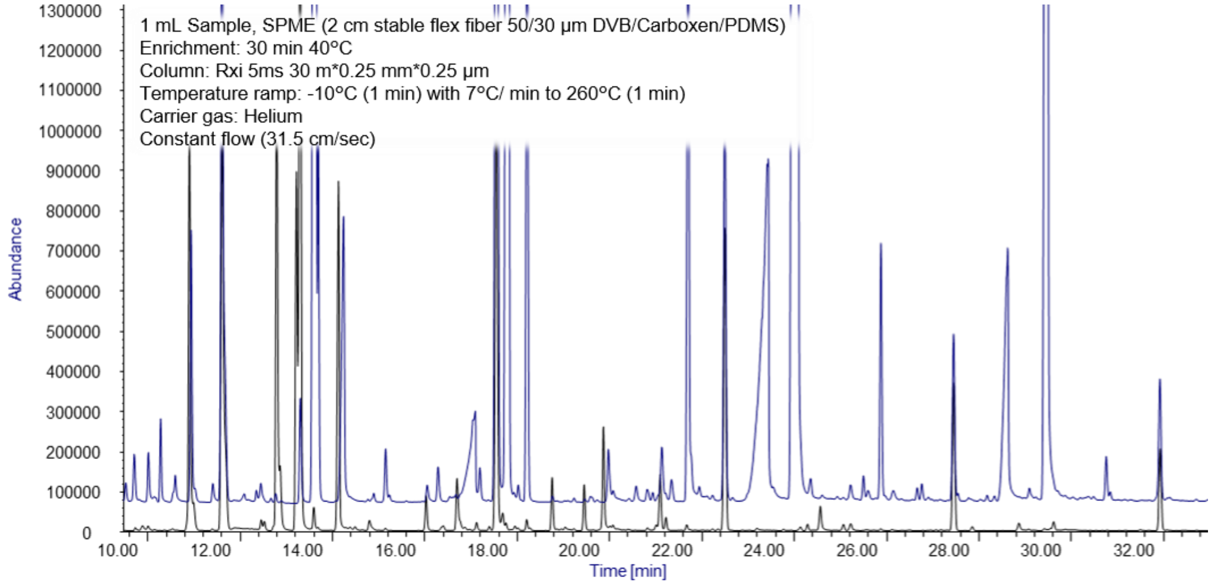
In this step the fermentable sugars in grape juice or must are converted into ethanol and carbon dioxide. As this is an energy producing reaction, heat is released during the fermentation. The whole process is carried out by microorganisms (MOs), mainly yeasts of the genus *Saccharomyces*, with *S. cerevisiae* being one of the most important ones (Bakker and Clarke 2012). In the following paragraphs, the process and its factors will be discussed. The focus will not only be on *S. cerevisiae*, but also MOs from other sources, either the vineyard or the cellar and the technology need to assure a safe fermentation.

Before the start, adjustments can be made to prepare the must, including addition of sugar or adjustment of the pH to optimize fermentation conditions (Grainger and Tattersall 2016). These adjustments have to comply with the regulations. Their extent can vary from country to country. In Austria for example, it is allowed to adjust the sugar content of the must to up to 18 g of unfermented sugar for Landwein and Qualitätswein. This is in accordance with the EU regulations (Bundeskelleriinspektion 11/17/2009).

The prepared must is filled into a fermentation vessel. Most of the fermentation tanks used today are made from stainless steel, which can be disinfected relatively easily and provides rapid heat transfer. Other materials for fermenters include cement and fiberglass. Before this, they were mostly made from different wood-types (Jackson 2014). The type of fermenter also depends on the wine type. While tanks for the production of white wine are relatively simple and mostly only require a cooling system, those for the production of red wine are more complex. During the fermentation, carbon dioxide is released, which, on its way to the surface of the fermenting must, catches in the solids. They float to the top where a pomace cap forms. This cap has to be broken up and submerged from time to time to enable the extraction of its constituents. In addition, the mixing minimizes the temperature difference over the whole fermenter and aids the yeast distribution. Particularly towards the end of the fermentation, the large surface area of the cap makes it prone to the growth of bacteria which can result in the formation of off-flavor compounds (Ribéreau-Gayon et al. 2005). There are different ways to archive the submersion of the cap, one is to regularly separate the fermenting juice into a different tank or take it from the bottom of the fermenter and pump it back into the fermentation tank to break up the cap or keep it submerged. Another way, mostly for smaller fermenters, is the manual punching down of the cap (Jackson 2014).

In winemaking fermentation is normally done in batches. Continuous fermentation is possible, but only used on an industrial scale. Using a 4,000 hl system, it is possible to produce 23,000 hl of wine over a period of three weeks. While this method can save space, labor and costs, there are several disadvantages. Using a continuous system is only profitable on a very large scale and has to be closely monitored, as the system can otherwise be prone to bacterial contamination. The wines produced resemble each other and no distinction based on variety or region of origin is possible (Ribéreau-Gayon et al. 2005).

The fermentation starts after the inoculation with microorganisms (MOs) of juice or must in the fermentation tank. For this, either a specific strain of yeast or a mixture is added in the form of activated dried yeast or as already fermenting must. Alternatively, fermentation can happen spontaneously with endemic MOs that naturally occur on the grape skins or originate in the cellar. The choice of MOs has a large impact on the final quality of the wine as many flavor compounds stem from the metabolic processes during fermentation that happen in addition to the conversion of sugar to ethanol (Fleet 2003). Figure 27 shows the aroma profile of a grape juice (black) and the wine (dark blue) produced from it. There is an obvious difference between the peaks in the chromatogram and the compounds present in the two samples. In addition to compounds that are derived from the fermentation process, others that are present in the grapes as odorless precursors are converted to their odor-active counterparts (Waterhouse et al. 2016). As a result of these changes, wine has more flavor than grape juice (Swiegers and Pretorius 2005).



1 mL Sample, SPME (2 cm stable flex fiber 50/30 µm DVB/Carboxen/PDMS)
Enrichment: 30 min 40°C
Column: Rxi 5ms 30 m*0.25 mm*0.25 µm
Temperature ramp: -10°C (1 min) with 7°C/ min to 260°C (1 min)
Carrier gas: Helium
Constant flow (31.5 cm/sec)

Figure 27: Aroma profile chromatograms of wine (blue) and grape juice (black) of the same grape variety

One of the most abundant products of fermentation is ethanol, which is produced from the sugars in the juice. Without access to oxygen, MOs, mainly yeast, transform the present sugars, mainly fructose and glucose, to ethanol and carbon dioxide (Equation 2) (Zamora 2009).

Equation 2: overall reaction of alcoholic fermentation

$$\underset{\text{sugar}}{C_6H_{12}O_6} \rightarrow \underset{\text{ethanol}}{2\,CH_3CH_2OH} + \underset{\text{carbon dioxide}}{2\,CO_2}$$

In the first step, glycolysis, the sugar is broken down to pyruvate. Over the course of this process, two molecules of ATP, the MOs main source of energy, are formed from ADP for each molecule of sugar. Pyruvate is then broken down to acetaldehyde and CO_2 by the enzyme pyruvate decarboxylase. When acetaldehyde is reduced to ethanol by alcohol dehydrogenase, NADH is oxidized to NAD^+, which is needed for glycolysis to take place (Figure 28). While this process produces less energy than aerobic fermentation, it is a way for the yeast to survive under non-ideal conditions (Zamora 2009; Jackson 2014).

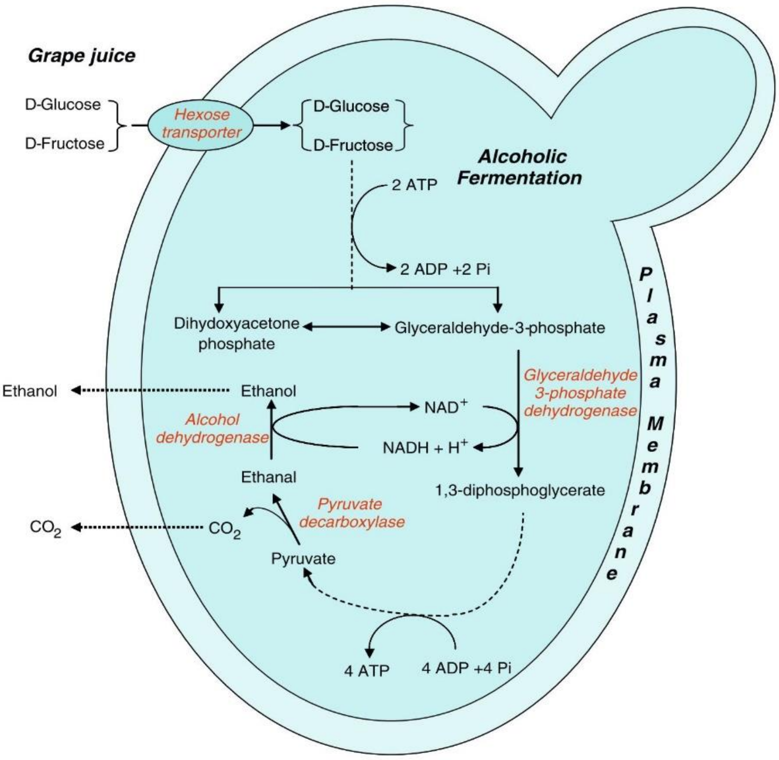


Figure 28: Alcoholic fermentation (Zamora 2009, p. 8)

Even though ethanol might be the main product of fermentation, all the additional processes happening transform the grape juice into wine, the complex beverage that has been fascinating people for ages.

4.3.1 Yeasts

Without a doubt, yeasts are the main players in the fermentation process of wine. Many have studied their influence on the final product. Yeast has been used in food production for a very long time, unknowingly in the beginning but more targeted with growing understanding of its impact on different production processes (Fleet 2005). In general, yeasts are classified as a group of single-celled fungi that reproduce either by budding or by fission (Jackson 2014). When yeast is mentioned in a fermentation context, the one that most likely comes to mind is *Saccharomyces cerevisiae*. While this species has a large part in the production of wine, in reality there are many that play their part in the production of wine. They can occur naturally on the grapes. In their review Lambrechts and Pretorius (2000) list different wine-related yeasts (Table 1), showing the large variety of endemic species.

Table 1: List of wine-related yeasts adapted from Lambrechts and Pretorius (2000)

Genus	Species	Genus	Species
Brettanomyces	*anomalus* *bruxellensis* *intermedius*	***Saccharomycodes***	*ludwigii*
Cryptococcus	*albidus*	***Debaromyces***	*hansenii*
Dekkera	*anomala* *bruxellensis*	***Hanseniaspora***	*uvarum*
Hansenula	*anomala* *kluyveri*	***Kloeckera***	*apiculata*
Kluyveromyces	*marxianus* *thermotolerans*	***Metschnikowia***	*pulcherrima*
Pichia	*kluyveri* *membranifaciens*	***Rhodotorula***	*glutinis*
Saccharomyces	*bayanus* *beticus* *capensis* *cerevisiae* *chevalieri* *ellipsoideus* *fermentati* *oviformis* *rosei* *uvarum*	***Candida***	*boidinii* *colliculosa* *guilliermondii* *hellenica* *krusei* *lambica* *oloephila* *pelliculosa* *pulcherrima sorbosa* *stellata* *valida* *vanrijiae*
Schizosaccharomyces	*pombe* *japonicus*	***Torulaspora***	*delbrueckii*
Zygosaccharomyces	*bailii* *bisporus* *florentinus* *rouxii*		

The influence of the yeasts found on the grapes on the final product varies depending on their properties. This includes tolerance to sugar and ethanol, their response to amount of added SO_2, as well as the ability of the yeast to assert themselves over other species. Other factors that influence the microbiological diversity are the type of harvest, transport of the grapes and their condition as well as the pre-fermentation treatments (Lambrechts and Pretorius 2000).

Most yeast species are less alcohol-tolerant than *Saccharomyces*, resulting in their decline over the course of the fermentation. Subsequently, yeasts of the genus *Saccharomyces*, especially *cerevisiae* and *bayanus*, were used for the inoculation of must. Since the 1980s, the importance of other species for the final product has started to be recognized. The influence on the aroma of the final product is, at least partially, dependent on the variety of MOs present. The transformation of non-volatile precursors can be facilitated by non-*Saccharomyces* yeasts that produce more enzymes capable of releasing odor compounds. One example is the cleavage of glycosidic bonds between certain terpenes and sugars with glycosidases. This can be important for the varietal characteristic of some grape varieties, like muscats and Riesling. Another group of aroma compounds that are released during fermentation are varietal thiols like 4-methyl-4-sulfanyl-pentan-2-one (4MSP), 3-sulfanylhexan-1-ol (3SH) and 3-sulfanyl hexyl acetate (3SHA). In the grapes, they are conjugated to cysteine. The bond is broken by cysteine lyase. This enzyme is produced by different strains of yeast to different extends, influencing the final amount of aroma active thiols. In addition to the two enzymes mentioned, many others originating from different yeasts also have influence on the final flavor of the wine. This influence can be positive, as with the examples pointed out before but can also contribute to off-flavors. A presence of some *Dekkera* species for example can facilitate the formation of volatile phenols, like 4-ethyl phenol and 4-ethylguaiacol resulting in a “Brett” off-flavor (Fleet 2008).

Another risk a winemaker might run into is the occurrence of sluggish or stuck fermentations. In these cases the fermentation slows down or comes to a halt even though only part of the sugar has been transformed into alcohol. There are multiple reasons, including inadequate cooling or stress factors like the lack of nutrients such as nitrogen or certain vitamins or extensive sugar, particularly in must from overripe or botrytised grapes. In juice from those grapes, an additional factor is the ratio between glucose and fructose. Out of these, glucose is metabolized faster, resulting in a constantly increasing fructose to glucose ratio, which has been linked to stuck fermentation. Other reasons include the presence of indigenous MOs and “killer yeasts”, which can stunt the growth of beneficial yeasts or replace them. While cooling has become less of an issue due to developments in winemaking equipment, stuck fermentation can still occur and result in low alcohol wines with high residual sugar that are prone to spoiling. To prevent this, an inoculation can be beneficial as well as ensuring a sufficient nutrient concentration in the beginning of the fermentation. Stuck fermentation can be restarted by re-inoculation with specific yeast strains, addition of depleted nutrients and other adjustments depending on the cause (Jackson 2014).

Saccharomyces

Of all the yeasts connected to winemaking probably the most important ones are of the genus *Saccharomyces*, with the most well-known one being *S. cerevisiae* as mentioned before. The knowledge of the characteristics of selected strains of this yeast has improved over time, allowing winemakers to guide the fermentation in more directed ways and in this way enhancing certain characteristics of the wine while preventing off-flavors (Ugliano and Henschke 2009). Overall, *S. cerevisiae* has become one of the best studied eukaryotic MOs over the last several decades (Rodicio and Heinisch 2009).

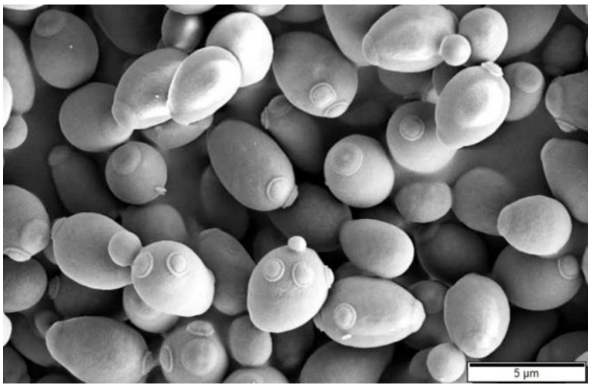


Figure 29: Saccharomyces cerevisiae (Murtey and Ramasamy 2016)

When it comes to alcohol production, *Saccharomyces* is very effective: from a starting material containing 22-24% sugar about 95% are turned into ethanol and CO_2, 1% to cellular material and the remaining 4% to other products (Lambrechts and Pretorius 2000). Over the course of a traditional batch fermentation, yeast cultures follow a relatively fixed growth pattern that can be divided into several parts: a lag or latency phase, in which the cells adapt to their new environment following inoculation; the exponential growth or log phase, in which the cells multiply at an exponential rate and the biomass increases until the conditions do not allow further expansion. This is followed by a (quasi-)stationary phase, in which new cells form and die at a similar rate, keeping the amount of biomass at a stable level; and finally the decline phase, in which the reproduction rate on cells falls below the death rate, until almost all cells are dead or inactive (Zamora 2009).

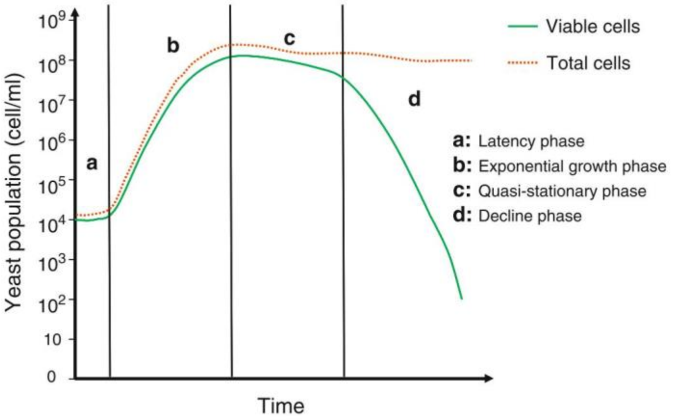


Figure 30: Growth cycle of yeast cells (Zamora 2009, p. 5)

Figure 30 shows the development of biomass over the course of a batch fermentation divided into the four described phases. Although following the same pattern, the yeast life cycle in wine can differ from the regular pattern, insofar that the lag phase is usually not longer than a few hours, the log and stationary phase can be shorter and the decline phase comparatively long, sometimes lasting several weeks or even months. This can result in up to 40% of ethanol production occurring during the last phase in the cycle (Jackson 2014). While the use of commercially available *S. cerevisiae* cultures for the inoculation has resulted in more controlled and predictable winemaking, some argue that it has also led to the loss of diversity and standardization of the resulting wines. Due to this, some winemakers might favor co-inoculation with local strains in order to give wines a distinct character. Tempère et al. (2018) debate this in a recent review. Overall, the amount of commercially available strains of *Saccharomyces*, as well as those occurring naturally on the grapes allow winemakers to create wines with different characteristics, depending on the base material and their personal preference and vison for the final product.

Other yeasts

Saccharomyces strains are a major factor in alcoholic fermentation, in particular due to their high tolerance to ethanol. Other, non-*Saccharomyces*, yeasts have long been considered undesirable in wine production. The presence of some has been linked to off-flavor production and unreliable, stuck or sluggish fermentation. In recent years however, winemakers have found the addition of some yeasts, like *Torulaspora delbrueckii*, to be beneficial for the organoleptic qualities final product. Mixed starter cultures have therefore gained increased interest (Tempère et al. 2018).

Some winemakers might choose to use only the MOs that are endemic to the grapes and forego the inoculation with *Saccharomyces*. The types of yeasts and their relative abundance depend on several factors. This includes the climatic conditions, the location of the vineyard, and the treatment of the grapes at all stages of maturation as well as the variety and maturity of the grapes and their condition. Non-*Saccharomyces* yeasts will dominate the early stages of fermentation. Several studies have for example found that *Hanseniaspora uvarum* (also known as *Kloeckera apiculate*) plays an important part during the first stages of spontaneous fermentation in different regions of the world including Brazil (Bezerra-Bussoli et al. 2013), Spain (Clemente-Jimenez et al. 2004), and Argentina (Combina et al. 2005). The presence of yeasts of this genus has been linked to higher concentrations of ethyl esters as well as acidic acid and 2-aminoacetophenone (Martin et al. 2018; Jackson 2014). Other yeasts found during earlier stages of fermentation include *Candida stellata*, which at least in one study was able to transform glucose up to an ethanol level typical for table wine without the presence of *S. cerevisiae* (Clemente-Jimenez et al. 2004). Table 1 lists a variety of other yeasts that have naturally been found on grapes. Over the course of the fermentation, the composition of yeasts will change, mostly based on their tolerance to ethanol and the later stages are usually dominated by *S. cerevisiae*. Using the natural flora of yeasts specific to certain vineyards can give the resulting wines unique characteristics, differentiating them from other wines of the same variety, but it can also have unpredictable and inconsistent outcomes or lead to the development of off-flavor and stuck fermentations especially under stressful fermentation conditions (Jackson 2014).

In order to avoid this, the winemaker can choose to inoculate the must with a mixed starter that contains other yeast alongside *S. cerevisiae*. When done well, this can combine the benefits of spontaneous and inoculated fermentations. The selection of yeast species used in starter cultures should be based on the desired outcome. There are two possible ways of inoculation: co-inoculation, where all strains of yeast are added at the same time, or sequential inoculation. In the second scenario the non-*Saccharomyces* yeasts are added first in order to allow them to have an impact on the aroma and *S. cerevisiae* is added at a later point to take over (Padilla et al. 2016).

4.3.2 Other MOs

Its low pH, usually between 3.0 and 3.5, restricts the types of MOs that are able to survive in grape must. In addition to different types of yeast, this includes lactic and acetic acid bacteria and some molds. While they can survive the low pH, the growth of the latter two is inhibited by the anaerobic conditions of the fermentation. Their presence in fermenting must is, in most cases, related to technological errors (Cantarelli and Lanzarini 1989) even though it has been found that acetic acid bacteria can use different hydrogen acceptors to remain viable inside of wine bottles and barrels (Acetic and Other Fermentations 2011). Neither acetic acid bacteria nor molds are desired in the vinification process, as their presence usually leads to the development of off-flavor. *Acetobacter*, the main type of acetic acid bacteria present in wine, for example, increases volatile acidity by oxidizing ethanol to acetic acid. To ensure the absence of MOs that negatively affect wine, control of the fermentation process is crucial. The addition of SO_2 can also help when it comes to controlling the presence of unwanted MOs (Guillamón and Mas 2009).

While the presence of acetic acid bacteria and molds is generally avoided, lactic acid bacteria are needed when a secondary fermentation is wanted.

Malolactic Fermentation (MLF)

Malolactic fermentation is a secondary fermentation occurring in wines. It is the conversion of malic acid to lactic acid by different species of lactic acid bacteria. This results in a de-acidification of the wine due to the direct decarboxylation of malic acid (Figure 31). This leads to wines having lower titrable acidity and a higher pH (Buglass 2011). And while it is, at least from a biochemical point of view not a real fermentation, it does result the emission of CO_2, winemakers therefore refer to it as MLF (Lonvaud-Funel 2010).

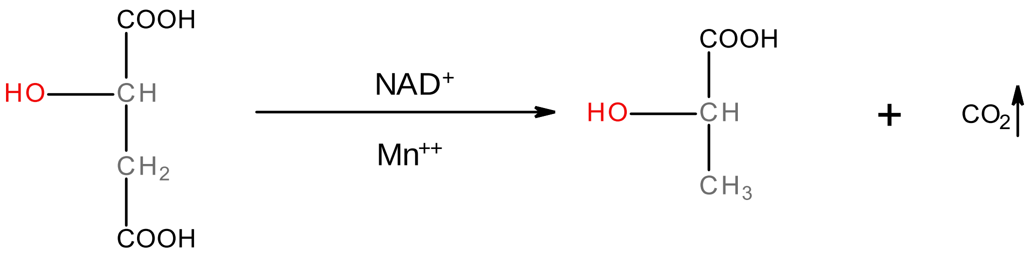


Figure 31: Conversion of malic acid to lactic acid (Boulton et al. 1996a)

MLF can take place during or after alcoholic fermentation. Some winemakers may choose to add the needed bacteria to the must to encourage faster completion, while other might add them after the alcoholic fermentation is completed or forego the addition altogether and rely on endemic bacteria (Bakker and Clarke 2012; Boulton et al. 1996c). Figure 32 shows one possible development of the population of lactic acid bacteria over the course of the winemaking process.

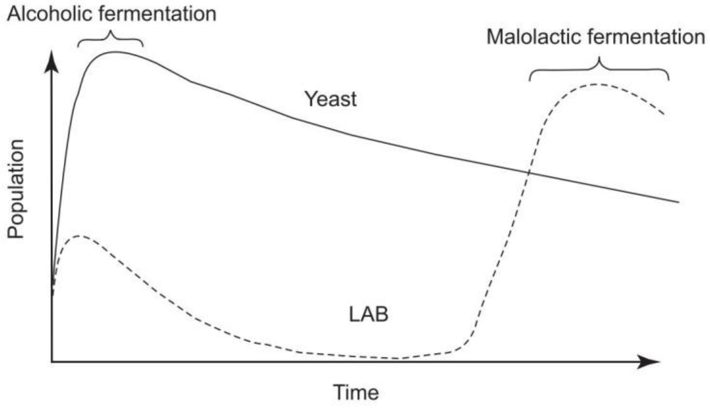


Figure 32: Development of lactic acid bacteria during winemaking (Lonvaud-Funel 2010)

With the conversion of malic acid to lactic acid, the overall titrable acidity is reduced. MLF is therefore often used to mediate excessively acidic wines, sometimes from colder climates. This also results in slightly higher pH. Changes of up to 0.2 units are possible depending on the starting pH, also taking the buffer capacity of the wine into account. In some cases, the lower pH might bring risk of microbiological spoilage. The higher pH can also influence the color of wine, and result in a loss of red pigment. Larger changes in pH may result in a bluish hue. In some cases, the acidity of the wine needs adjustment following malolactic fermentation. In general, this is done by adding tartaric acid, but not citric or malic acid, as remaining bacteria can transform the latter two (Boulton et al. 1996a).

Most commercially available red wines and some white wines have undergone MLF. As with most biochemical processes involving MOs, the choice of strain is vital as wrong strains can result in the production of off-flavors.

As briefly mentioned before, the lower acidity and higher pH can, in some cases, increase the growth risk of some spoilage bacteria, mainly other strains of lactic acid bacteria. Overall, MLF causes microbiological stabilization due to the depletion of nutrients. In addition, MLF has been found to reduce the amount of off-flavors related to spoilage yeasts, like *Brettanomyces*, as it can reduce the amount of 4-ethylguaicol and 4-ethylphenol (Gerbaux et al. 2009).

MLF also causes the formation of diacetyl, which in concentrations above its sensory threshold results in a buttery smell. This is more obvious in white wines, as their aroma is generally more subtle. For example, some Californian Chardonnays exhibit this characteristic. The formation is mostly a result of transformation of citric acid, which is used as substrate after malic acid, but it also depends on the strain of bacteria and availability of oxygen (Boulton et al. 1996a).

MLF can also result in the reduction of varietal aroma characteristics, which can be more pronounced in white wines, as those usually associated with more delicate aromas that can more easily be overwhelmed by the changes introduced by MLF. This is less likely in red wines, as they typically have more flavor (Boulton et al. 1996a; Jackson 2014).

In red wines, particularly from colder climates, with higher acidity, MLF can be employed to reduce the acidity. In wines from warmer climates, it can be used for stabilization, as bacteria consume most of the nutrients and, therefore, inhibit new MOs from growing. When red wines are bottled young without MLF, winemakers need to take steps to prevent secondary fermentation in the bottle as this can result in turbid and gassy wines (Boulton et al. 1996a).

In white wines, MLF is relatively rare. In most cases, winemakers take steps to prevent MLF from happening as the impact on the aroma is usually unwanted. It has become more common in recent years, using strains that do not or only minimally influence the aroma of the finished product. The choice to use MLF strongly depends on the climate of the winegrowing region and the acidity of the wines. The *sur lies* storage of Chardonnay wines from France results in MLF. This effect has been reproduced in other areas like California.

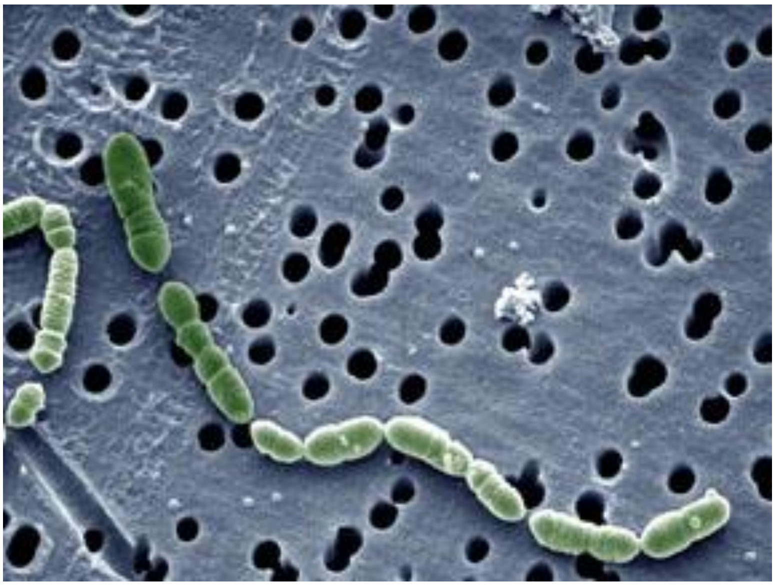


Figure 33: Oenococcus oeni (Wikimedia Commons 1/15/2007)

The lactic acid bacteria strain mainly found in wine and utilized for MLF was first identified in the mid-1960s and named *Leuconostoc oenos*. Later, after the introduction of molecular techniques, it was reclassified to *Oenococcus oeni* (Figure 33). Over time, it has adapted to the low pH, high alcohol, low oxygen and, limited nutrient environment of wine. Other types of lactic acid bacteria belong to the categories of *Leuconostoc*, *Lactobacillus* and *Pediococcus*.

The latter two can be responsible for the development of off-flavors (Buglass 2011). Over the course of the MLF, the energy source of the bacteria can change, starting with residual sugars during the exponential growth phase. During the early stationary phase, malic acid becomes the main source and towards the end citric acid is utilized. As already mentioned before, the fermentation of citric acid can result in the production of diacetyl and other compounds that can have an effect on the final aroma of the wine (Jackson 2014).

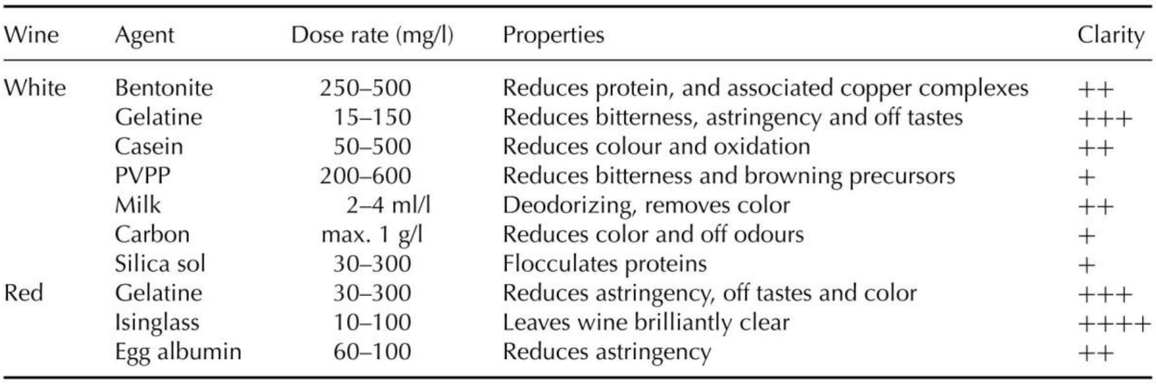
Different steps can be taken in case MLF is not desired by the winemaker. These include storage of the wine at temperatures below 10°C, frequent racking and early clarification, lowering the pH, short maceration times, avoiding maturation on lees as well as a sufficiently high concentration of SO_2. To prevent in-bottle MLF different steps have to be taken. For example, wines can be sterile filtered into sterilized bottles and more SO_2 can be added in order to avoid off-flavors, clouding of the wine and the solution of CO_2 in the wine leading to petillance (Jackson 2014).

4.4 Stabilization

Which clarification and stabilization steps need to be taken, depends on the compounds causing the wine to be hazy. This ranges from insoluble particles, like yeast cells, dust and grape material, to soluble compounds including tartrates, tannins and proteins whose precipitation depends on the properties of the wine like ethanol content and temperature. The removal of the first group is generally referred to as clarification while the process for the second group is called fining (Boulton 1996).

The easiest way for the removal of insoluble particles is natural settling using gravity and time. The speed depends on several factors like the size of the particles, the viscosity of the liquid and the density difference between particle and liquid. Settling will take longer for smaller particles, higher viscosity and low density differences. The process can be enhanced by adding settling aids that promote the formation of larger particles and drag smaller particles along, like silica sols or gelatin. To remove the settled particles (lees), the liquid is moved to another vessel. This process is called racking and can be performed as often as necessary. In addition to removing lees, racking also provides aeration, removes CO_2 and homogenizes the solution. Centrifugation is another possibility to remove insoluble particles, particularly in wines intended for early consumption as it significantly speeds up the process. The main downsides of this process are the initial costs of the system, as well as the fact that not all particles can be removed. Centrifugation of wine is usually done under a protective atmosphere to minimize the oxygen contact of the wine. Filtration is another way to remove insoluble particles. With the use of filters with different properties, it is possible to remove larger particles down to microbes with diameters below 1 µm. Depending on the openness, filters can be divided into different categories: rough, polish, tight and sterile. Filters can also be categorized by material, mainly those made from diatomaceous earths (Kieselgur), pad, membrane and cartridge filters. It is important to first remove larger particles as not to overload and clog the finer filters. To avoid changes in flavor, filters can be rinsed with an acidic solution or a small quantity of wine, which is discarded afterwards (Boulton 1996; Bakker and Clarke 2012; Buglass 2011).

Fining requires the addition of a fining agent to aid the removal of soluble compounds that can cause the wine to become hazy after bottling. The more traditional fining agents are protein based and include gelatin, milk and albumin from animal sources like egg white or blood. The use of blood as fining agent has been banned by the EU and in the US in 1997 due to the increase in mad cow disease. Today, a wide range of fining agents is available. All of them function in a similar way by reacting with some compounds in the wine, either chemically or physically, and forming new, mostly larger compounds that can be separated from the wine more easily using clarification methods (Buglass 2011). Fining agents can be grouped based on their general nature. This includes earths, like bentonite and kaolin, proteins, like gelatin and albumin, polysaccharides, like alginates, carbons, synthetic polymers and others (Zoecklein et al. 1999). Figure 34 lists the properties of some commonly used fining agents.



Wine	Agent	Dose rate (mg/l)	Properties	Clarity
White	Bentonite	250–500	Reduces protein, and associated copper complexes	++
	Gelatine	15–150	Reduces bitterness, astringency and off tastes	+++
	Casein	50–500	Reduces colour and oxidation	++
	PVPP	200–600	Reduces bitterness and browning precursors	+
	Milk	2–4 ml/l	Deodorizing, removes color	++
	Carbon	max. 1 g/l	Reduces color and off odours	+
	Silica sol	30–300	Flocculates proteins	+
Red	Gelatine	30–300	Reduces astringency, off tastes and color	+++
	Isinglass	10–100	Leaves wine brilliantly clear	++++
	Egg albumin	60–100	Reduces astringency	++

Figure 34: Fining agents and their recommended doses and properties (Buglass 2011)

It is important to use only the minimum amount of fining agent necessary to achieve the wanted effect. Fining can have an influence on the overall flavor of a wine, mainly the impression on the palate. Too much fining agent can reduce the amount of molecules contributing to the body and volume of the wine and make a wine seem thinner. The influence on the aroma depends on the fining agents used, as some may have an affinity to certain volatile compounds, but overall the effect is normally very limited if the right amount of agent is used. Too much fining agent can result in over-fining, where not all of the added proteins have separated. An over-fined wine will be clear but can become hazy when tannins are added, for example when blending with another wine with a higher tannin content or due to the addition of enological tannins. To avoid this, it is recommendable to bypass the use of gelatin for fining red wines and white wines with high tannin content as well as egg whites for white wines, as they need a larger amount of tannins to flocculate. To avoid this, fining agents have to be matched to the wine and the wanted result and the contact time should be limited. They also need to be free of any impurities and unwanted odors as those can affect the final wine (Ribéreau-Gayon et al. 2006; Zoecklein et al. 1999).

In addition to fining, other stabilization measures are performed to remove tartrates, which can form solid residues that collect at the bottom of the bottle or on the cork. While they are harmless, consumers may find their presence disturbing or mistake them for foreign objects, like glass shards. In a process called cold stabilization the wine is stored close to freezing temperature for up to two weeks. This encourages the crystallization. The removal of the crystals can be done by racking, centrifugation or filtration while the wine is still cold, as warmer temperatures can result in decrystallization, rendering the process pointless (Bakker and Clarke 2012).

To archive the best stability and avoid unwanted effects, stabilization, fining and clarification processes have to be matched to the wine and to each other.

4.5 Maturation

The phase between the end of the alcoholic fermentation and bottling is called maturation or bulk storage. It can last from less than two months up to several years for red wines and even span decades for fortified wines. Some of the processes discussed in previous parts like secondary fermentation and stabilization processes occur during this time. Adjustments can be made to correct and prevent problems in the final wine. The extent of these is often regulated by law to prevent fraud. In the European Union, the allowed adjustments are detailed in Regulation (EU) 2019/934 (European Commission 6/7/2019). Possible alterations can be made to pH, acidity, sugar and the alcohol content, mainly the removal for the production of low- or non-alcoholic wines. Other changes in the composition of the final product occur due to the choice of maturation vessel. The main option are stainless steel tanks and wooden barrels. Both options and their influences will be discussed in greater detail in a later chapter (Jackson 2014; Buglass 2011; Boulton 1996).

Bottle aging starts once the wines have been filled into bottles or similar storage containers. The benefits of aging wines have been known since Roman times and are still today one of the most intriguing properties of some wines. Over time, the aroma of red wines produced from varieties like Shiraz, Nebbiolo, Cabernet Sauvignon, Tempranillo and Pinot noir and some white wines from varieties like Sauvignon Blanc, Riesling and Chardonnay shows beneficial changes. While the fruitiness of the wines declines, it is replaced by a so-called aged bouquet. In addition to the aroma, aged red wines show a smoother texture due to changes in the phenolic composition. Ageing also has an effect on the color of the wine. The aging potential of wine has been and is still a topic of much debate but will ultimately depend on the personal preferences of the consumer. Some might prefer the stronger astringency of younger red wines while other will prefer the smoother texture and more subtle flavor of aged red wines. Some wine drinkers will store their wines in cool cellars for several years or even decades to allow for the development of an aged bouquet. Overall, a wine is said to have aging potential when the flavor that develops during prolonged bottle storage contribute in a positive way to the aroma and tasting experience. Several chemical reaction happen to make this possible, including oxidation, reduction, structural rearrangement, polymerization, volatilization, and others (Bakker and Clarke 2012; Jackson 2014, 2009). Figure 35 shows the possible flavor development during aging of three wines of different styles with the development of the overall aroma (dashed line), the fermentation aroma, the fresh, fruity notes of young wines (solid line) and the aged bouquet (dotted line). The first one (A) is a wine that is sold in the year of its harvest, like a Styrian Junker or the Italian Novello, French Nouveau or the Spanish Joven (wein.plus 2020). Wines like these are intended to be drunk young and will lose their fresh aroma during prolonged storage. The second (B) is a standard quality red wine, which will reach its aroma plateau after a few years and should be consumed within 5-10 years after bottling. And the last graph (C) depicts the aging potential of a high quality red wine, where the fermentation bouquet is overtaken by the aging bouquet after several years and an extended aroma plateau, lasting more than ten years. Wines like this one can theoretically be stored for several decades under the right conditions (Jackson 2009). These are only recommendations, because, as mentioned before, the most important factor is personal preference and even experts do not agree on when the best time for the consumption of any specific type of wine is and recommendations are highly speculative (Jackson 2014).

In this part the focus will lie on the ways of maturing a wine before bottling and the types of storage vessels, including stainless steel tanks, wooden barrels and other methods.

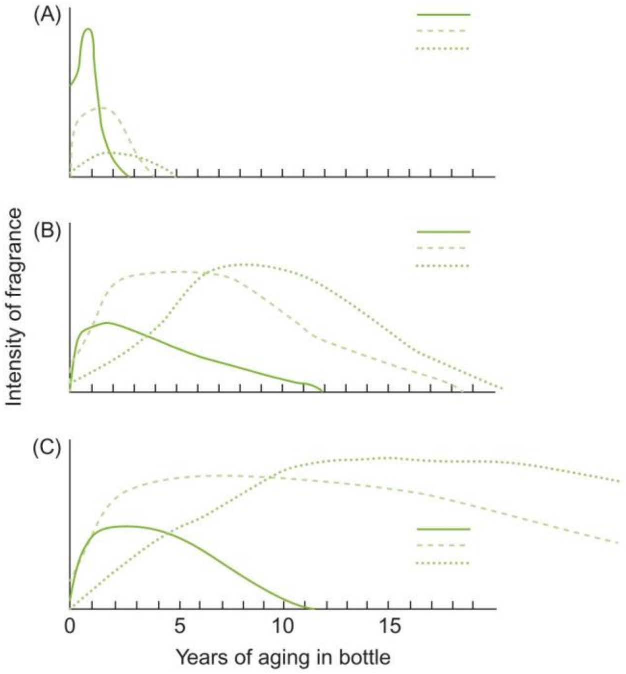


Figure 35: Aging potential for different wines: (A) nouveau-type wine, (B) standard quality red wine, and (C) premium quality red wine (solid line: fermentation bouquet; dashed line: aroma; dotted line: aged bouquet) (Jackson 2009, p. 370)

4.5.1 Technology

The ideal container for bulk maturation of wine has to fulfil several requirements. It should be durable, inert, easy to clean, convenient and cheap. If it imparts flavor to the wine, they should be beneficial. No container will be able to fulfil all the requirements and compromises will have to be made when choosing the material in which the wines will be stored until they are ready for bottling. Containers have been made from different materials over the years, including ceramics, metals, wood and plastic. The choice strongly depends on the wine type and wine style (Kunkee and Großmann 2003). Most higher-quality red wines will have been barrel aged at least for some time, usually lasting between 9 and 22 months (Grainger and Tattersall 2016). For white wines this is relatively uncommon but is done in some cases if the winemaker thinks that the wine will benefit from it. White wines are usually matured in stainless steel tanks to maintain their characteristic taste (Jackson 2014). While other techniques including archaic techniques like the Georgian qvevri are available, the choice for winemakers is usually between stainless steel and wooden barrels. In the following some of the maturation techniques will be described further.

Wooden Barrel

When it comes to wine storage and maturation the most common image in people's mind are large wine cellars filled with wooden barrels of different sizes, but in reality depending on the source only about 2-10% of all wines are aged in barrels (Fédération des Tonneliers de France 2019; Bakker and Clarke 2012; Crisaldi 2018). The use of wooden containers for the storage and transport of wine can be traced back to antiquity, with reports from the 5th century BC mentioning the transport of wine in palm-wood containers (Jackson 2014).

The types of wood species used for the production of barrels have declined over the years. From the 16th century on it was basically reduced to chestnut and oak, as they imparted favorable aromas to the liquid stored inside. Today, they are the only ones approved for the storing of wine by the OIV (Martínez-Gil et al. 2018).

Figure 36 depicts the stages of the barrel manufacturing process. After the cooper (barrel maker) has chosen the wood, it is cut or split into quarters and then into staves which are then dried and aged for around three years. Alternatively, they can be dried kiln but this can result in harsh green notes in the wine. After the drying process, the staves are cut and bent to form barrels by wetting the outside of the wood while heating the inside. The barrels are then toasted, either by a fire or a gas burner for a certain amount of time depending on the desired level. After the head pieces are added to both ends of the barrel, the final metal hoops are fitted. The finished barrels are tested for integrity and impermeability with hot water (Jackson 2014).

Figure 36: Barrel manufacturing process (Carpena et al. 2020, p. 6)

Most of the barrels used to store and age wine are made from different types of white oak (*Quercus*). The barrels are usually classified by the origin of the wood used in their production, mostly French or American (Bakker and Clarke 2012). Most of the American oak used in the production of barrels originates from Kentucky, Missouri, Michigan and Arkansas (Jackson 2014). The wood, mainly from *Quercus alba,* is significantly less porous compared to European oak and has a stronger flavor. For this reason, it was typically used for the aging of stronger red wines like Spanish Rioja and Australian Shiraz. Today, American oak barrels are used more often due to the improvements in barrel making techniques (Robinson and Harding 2015). The main species for the production of French barrels are *Q. robur* and *Q. petraea* (previously *Q. sessilis*). The barrels vary mainly in chemical composition with the latter showing higher amounts of extractable aromatics and is the preferred type of wood for wine barrels (Jackson 2014). According to the Fédération des Tonneliers de France, there were 670,000 barrels produced from French oak in 2018 with more than two thirds being exported, mainly to the United States (Fédération des Tonneliers de France 10/14/2019).

The typical barrel size depends on the wine region. In some parts of Europe, including Germany and Austria, wine was traditionally aged in larger wooden containers, fitting more than 1,000 liters. These barrels were conditioned with hot water or steam to remove most of the extractable compounds as to not transfer any flavor into the wine. Until the 1980s, any wood-derived aromas were considered faults (Ilgen 2020). Since then, the opinion of many wine drinkers has changed and aging wine in smaller barrels gained popularity as the flavor of wood became more accepted and an indicator for fine wines. The amount of compounds extracted from the barrel depends on several factors, one of them being the size of the barrel. Smaller barrels generally offer a larger surface area to volume ratio. This allows more compounds to be extracted into the wine. A 2003 study compared wines matured in different size oak barrels (220, 500 and 1,000 liters). Wines stored in the smallest barrels showed higher concentrations of characteristic oak-derived compounds, like oak lactone, while for other compounds like vanillin and furfural there was only a small difference between the small and the medium sized barrel, but significantly larger than the concentration of the wines stored in the largest barrels (Pérez-Prieto et al. 2003). Figure 37shows some barrel sizes including one of the most popular among winemakers, the 225-liter barrique, which is the typical barrel size for wines from the Bordeaux region in France.

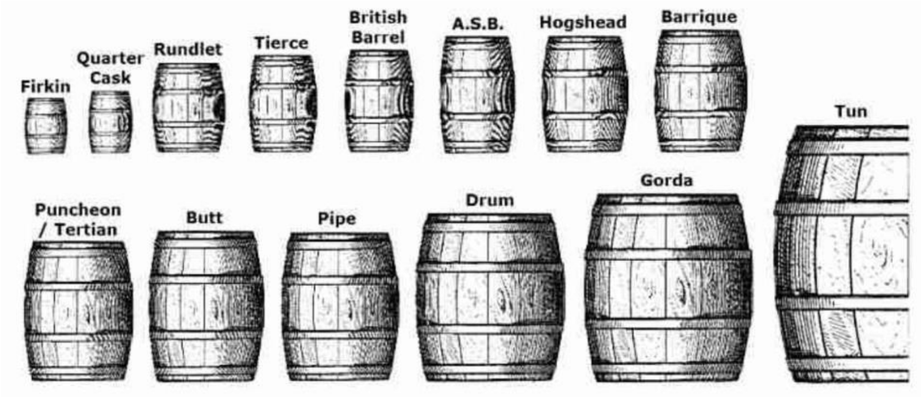


Figure 37: Different barrel sizes (not to scale) (Drinking Cup 2015)

Over the years, there have been several scientific publication comparing different wines aged barrels made from different origin oak. In all studies there were significant differences between wines aged in French and American oak barrels, and while in some cases a sensory panel was in two out of three different aging duration tests not able to distinguish between the two types (Aiken and Noble 2016), the others found a significant analytical differences between them (Rous and Alderson 1983; Cerdán et al. 2002; Fernández de Simón et al. 2003; Pomar and González-Mendoza 2016; Simón et al. 2003; Waterhouse and Towey 1994). American oak generally possesses less tannins but higher concentrations of some aromatic compounds like oak lactone. While this is true in most cases, the levels can vary as this is a natural product and prone to differences (Jackson 2014).

In addition to the origin of the wood, there are several other factors that influence the impact of the barrel on the wine. The duration and conditions of the maturation, which in most cases is between 3 and 24 months, the toasting level of the wood, the age of the barrel and its size also have an impact on the extent and the kind of the oak aroma imparted (Waterhouse et al. 2016). New oak barrels, especially from French oak, have the largest influence on the wine. In one study, the amount of total extracted phenolic compounds on the second fill of French oak barrels was only about 25% of that from the first fill after the same maturation period. For American oak the same study found only a small difference in the extracted phenols between the first and second fill (Rous and Alderson 1983). The toasting level of the wood has an influence on the concentration of volatile phenols, like guaiacol, and carbohydrate degradation products, like furfural. There are three toasting levels: light, medium and heavy, though they are not standardized across different producers. The variability of the influence can vary greatly between different barrels as the toasting process is not homogenous, even among barrels from the same manufacturer (Towey and Waterhouse 1996). As far as barrel size is concerned, larger barrels will have a smaller impact on the wine than smaller containers as there is less contact area, as mentioned before (Bakker and Clarke 2012).

The maturation in barrels also allows wine to come into contact with minimal amounts of oxygen. Minimal amounts of oxygen are considered beneficial for the development of red wines, as it promotes color stability as a result of the polymerization of anthocyanins. To minimize the oxygen contact of the wine, barrels can be stored with the opening (bung) tightly closed and turned to the side. Over the course of the maturation process, some of the liquid is lost, either to evaporation or to the wood itself. Depending on the tightness of the barrel this can amount to the loss of 4-10 liters of wine every year for a standard 225-liter barrique (del Alamo-Sanza and Nevares 2018). The empty space left behind is called ullage. The parts above this can dry out and increase the permeability of the wood. Regular topping up is therefore unavoidable to ensure minimal oxygen contact as too much can lead to off-flavor development, like excessive oxidation and high volatile acidity (Robinson and Harding 2015; Jackson 2014).

The maturation of wine in wooden cooperage is more common for structure rich red wines with high aging potential. When a winemaker chooses to work with wood in combination with white wines, the whole vinification process can be carried out in the barrel and not just the maturation phase. This is possible due to the fact that white wines are fermented without grape solids, like skins and seeds. After the alcoholic fermentation is finished, the yeast residue (lees) that collects at the bottom of the barrel can be stirred from time to time, resulting in fuller-bodied white wines. This is not possible for red wines, as all steps would be complicated by the presence of grape solids and the cleaning of the barrel after the removal of the wine would be highly problematic (Dominé et al. 2008).

The maturation of wine in wooden barrels is a cost and work intense process. For premium wines it can be very beneficial and is generally seen as desirable, whereas it can overpower wines with more neutral and delicate aroma. The results can vary significantly from one barrel to the next, resulting in the need for regular control and adjustments. Empty barrels need to be cleaned and maintained to avoid the development of spoilage bacteria and the accumulation of residues that can negatively impact any future fillings. In the end, the decision lies with the winemaker and the desired wine style (Jackson 2014; Dominé et al. 2008).

A cheaper alternative to traditional barrel maturation is the use of oak in other forms, including chips, planks and powder. The achieved effect is not the same and will depend on the size, toasting level and wood origin of the oak substitutes. Their use can be an economical alternative for younger wines where slight wood notes are desired (Carpena et al. 2020).

Stainless Steel

Stainless steel tanks are used as a fermentation and maturation vessel in most wineries today, but only gained popularity in the late 1970s. Before that, wood and cement were the preferred materials (Grainger and Tattersall 2016).

The use of stainless steel in winemaking has many advantages: the material is inert, relatively cheap and easy to clean. Tanks made from stainless steel are very durable and can be used for many years, if they are cared for correctly. The inert material does not impart flavor to the wine, therefore preserving the characteristics of the wine (Boulton 1996). When using stainless steel tanks, it is possible to completely exclude oxygen from entering into the system. Inside the tanks, the headspace can be filled with inert gas to even further minimize the oxygen contact. Red wines generally need a certain amount of oxygen during maturation to soften phenolic compounds, a complete exclusion can also result in the development of reductive aroma and the formation of sulfur compounds that negatively affect the wine. To avoid this, winemakers can use careful racking or micro-oxygenation methods. This process requires monitoring and regular tastings of the wine development by the winemaker as there are no strict rules (Robinson and Harding 2015; Bakker and Clarke 2012).

Cooling systems and the heat transfer capabilities of stainless steel allow for precise control of the storage temperature. For white wines bulk storage temperatures should be kept around 13°C and 15°C for red wines (Jackson 2014; Boulton 1996).

Figure 38: Stainless steel tanks for fermentation and storage of wine in Styrian winery

Other maturation techniques

There are several other materials used in wine maturation cooperage. Some, like the Georgian Qvevri, have been used for thousands of years and others, like fiberglass, have only been introduced several decades ago.

Some of these techniques have experienced a renaissance over the last years in combination with the raise of the natural wine movement. Winemakers embrace ancient techniques and fermentation vessels, mostly made from clay. These can take the shape of qvevri, amphorae or similar vessels that are stored above or below ground. In most cases, they do not only serve as maturation container, but are also used for the fermentation (Robinson and Harding 2015).

Traditional Georgian Qvevri (also Kvevri or Quevri), if maintained correctly, can be used for centuries once they are installed underground, providing natural temperature control. The porous material is sealed with beeswax, which still allows for some oxygen to enter while waterproofing the inside and making it more easy to clean. They are used for the production of white and red wines, with the wines often being fermented with skins, seeds and stems, depending on the region resulting in tannin rich red and white, or rather orange, wines (McKirdy 2018). Qvevris come in sizes from 50 to 4000 liters depending on their field of application (Robinson and Harding 2015). Diaz et al. (2013) compared the conventional wines to Qvevri wines and found that while one could expect higher mineral content due to the storage in the clay vessels, this was not the case. The white wines did, however, show a higher concentration of phenolic compounds related to the prolonged skin contact. Figure 39 and Figure 40 show traditional Georgian Qvevri.

Figure 39: Qvevris in Chateau Zegaani (Totosashvili 2009)

Figure 40: Georgian Qvevri (Gokadze 2013)

Concrete has been used in winemaking since the 19th century but its use declined during the second half of the 20th century. In the last years, the popularity of concrete tanks has increased again. The tanks come in many different styles, including eggs (Figure 41) and pyramids. For wines that are matured on the lees, egg-shaped tanks offer a large surface area increasing interaction. Concrete tanks can be coated on the inside with epoxy resin or remain unlined except for a coat of cream of tartar to allow for more oxygen exchange. Other than the minimal oxygen contact, the material does not affect the flavor of the wine due to its inertness. Concrete tanks have good insulation properties, are relatively cheap and durable (Robinson and Harding 2015; Schiessl 2018; Grainger and Tattersall 2016; Denig 2019).

Figure 41: Egg shaped concrete tanks (Grainger and Tattersall 2016)

Fiberglass containers (Figure 42) are used as a cheaper alternative to stainless steel. Their use has several disadvantages, including the possibility for light to enter for some models, the possibility of residual styrene stemming from the polyester binding agent to dissolve in the wine. Their rough surface area makes them more difficult to clean and they have a lower heat conductivity. The tank sizes range from several hundred to more than 20,000 liters. The porous material can allow small amounts of oxygen to enter the system, which can be desired in some cases. In the last years, the technology has improved, but fiberglass and other plastic vessels are still rather uncommon, especially for the production of higher quality wines (Denig 2019; Jackson 2014; Grainger and Tattersall 2016; Carey 2009).

Figure 42: Fiberglass tanks(Vetroresina Toscana 2018)

5. Composition of wine

Water and ethanol make up around 97% of every wine, the ethanol content will vary depending on the type of wine from 5% in Moscato d'Asti to more than 20% in fortified wines like port and sherry (Puckette and Hammack 2015). The remaining three percent are a mixture of volatile and non-volatile compounds that are different for each wine. Figure 43 shows the chemical composition of an average wine. This chapter will focus on those three percent and some of the compounds that give each wine its own unique character.

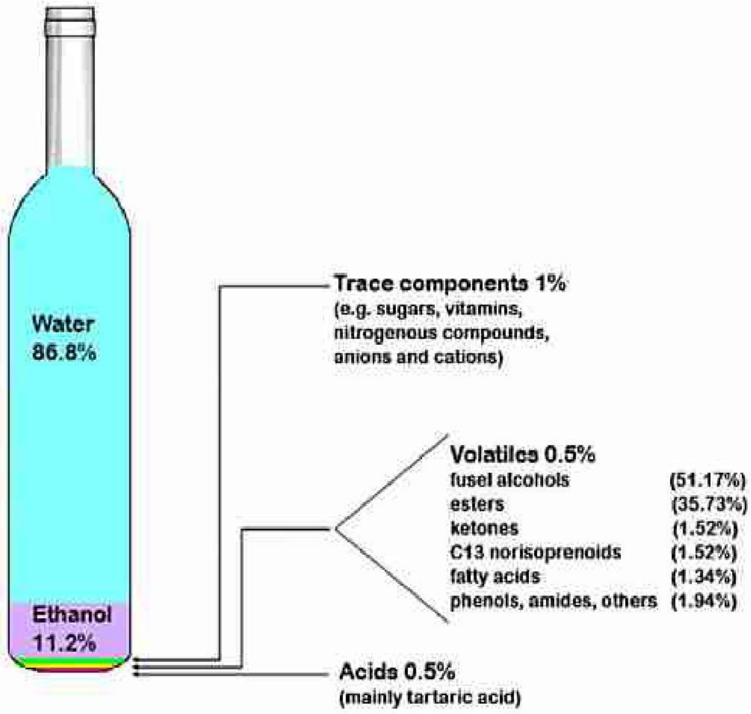


Figure 43: Chemical composition of wine in weight per volume, 11.2% w/v ethanol are equivalent to 14% v/v, composition will vary depending on the type of wine, its origin and several other factors, red wines additionally have about 0.5% w/v of different tannins (Sumby et al. 2010, p. 2)

The flavor of food is made up of two main components: volatile and non-volatile compounds. Volatile compounds contribute smell or aroma, depending on their combination and concentration. Non-volatile compounds mainly contribute to the taste and mouthfeel (Schreier 1979). Table 2 lists some sensory modalities in wine and some compounds causing them.

Table 2: Flavor sensations and some example compounds in wine (Polásková et al. 2008)

Sensory modality	**Attribute**	**Example chemical compounds in wine**
Taste	Sweet	Glucose, fructose, glycerol
	Sour	Tartaric acid
	Salty	Sodium chloride, potassium chloride
	Bitter	Catechin
Smell	Floral aroma, Earl Grey tea	Linalool
	Banana-like aroma	Isoamyl acetate
Chemesthesis	Mouth-warming/ heat	Ethanol
Tactile	Viscosity	Glycerol, polysaccharides
	Astringency	Tannins
Vision	Red	Malvidin-3-*O*-glucoside

5.1 Non-volatiles

The focus of this sub-chapter will be non-volatile constituents of wine that contribute to taste and tactile sensations of wine. In addition to a description of the compounds and their impact on the wine, there will be a short description of some of the methods for their quantification based on the *Compendium of International Methods of Analysis* published by the International Organisation of Vine and Wine (Organisation Internationale de la Vigne et du Vin 2020a).

The total dry extract is defined as all matter that is non-volatile under certain conditions. These conditions have to be such, that all matter undergoes as little physical and chemical changes as possible for the duration of the test. It includes a mixture of glycerol, sugars, non-volatile acids, glycols, minerals, phenolics, some nitrogenous compounds and traces of gums, pectins and proteins. In some cases, the sugar free extract, or non-reducing extract, is given which excludes fermentable sugars. Red wines have higher extract levels than white wines due to their higher concentration of tannin. Dry white wines reach non-reducing extract concentrations between 18 and 28 g/L. In red wines, the concentrations are a few grams higher. For sweet wines, the extract levels can reach up to 100 g/L, depending on the amount of residual sugar. Wines with lower extract levels (below 20 g/L) can appear light-bodied or thin in some cases, whereas wines with higher levels (above 30 g/L) will seem fuller-bodied. The determination of the extract used to be done by distilling the wine and measuring the residue of non-volatile substances that remained. Today, the criterion benchmark method (category I in the OIV methods) is OIV-MA-AS2-03A. For this, 10 mL of wine (or must) are added to a to a stainless steel dish containing 4-4.5 g of a washed and air dried filter paper. Once all the liquid is absorbed, the dish is placed in a 70°C oven for two hours under a defined airflow. Another method (type IV, auxiliary method) is OIV-MA-AS2-03B, which is faster but can be less accurate. It is a densimetric determination of the alcohol-free wine (d_r) using the specific gravity of the wine corrected for volatile acidity (d_v) and a water-alcohol mixture of the same strength (d_r) at 20°C (Equation 3). The result is determined by using a reference table (Robinson and Harding 2015; Weinhalle 2020; Zoecklein et al. 1999; Ribéreau-Gayon et al. 2006; Organisation Internationale de la Vigne et du Vin 2020a)

Equation 3: Determination of the specific gravity of alcohol-free wine

$$d_r = d_v - d_a + 1.000$$

The most abundant non-volatile substance is glycerol (Figure 44). It is formed during fermentation and can be found in a wide range of concentrations, which is generally higher in red wine (~10 g/L) than in white wine (~7 g/L). The amount depends on various fermentation factors, including yeast strain and fermentation temperature and can be higher in ice and botrytised wines, reaching up to 42.4 g/L in botrytised wines and between 7.06 and 25.04 g/L in ice wines. While it used to be believed that glycerol contributes to mouthfeel, this is unlikely, as studies have shown that concentrations above 25 g/L are needed to cause a detectable difference in model wine. It can contribute to the sweetness when present above its taste threshold, which was determined at 5.2 g/L in white wines and 7.5 g/L in dry red wine (Noble and Bursick 1984; Gawel and Waters 2008; Nurgel and Pickering 2005; Hufnagel and Hofmann 2008). Different methods have been used for the quantification, including High Performance Liquid Chromatography (HPLC, OIV-MA-AS311-03) and colorimetric methods (OIV-MA-AS312-04; OIV-MA-AS312-05), which are all type IV methods, as well as GC (Ough et al. 1972; Organisation Internationale de la Vigne et du Vin 2020a).

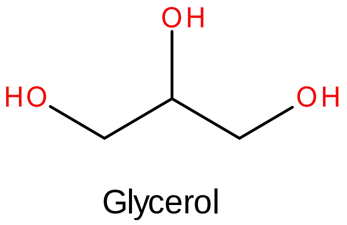


Figure 44: Structure of glycerol

When Hufnagel and Hofmann (2008) reconstructed the non-volatile sensometabolome of a dry red wine, they found that the only other compound contributing to the perceived sweetness of the wine was fructose. Together with glucose, it makes up most of the fermentable sugars that are found in grape juice and subsequently wine. Fructose has a higher relative sweetness and is generally present in higher concentrations in the final wine. This is related to the fact that most yeast strains are glucophilic and therefore first ferment the available glucose (Bakker and Clarke 2012; Kunkee and Eschnauer 2003). The quantification of glucose and fructose can be done photometrically using an enzymatic assay (OIV-MA-AS311-02), HPLC (OIV-MA-AS311-03) (Organisation Internationale de la Vigne et du Vin 2020a) or with the use of a test kit (for example Clinitest®). Depending on the concentration of residual sugars, wines are divided in four categories: dry (up to 4 or 9 g/L, depending on the acidity level of the wine), medium dry (up to 12 or 18 g/L, also depending on the acidity), medium sweet (12 to 45 g/L) and sweet (more than 45 g/L) (Österreich Wein Marketing GmbH 2018a).

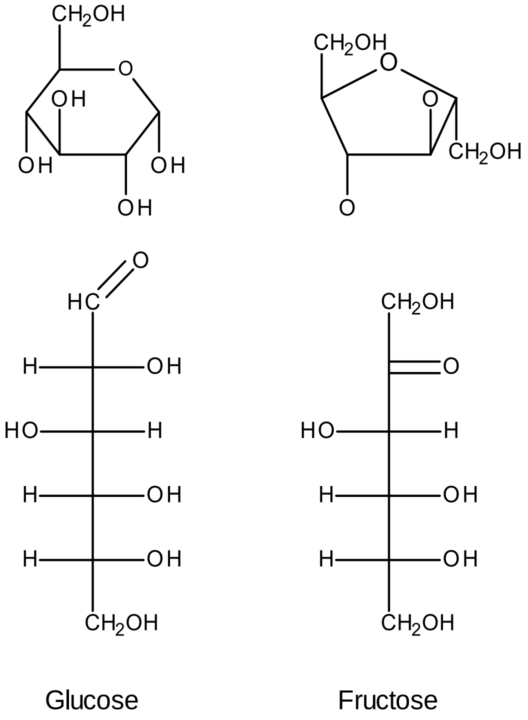


Figure 45: Structure of glucose and fructose in Haworth (top) and Fischer (bottom) projection

Other groups of carbohydrates are sugar alcohols, including glycerol, sugar acids and polysaccharides. The first two groups have a negligible effect on the sensory properties of wine in most cases as most of the compounds are present in concentrations below their sensory threshold (Waterhouse et al. 2016). The third group, polysaccharides, can be divided into two groups; the first are grape derived polysaccharides, including those rich in arabinose and galactose, like rhamnogalacturonans I and II and arabinogalactan-proteins (Martínez-Lapuente et al. 2013). The second group are those derived from microorganisms. The main group are mannoproteins, which are derived from the cell walls of yeasts. Their concentration is higher in wines that have been aged on lees. Due to their interaction with tannins polysaccharides can influence astringency and in white and rosé wines they can prevent the formation of protein haze and tartrate crystals (Waterhouse et al. 2016; Robinson and Harding 2015).

The main contributors to the sourness of a wine are organic acids. Only six different acids are responsible for more than 95% of the total organic acids. They are non-volatile, with the exception of acetic acid. The most abundant are tartaric and malic acid, with typical concentrations between 2 and 7 g/L each. The concentration of malic acid depends on two main factors: The ripeness of the grapes at harvest and the occurrence of malo-lactic fermentation. Unripe grapes increase the abundance, while malo-lactic fermentation reduces the concentration as malic acid is metabolized by lactic acid bacteria into the less sour lactic acid (see 4.3.2). Tartaric acid is the other main acid originating in the grapes. Unlike malic acid, its concentration is relatively stable over the ripening process and unlikely to be metabolized during fermentation. While it is produced in many plants, it is mainly member of the Vitaceae family that produce it in significant amounts. Its salts have been used to identify vessels that at some point most likely contained wine from the Neolithic era (McGovern et al. 2017). During the aging of wine, potassium or calcium tartrate crystals can form a precipitate. This process can be accelerated by cooling (see 4.4). The other four are lactic, acetic, citric and succinic acid, which are produced during primary or secondary fermentation. Small amounts of citric acid can also be present in the grapes (Waterhouse et al. 2016; Jackson 2014). For the determination of the total acidity, the OIV method (OIV-MA-AS313-01, type I) is titration, either potentiometric or using bromothymol blue as indicator against an alkaline sodium hydroxide solution (Organisation Internationale de la Vigne et du Vin 2020a).

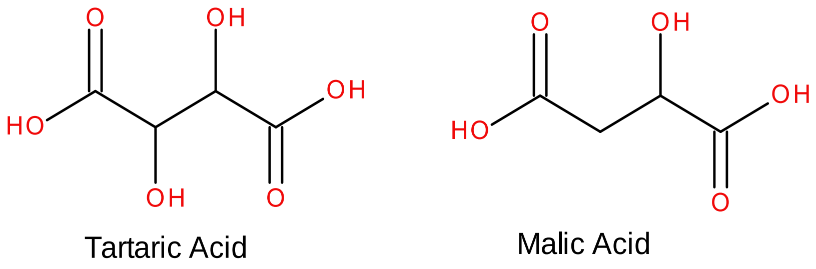
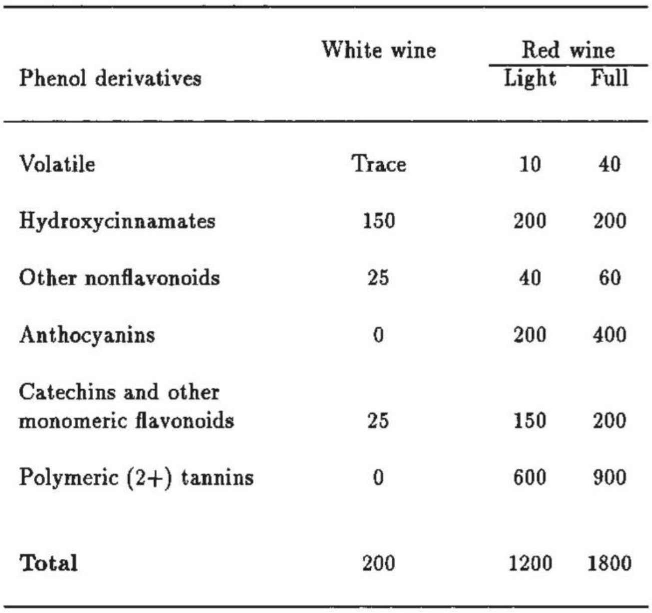


Figure 46: Structure of tartaric acid and malic acid

Another important group are non-volatile phenolic compounds. They are responsible for the astringency, bitterness of wines and for the color of red and, to a lower degree, white wine. In addition, they serve as oxygen reservoirs and substrates for browning reactions. They are present in both, red and white wines, but are a lot more abundant in red wines. The levels in red wines are commonly more than five-times higher than the ones in white wines (Figure 47), due to the prolonged skin contact time for red wines. Most phenolic compounds originate in the grapes and are located mainly in the skin and the seeds of the berries. A few can be extracted from oak cooperage. While the understanding of some phenolic compounds, mainly red pigments and tannins, has increased in recent years, there are still many unknown or only partially understood phenomena, like their production, stability and the relevance of individual compounds. It is therefore not surprising that phenolics have been the focus of various research efforts since the late 19th century (Waterhouse et al. 2016; Kennedy et al. 2006).



Phenol derivatives	White wine	Red wine	
		Light	Full
Volatile	Trace	10	40
Hydroxycinnamates	150	200	200
Other nonflavonoids	25	40	60
Anthocyanins	0	200	400
Catechins and other monomeric flavonoids	25	150	200
Polymeric (2+) tannins	0	600	900
Total	200	1200	1800

Figure 47: Concentration of different phenols in young wines, simplified, in gallic acid equivalents (GAE) (Singleton 1992)

Phenols are compounds that have a hydroxyl group attached to a benzene ring. When the compound includes more than one phenol ring they can be described as polyphenols. Polymeric polyphenols are often called tannins, which stems from the ability of these compounds to tan animal hide to produce leather. Another way of grouping phenolic compounds in wine is into flavonoids and non-flavonoids. Flavonoids have a distinct basic structure (Figure 48) including two phenyl rings (A and B) and an oxygen containing pyran-bridge. This group includes three main types of compounds: flavan-3-ols (catechins), anthocyanins (red colored polyphenols) and flavonols (Kennedy et al. 2006; Waterhouse et al. 2016).

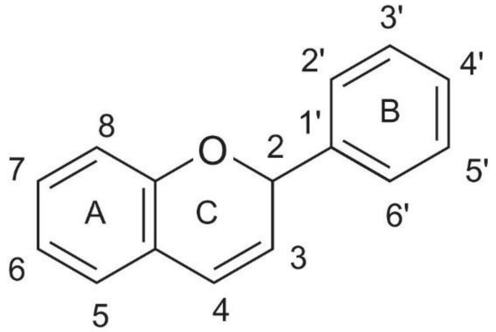
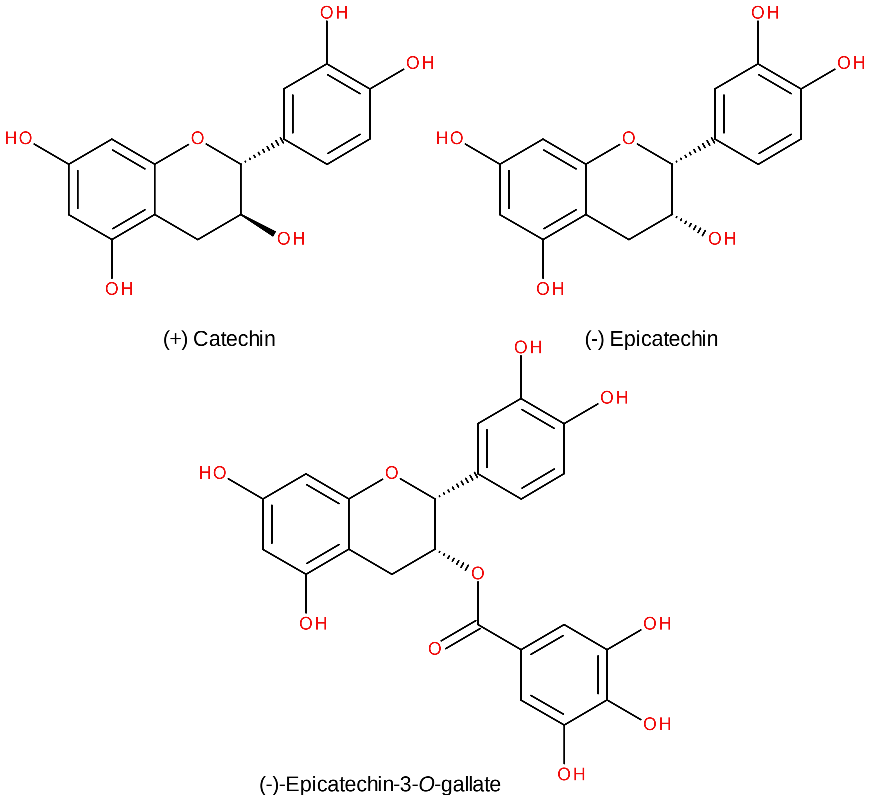


Figure 48: Basic flavonoid skeleton (Jackson 2014, p. 360)

In grape berries, flavan-3-ols are present in both, skin and seed. The three main monomeric representatives in wine are (+)-catechin, (-)-epicatechin and (-)-epicatechin-3-*O*-gallate (Figure 49), with their distribution depending greatly on the grape variety. In red wine between 25% and 50% of the phenolic compounds in a wine are oligomers and polymers of flavan-3-ol units. They are most probably the result of biochemical condensation of monomer units. The resulting oligomers and polymers (condensed tannins) are called proanthocyanidins. Their sensory impact on the wine depends on the degrees of polymerization. The monomers are bitter and astringent, with increasing size the bitterness decreases and the astringency increases (Singleton 1992; Waterhouse et al. 2016). During aging, self-polymerization with flavan-3-ol monomers, as well as condensations with other compounds, including polysaccharides and anthocyanins can happen. With increasing size, precipitation of the molecules becomes more likely due to a decrease in solubility (Jackson 2014).



(+) Catechin

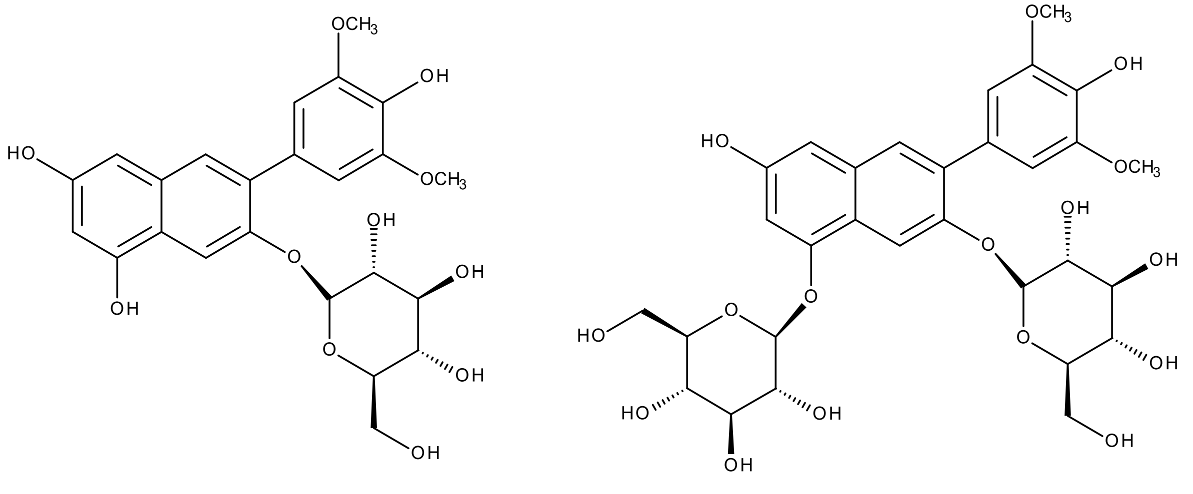
(-) Epicatechin

(-)-Epicatechin-3-*O*-gallate

Figure 49: Structure of the three main flavan-3-ols

Anthocyanins are the glycosylated form of the anthocyanidin, which is unstable in its natural state. This group of compounds is responsible for the color of red grapes and therefore red wine. In *vitis vinifera* cultivars the main representatives are the 3-*O*-glucosides while in other vitis species and their hybrids 3,5-di-*O*-glucosides, for example malvidin-3,5-di-*O*-glucoside, are present as well (Figure 50). Van Buren et al. (1970) compared the anthocyanin composition in 151 cultivars, including first generation hybrids like Isabella (55% diglucoside pigments) and

Concord (40% diglucoside and 20% acylated pigments). For this reason, the presence of non-monoglucosilated pigments has been used as marker for the presence of non-vinifera grapes in wine production. Most recent hybrid cultivars do not produce diglucosidic pigments, due to the high amount of *vinifera* cultivars in their pedigree. In most vinifera cultivars, the main anthocyanin is malvidin-3-*O*-glucoside. In their free form, anthocyanins are not very stable. In young wines, there are five types of anthocyanins that are in a dynamic equilibrium with each other. Out of those five, three are colorless, one is blue violet, one pale yellow and only one is red: the flavylium cation of anthocyanins. Its concentration depends on several factors including the pH and the amount of free SO_2. When the level of one of the factors raises, the equilibrium is shifted towards the other forms. Over time, larger complexes with catechins, proanthocyanidins and other compounds present in wine form and new, stable (polymeric) pigments occur. When the interaction between the anthocyanins and other phenolic compounds are non-covalent, this is known as co-pigmentation. The newly formed pigments are more stable towards bleaching by bisulfite, less likely to degrade during storage and have a higher light absorbance at the natural pH of wine (Waterhouse et al. 2016; Jackson 2014).



Malvidin-3-*O*-glucoside

Malvidin-3,5-di-*O*-glucoside

Figure 50: Structure of Malvidin-3-O-glucoside and Malvidin-3,5-di-O-glucoside

Flavonols make up between 1-10% of the total phenolic compounds in red wine, the smallest fraction of the flavonoid compounds. In the grapes, they, together with anthocyanins, function as a sunscreen for the berries. As such, they are mainly found in grape skin. Studies show that higher sun exposure leads to increased levels of flavonol compounds in the fruit (Price et al. 1995; Matus et al. 2009). The main representatives are kaempferol, myricetin and quercetin (Figure 51). Due to their low concentration it is unlikely that flavonols, which have a bitter taste, impact the overall flavor of wine (Jackson 2014; Waterhouse et al. 2016).

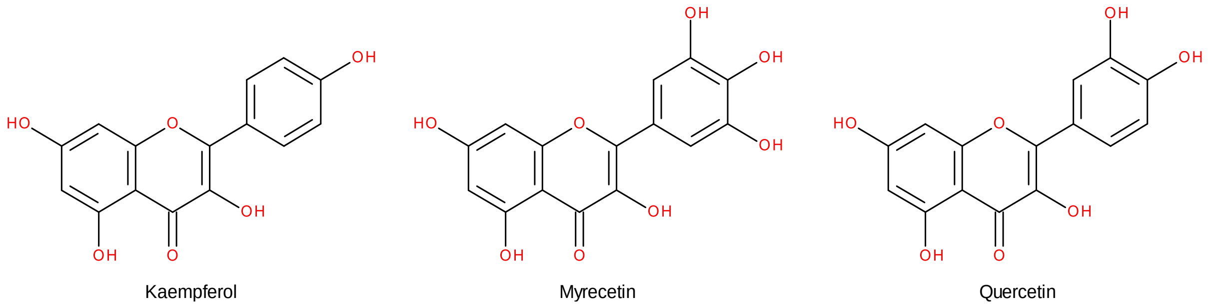
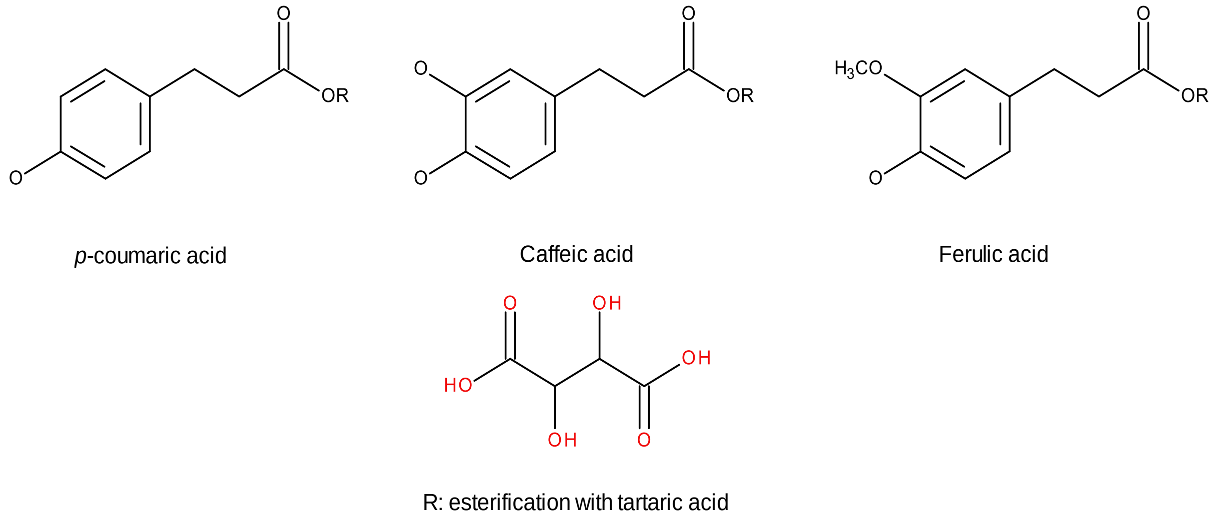

Kaempferol Myrecetin Quercetin

Figure 51: Structures of the three most abundant flavonols: kaempferol, myricetin and quercetin

Unlike flavonoids, non-flavonoids are extracted from the pulp. Therefore, they are present in similar levels in red and white wines, but these levels are usually below the respective sensory threshold of the compounds (Vèrette et al. 1988). Some of the important subclasses of non-flavonoids are stilbenes, including resveratrol that has been associated with health benefits (Jang et al. 1997), benzoic acids and hydroxycinnamates. The latter are present in the highest concentration of the non-flavonoid compounds and make up the largest group of phenolics in white wine. They are generally present esterified with tartaric acid and not their free acid form, with the three main ones being coumaric, caffeic and ferulic acid tartrate ester (Figure 52) (Zoecklein et al. 1999; Waterhouse et al. 2016; Kennedy et al. 2006).


p-coumaric acid Caffeic acid Ferulic acid

R: esterification with tartaric acid

Figure 52: Structure of the three main non-flavonol phenolic compounds in wine

Nitrogen is one of the most abundant elements on the planet and an important nutrient for plants and microorganisms. As they cannot process elemental nitrogen, it is usually taken up in the form of nitrate, ammonia or urea. In the finished wine, nitrogen is present in several different forms, mostly related to amino acids like oligo- and polypeptides and proteins.

Amino acids present in grape juice serve as the main source of nitrogen during fermentation. Deficiencies in free *alpha*-amino nitrogen have been linked to sluggish or incomplete fermentation. The amino acids proline, arginine, valine and alanine are the most abundant in must. Proline, as a secondary amino acid, is not utilized by yeast under anaerobic conditions, therefore its concentration remains mostly stable over the course of the winemaking process (Zoecklein et al. 1999). Some amino acids have taste properties, however, Hufnagel and Hofmann (2008) found no amino acid that exceeded its respective threshold levels. While they might not participate in the flavor of the wine, they can be an important source for some flavor compounds, like aldehydes, organic acids, lactones and higher alcohols (Jackson 2014).

An oligopeptide is defined as a molecule containing up to 4 amino acids. Of the oligopeptides occurring in wine, the best studied is glutathione, which is a tripeptide containing glycine, cysteine and glutamine. Glutathione (Figure 53) occurs in the grapes and is additionally produced during fermentation by yeast and helps with the prevention of browning and other oxidative reactions (Waterhouse et al. 2016; Ribéreau-Gayon et al. 2006).

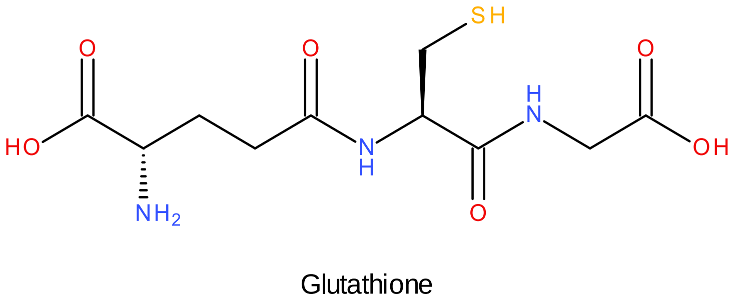


Figure 53: Structure of glutathione

Proteins are large polypeptides, usually containing more than 100 amino acid monomers. Their main source are the grapes, the pulp in particular. Red wines have been shown to contain lower concentrations of proteins than white wines as proteins tend to bind to tannins and precipitate. In wines with lower tannin concentrations, white and rosé wines, concentrations of several hundred mg/L of proteins have been found. Their presence can become a problem as they tend to precipitate and form a hazy “protein casse”, especially when the wines are stored at elevated temperatures. To prevent this, different fining agents, including bentonite and silica sol, can be used to reduce the amount of proteins present (4.4 above) (Zoecklein et al. 1999; Waterhouse et al. 2016; Jackson 2014).

Another group of nitrogen containing compounds that can be found in wine are biogenic amines, out of which the best known and probably most harmful one is histamine (Figure 54). In high concentrations their presence has been linked to headaches, allergic reaction and other adverse reaction in some people. They are a result of the decarboxylation of amino acids during the metabolism of lactic acid bacteria including some strains of *Oenococcus oeni*. Their concentration can be significantly reduced by fining the wine with bentonite (Lonvaud-Funel 2001; Jackson 2014). The OIV standard method (type II) for their determination and quantification is an HPLC after the derivatization of the compounds (Method OIV-MA-AS315-18 (Organisation Internationale de la Vigne et du Vin 2020b).

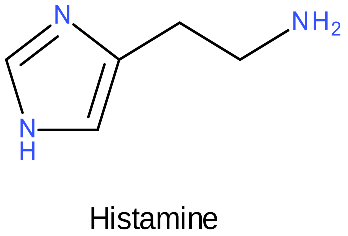


Figure 54: Structure of histamine

Ethyl carbamate (Figure 55) is another nitrogen containing compound with medical relevance as it is a known carcinogen. In wine, it has two formation pathways; the main one is from urea which is the result of the degradation of arginine by yeast, and the second one is from arginine degradation to citrulline by lactic acid bacteria. In the EU and Canada, the ethyl carbamate concentration has to be below 30 µg/L (Waterhouse et al. 2016; Stevens and Ough 1993). The OIV method (type II) is a selective detection using gas chromatography with mass spectrometry (Method OIV-MA-AS315-04 (Organisation Internationale de la Vigne et du Vin 2020b). Volatile nitrogen compounds including pyrazines will be discussed later.

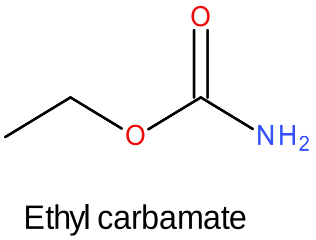


Figure 55: Structure of ethyl carbamate

The ash of a wine describes the total content of non-volatile inorganic compounds. It is the result of the careful ashing of the extract and includes carbonates and oxides formed during the process, as well as those that are naturally present. The composition of the ash depends on several factors, some are listed in Figure 56. In general, the total concentration of inorganic compounds is analyzed. This is made up of the primary concentration of inorganic material that enters the grape through the roots of the grapevine and the secondary concentration, which stems from contamination, either natural or man-made (anthropogenic). The differentiation between primary and secondary concentration is almost impossible, as one would have to ensure that every step of the wine production was contamination free. The primary concentration is usually the larger part and depends on the specific soil the vines are grown on. In addition, the concentration will depend on climatic conditions; wines from warmer climates show higher potassium levels compared to wines from cooler climates.

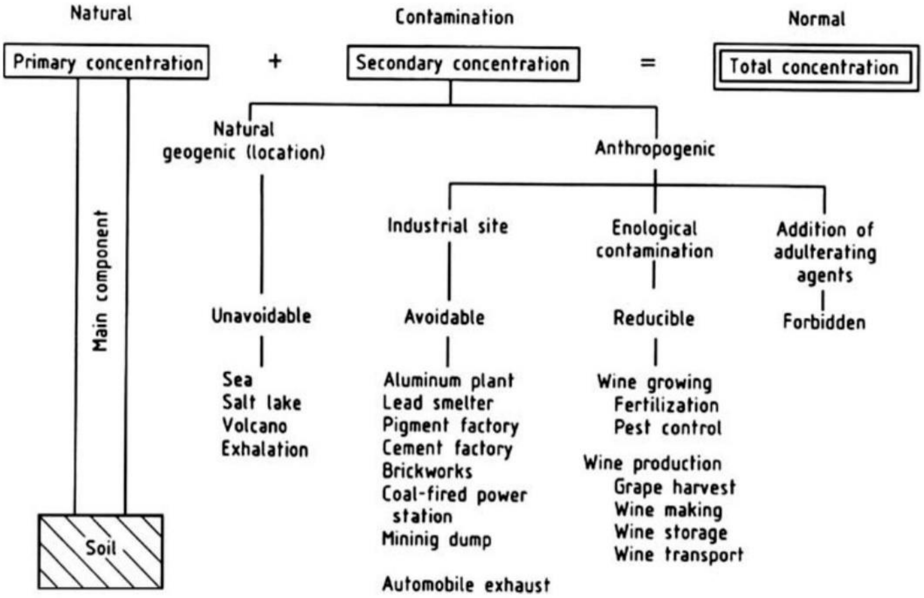


Figure 56: Primary and secondary sources of minerals in wine (Kunkee and Eschnauer 2003, p. 10)

The inorganic fingerprint of wines has been used in the past to verify their place of origin with different analytical techniques including atomic absorption spectrometry, inductively coupled plasma (ICP) optical emission spectrometry and ICP mass spectrometry. The natural part of the secondary concentration is almost unavoidable and depends on natural phenomena around the vineyard, like seaside, volcanos and others. The anthropogenic contamination can be caused by industrial sites close to the vineyard, like different factories, highways and cities. It can also be used as a term to describe inorganic substances added with enological treatments like fertilizers and plant protection agents, although the uptake of contamination is minimal, as the roots of the plant act as a natural filter, especially for heavy metals. Other ways for inorganic contamination are the metal containers and fining agents, as well as the addition of illegal additives. Some metals present are, at their natural levels, important co-factors for some vitamins, others, like mercury, lead and cadmium, pose a potential health risk, if they are present in high concentrations. Other metals can cause problems if they are present at higher than normal levels, causing haziness (copper and iron) or the precipitation of tartrate crystals (calcium). Impacts on the sensory properties are also possible, but only at very high concentrations. Fining techniques for the removal of different metals are available: In Möslinger or blue fining, metals are almost completely removed (iron, copper, cobalt and zinc) or to a large amount (cadmium, manganese, lead and vanadium) with potassium ferrocyanide. This is only done in few cases as improper conduction can result in the formation of hydrogen cyanide (Kunkee and Eschnauer 2003; Jackson 2014; Rodrigues et al. 2011).

5.2 Volatiles

The composition of volatile compounds is responsible for the unique smell of a wine. More than 800 different compounds have been identified, which results in the great complexity of wine aroma (Belitz et al. 2012; Rapp 1990). To be volatile, a compound has to fulfill certain requirements, like low boiling point and molecular weight. Most volatile compounds do not exceed a molecular weight of 300u (Buettner 2017). This applies to a large number of compounds, but not all volatiles are also odor active. In fact, less than 5% of the volatiles actually contribute to the overall impression (Grosch 2000). For a compound to be odor active there has to be a corresponding odorant receptor for the compound to bind to. When the compound binds to the receptor, a signal is transmitted to the brain. That signal then has to be recognized by the brain and interpreted (Pickenhagen 2017). With a limited set of odor receptors that is specific to each individual person, the perception of the aroma of any wine can differ greatly. In addition to the individual differences in perception, there are sensory phenomena that have an impact on the perception of a wine. Some compounds, mainly those that cause wine faults, will have a masking effect, while others will enhance each other in synergistic and additive effects, which is the case for some esters as well as some sulfur and terpene compounds. For untrained people it is almost impossible to determine the constituents of a mixture of three or more aroma compounds. Figure 57 shows the results of a study done by Laing et al. (1991), illustrating the decrease of correctly identified odors with increasing number of compounds in one mixture.

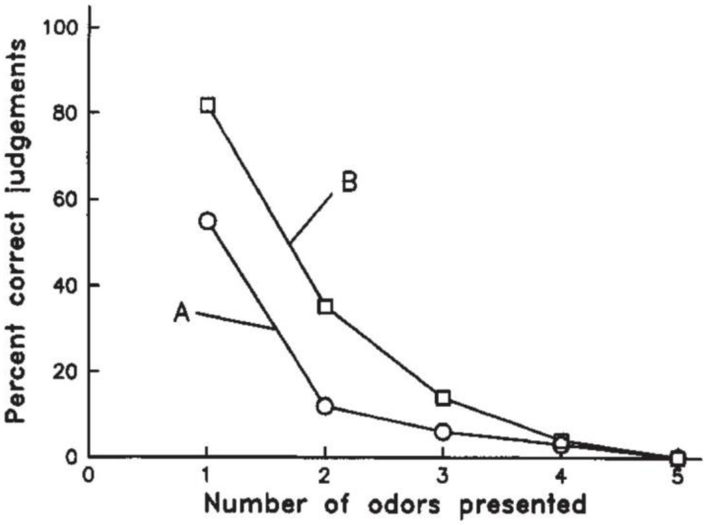


Figure 57: Number of correct judgments on mixtures of different aroma compounds, completely correct (A) and with one or more correct odors chosen including incorrectly chosen ones (B) (Laing et al. 1991, p. 247)

The final odor impression of a wine is the sum of many complex interaction phenomena that sometimes make it difficult to evaluate the impact one single compound has on the overall smell. This does not only depend on the compound's concentration, but also on its sensory threshold. An indicator that can be used is the OAV, which has been described on page 4. Another influence factor on how wine aroma is perceived, are the constituents of the non-volatile extract by increasing the volatility of some and others by influencing the overall flavor of the product (Darriet and Pons 2017).

Every step in the winemaking process, from the age of the vines, their location and the cultivar to the choice of maturation to storage conditions and the fining agents used, can have an influence on the volatile composition of wine. Depending on their origin in the winemaking process, volatile compounds are usually divided into three groups: Primary, secondary, and tertiary compounds. Primary aroma compounds are present in the grapes either in their free form, like methoxypyrazines and monoterpenoids in some cultivars, or as non-volatile precursor compounds, like it is the case for varietal thiols, which are present as *S*-conjugates and different others that are present as glycol conjugates. These precursor compounds are released during the winemaking process and contribute to the final aroma (Baumes 2009; Waterhouse et al. 2016; Ilc et al. 2016). Most other aroma compounds present in grapes and grape juice, are less relevant for the final aroma of wine. Some C_6 compounds and aldehydes, which form enzymatically due to lipid oxidation caused by mechanical damage, can, however, function as precursor compounds for fermentation derived esters, including acetate and ethyl esters (Dennis et al. 2012; Waterhouse et al. 2016). Secondary compounds are generated during the winemaking process as a direct result of fermentation. This is generally the largest group of volatile compounds present in the highest concentrations. This group includes esters, higher alcohols and volatile fatty acids among others that are present in all wines. Therefore, they are sometimes referred to as the backbone of a wine. Depending on the microorganisms carrying out the fermentation the concentration of these compounds can vary greatly (Ugliano and Henschke 2009). And finally, the tertiary compounds that are associated with maturation and aging of wines. They can be derived from the cooperage or develop over time as degradation products of other compounds including carotenoids (Pérez-Coello and Díaz-Maroto 2009).

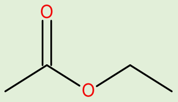
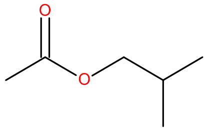
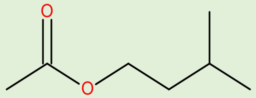
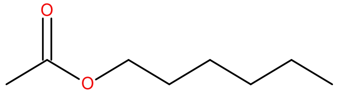
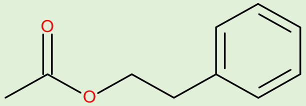
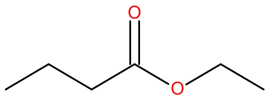
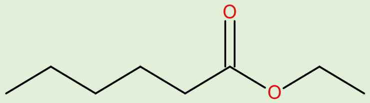
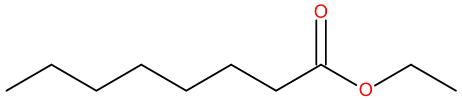
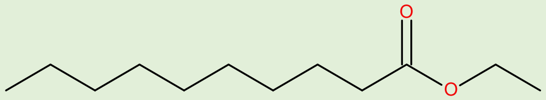
5.2.1 Backbone of wines

Some of the most abundant volatile compounds present in wine are fermentation derived. Depending on the microorganisms the composition and the concentration of the resulting compounds will vary, even the use of different strains of the same organism can give significantly different results. Chapter 4.3 discusses some of the microorganisms and how they carry out the transformation of hexose sugars to ethanol and carbon dioxide. This part will focus on some of the volatile compounds that are produced during the process, the secondary aroma compounds. Additionally, some of the compounds that are the result of the maturation process, tertiary compounds, will be discussed.

Some of the most abundant volatile compounds in wine are fermentation-derived esters, in particular ethyl esters and acetate esters. They contribute to the fruity aroma of wines, in particular in younger wines as the concentration of esters decreases over time due to hydrolysis (Ugliano and Henschke 2009). Table 3 lists some of the most important esters in wine and their properties. Esters form as condensation products of alcohols and different acids. Some of the most common esters are products of the esterification of acetic acid with higher alcohols and those of ethanol and fatty or other non-volatile organic acids. These reactions result in the formation of acetate esters and ethyl esters, respectively. However, the direct reaction is too slow to account for the ester concentration in wine. The formation of straight chain (aliphatic) esters, the acids have been activated with acetyl-S-CoA, while the synthesis of ethyl esters of branched, short chain fatty acids might be direct. Due to the large variety of alcohols and acids, the amount of possible esters is considerable. (Lambrechts and Pretorius 2000; Miller et al. 2007; Jackson 2014). Depending on the strain(s) of yeast(s) used, the concentration of esters will vary significantly (Mateo et al. 1992; Miller et al. 2007). Additionally, the final ester composition depends on several other factors, including grape variety and fermentation temperature (Jackson 2014).

The most investigated among the esters is ethyl acetate, the reaction product of acetic acid and ethanol. Its influence on the wine aroma dramatically changes depending on the concentration. While its presence contributes to the complexity of a wine at low concentrations (below 50 mg/L), in higher concentrations, generally above 150 mg/L, it produces a vinegary or nail polish remover off-flavor. High concentrations are a sign of bacterial contamination, as bacteria not only produce ethyl acetate directly but can also produce acetic acid, which can react with ethanol, resulting in even higher concentrations (Jackson 2014).

Table 3: Important esters and some of their properties (Waterhouse et al. 2016; Lambrechts and Pretorius 2000)

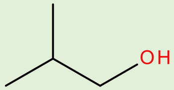
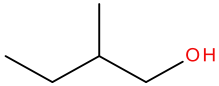
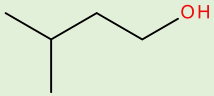
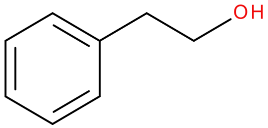
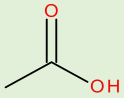
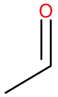
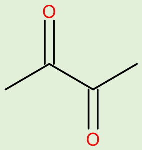
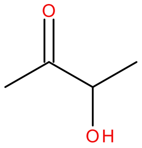
Name	Odor description	Typical concentration [µg/L]	Sensory Threshold in model wine [µg/L]	Structure
Ethyl acetate	Nail polish remover, fruity	5,000-63,000	12,000	
Isobutyl acetate	Banana, cherry	ND-800	1,600	
Isoamyl acetate	Banana	30-8,100	160	
Hexyl acetate	Green apple, sweet	ND-260	1,800	
2-Phenylethyl acetate	Honey, rose	ND-4,500	2,400	
Butanoic acid ethyl ester	Fruity	10-1,800	20	
Hexanoic acid ethyl ester	Apple peel, fruity	150-3,400	14	
Octanoic acid ethyl ester	Fruity, fat	140-3,800	5	
Decanoic acid ethyl ester	Grape	14-2,100	200	

Another quantitatively important group of fermentation derived compounds are higher or fusel alcohols. These are alcohols with more than two carbon atoms (Jackson 2014). Some of the most important representatives are either aliphatic, like 2-methylpropanol (isobutanol), 2- and 3-methylbutanol (active amyl and isoamyl alcohol), or phenolic, like 2-phenylethanol (Ugliano and Henschke 2009) (Table 4). While higher alcohols can be detrimental to wine quality above a certain concentration (>400 mg/L), below 300 mg/L they contribute to the complexity of wine flavor (Rapp and Versini 1995). Because of its distinct floral rose aroma, this is not necessarily true for 2-phenylethanol. It is therefore generally not considered when referring to fusel alcohols (Kunkee and Eschnauer 2003). The concentration of higher alcohols heavily depends on the yeast strain used, as for all fermentation derived compounds, as well as the must composition, including the concentration of amino acids, which serve as precursor compounds, and fermentation conditions (Swiegers et al. 2005).

Volatile straight and branched chain organic acids of different lengths are also generally part of the aroma of wines. The chain lengths range from short (C_2-C_4) over medium (C_6-C_{10}) to long chain (C_{12}-C_{18}). Their smell changes depending on the chain length. Longer chain fatty acids are less volatile and have odors reminiscent of cheese, sweat and goat. Shorter chain fatty acids have rather particular smells, for example of vinegar (acetic acid, C_2) or of rancid butter and sweat (butyric acid, C_4). Fatty acids also serve as precursor compounds for different esters. The most abundant compound in this group is acetic acid. It usually accounts for more than 90% of the volatile acidity of wine (Ugliano and Henschke 2009). In healthy wines, the concentration of acetic acid is usually close to its sensory threshold. Higher concentrations are an indicator for bacterial spoilage (Waterhouse et al. 2016).

The last group of fermentation derived aroma compounds discussed here, are carbonyl compounds, like aldehydes and ketones. the main aldehyde in wine is acetaldehyde, which accounts for more than 90% (Jackson 2014). When free SO_2 is present in wine, most of the acetaldehyde is bound and less than 1% is present in its free form. When present in concentrations above its sensory threshold, the odor description changes with increasing concentrations from fruity at low concentrations to rotten apple at higher levels. Saturated aldehydes, with a chain length between C_3 and C_9, can contribute herbaceous and grassy, as well as fruity and sometimes pungent aromas. Other aldehydes found in wine, are derived from the oxidation of higher alcohols and are especially prevalent in aged wines (Waterhouse et al. 2016; Ugliano and Henschke 2009).

Table 4: Other fermentation derived compounds and some of their properties (Swiegers et al. 2005; Lambrechts and Pretorius 2000; Waterhouse et al. 2016)

Name	Odor description	Typical concentration [µg/L]	Sensory Threshold in model wine [µg/L]	Structure
Isobutanol	Fusel, spirituous	9,000-174,000	40,000	
Active amyl alcohol	Solvent, fusel	15,000-150,000	65,000*	
Isoamyl alcohol	Harsh, nail polish	6,000-490,000	30,000	
2-Phenylethanol	Floral, rose	4,000-197,000	10,000	
Acetic Acid	Vinegar, sour	2,500-500,000	400,000	
Acetaldehyde	Sour, green apple	ND-211,000	100,000	
Diacetyl	Butter	5-7,500	100	
Acetoin	Butter, cream	100-60,000	150,000	

* in beer

One of the ketones most connected to wine is formed from acetaldehyde during malolactic fermentation: Diacetyl. It is responsible for the buttery, nutty aroma of wines that underwent MLF. When the concentration reaches levels above 2-5 mg/L, this changes to undesirable rancid butter notes. Diacetyl is also formed during alcoholic fermentation with yeast. In this case, however, most of it is further metabolized to acetoin and then to 2,3- butanediol. Both of these compounds have lower sensory threshold and, therefore, a lower impact on the aroma (Hirst and Richter 2016; Bartowsky and Henschke 2004).

Another group of aroma compounds, next to those derived from fermentation, which are present in a wide variety of wines, are those that develop during maturation and aging. Both processes have been discussed in chapter 4.5, including different possibilities of cooperage for maturation, like oak and stainless steel. The processes occurring during maturation are also referred to as oxidative aging, while those in the bottle are called reductive aging (Darriet and Pons 2017).

During maturation, wine can be exposed to different amounts of oxygen, which depends on the storage conditions, as well as the type of cooperage. Small amounts of oxygen can be beneficial for the stabilization of color in red wine (Jackson 2014), but also for the formation of some oxidation related compounds that can contribute to the complexity of wine at low concentrations. At higher concentrations, this changes and too much exposure generally results in a negative effect (Oliveira et al. 2011). Oxidative conditions will result in increased concentrations of aldehydes, including acetaldehyde. Their formation depends on different factors, including the levels of oxygen present, the age of the wine, as well as the available SO_2 and polyphenols (Bueno et al. 2018; Culleré et al. 2007). One of the compounds found in the aroma of wines that have been exposed to a high amount of oxygen is sotolon (3-hydroxy-4,5-dimethyl-2(5H)-furanone). In high concentrations, this compound has a distinct curry aroma. When diluted, the odor description can change to nutty, dry fruit and typical aromas of fortified wines, like Port and Madeira. It was also found in white wines produced from botrytised grapes (Silva Ferreira et al. 2003a; Darriet and Pons 2017). In table wines, a high concentration of this compound and others, like methional (3-Methylsulfanylpropanal) and phenylacetaldehyde, are part of oxidation related off-flavors (Silva Ferreira et al. 2003b).

During bottle aging, one of the main changes that occur is the partial loss of fruity fermentation aromas, mostly related to the hydrolysis of different esters. The concentration of fatty acid ethyl ester remains fairly constant during wine maturation due to their slow hydrolysis. This is not true for acetate esters, which are hydrolyzed more rapidly. The concentration of some important esters, like hexyl and isoamyl acetate, reduces by up to 80% over the span of two years when the wines are stored at 30°C (Rapp and Mandery 1986; Ramey and Ough 1980; Jackson 2014).

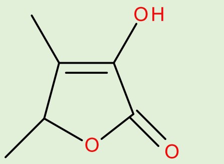
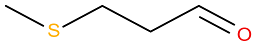
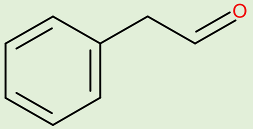
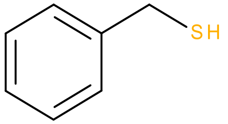
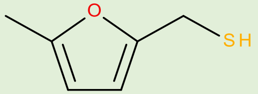
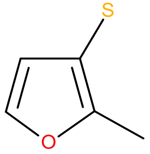
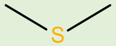
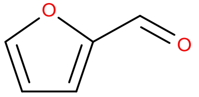
The aging of wines is also connected to the loss of certain varietal flavors, in particular terpenes, which are important for wines from Muscat cultivars but are present in others too. In Riesling wine, for example, a loss of more than 80% of linalool to below its sensory threshold was observed over the course of three years (Jackson 2014). While the concentrations of some terpene compounds, including linalool, nerol and geraniol, decrease, concentrations of α-terpineol, 1,8 and 1,4 cineol and different linalool oxides increase (Marais 1983; Slaghenaufi and Ugliano 2018). The aroma of these compounds is less floral and in most cases their sensory thresholds are higher, 100 µg/L for linalool compared to 3,000 to 5,000 µg/L for different linalool oxides (Rapp 1988). Another group of compounds related to the loss of varietal character are thiols due to oxidation (Herbst-Johnstone et al. 2011). Both, terpenes and thiols, will be discussed in the next part about varietal compounds (5.2.2).

A compound that has been found in aged wines from different cultivars and toasted oak is 2-furanmethanethiol. Due to its low sensory threshold of 0.4 ng/L its presence, even in small concentrations, can cause a strong roast coffee aroma (Tominaga et al. 2000b). It was found alongside 2-methyl-3-furanthiol in Champagne and other red and white wines from different French appellations. This compound, which on its own has an aroma of cooked meat, was found to contribute to the toasty aroma of some barrel aged wines after bottle aging (Tominaga and Dubourdieu 2006). Another sulfur compound related to the reductive aging aroma in wine is dimethyl sulfide. Its contribution strongly varies, depending on concentration. Its aroma has been described as asparagus, molasses and corn (Goniak and Noble 1987), but at lower concentrations it can have a black currant aroma (Segurel et al. 2004) and enhance other fruity aromas in wine at below sensory threshold concentration (Escudero et al. 2007). How much dimethyl sulfide is released over the course of bottle aging depends on various factors, storage temperature being one of the most important ones (Segurel et al. 2004; Ugliano 2013). This compound, when present together with 2-furanmethanethiol, is partially responsible for the aroma related to aged Bordeaux red wines (Picard et al. 2015; Darriet and Pons 2017).

Furfural is a compound that is mostly related to baked wines, like Madeira, and is also found in wines aged in oak barrels (Waterhouse et al. 2016). Over time, it will also develop in some wines stored at cooler temperatures due to acid catalyzed carbohydrate degradation (Jackson 2014; Rapp et al. 1985).

Overall, the development of wine aroma during maturation and bottle storage strongly depends on the amount of available oxygen and proper storage conditions. In addition, the concentration of many compounds that develop during this time can drastically alter their contribution to the final aroma. While some compounds are generally perceived as spoilage at high concentrations, in small concentrations they can contribute to the wine's complexity (Ugliano 2013).

Table 5: Aroma compounds related to oxidative and reductive aging of wine and some of their properties (Darriet and Pons 2017; Culleré et al. 2007; Escudero et al. 2000; Waterhouse et al. 2016)

Name	Odor description	Typical concentration [µg/L]	Sensory Threshold in model wine [µg/L]	Structure
Sotolon	Curry, madeira, nutty	ND-1,000	2 (racemic mixture)	
Methional	Boiled potato	ND-140	0.5	
Phenylacetaldehyde	Honey, old rose	ND-53 (white)/>90(red)	1	
Benzenemethanethiol	Gunflint, smoky	ND-0.4	0.0003	
2-Furanmethanethiol	Roasted coffee	ND-0.5	0.0004	
2-Methyl-3-furanthiol	Cooked meat	ND-0.2	0.002-0.008	
Dimethyl sulfide	Quince, truffle, cabbage	ND-480	25 (white)/ 65 (red)	
Furfural	Caramel	ND-5,000	15,000	

Some other compounds, like 1,1,6-trimethyl-1,2-dihydro-naphthalene (TDN) and other norisoprenoids, that are commonly related to the aging of wine will be discussed in later chapters as some of them fall into the category of varietal compounds.

5.2.2 Varietal compounds

The term varietal is used to describe certain characteristics that are related to one or more specific grape varieties and the resulting wines. This group of compounds commonly includes monoterpenes, methoxypyrazines, some volatile sulfur compounds, mainly varietal thiols, norisoprenoids and several others (Ribéreau-Gayon et al. 2006). These compound are present in the grapes, either as non-volatile precursor compounds or in their free, aromatic form (Francis and Newton 2005). In Sauvignon blanc grape juice for example, varietal thiols are present as odorless cysteine-bound conjugates, unlike methoxypyrazines that are present in their volatile form (Swiegers et al. 2009). They can therefore by classified as primary aroma compounds.

While some of the compounds associated with varietal aroma can be found in “neutral” grapes, like Pinot blanc, Chardonnay, Airén or Welschriesling, they are usually present at lower concentrations compared to “aromatic” cultivars (Ferreira 2010; Belitz et al. 2012). Monoterpenes, for example have been found in most grape varieties, but are most abundant in Muscat, Traminer and Riesling wines (González-Barreiro et al. 2015). The reason for these differences can be genetic, like a variation of a terpenoid biosynthetic gene in Gewürztraminer and Muscat cultivars or a different expression of a *O*-methyl transferase gene in Sauvignon blanc cultivars, which result in the accumulation of terpenes and methoxypyrazines, respectively, in wines produced from their grapes, but this is still a subject of investigation (Ilc et al. 2016). The utilization of different yeast strains can additionally influence the final aroma, for example by altering the amount of varietal thiols released during the fermentation of Sauvignon blanc (Swiegers et al. 2009; Murat et al. 2001a).

In very few cases, it is only one compound that is responsible for the aroma of a wine. In this cases, one “character impact odorant” would be responsible for most of the sensory impact and have a smell very closely resembling that of the product. For wine in most cases, it is a complex mixture of many compounds with an OAV greater than one that will contribute to the final aroma, though be it at different levels. Still, in some wines, some Muscat wines for example, it is possible that the overall odor impression is dominated by one or a few compounds (Ferreira and Cacho 2009). This can also be the case for wines from other cultivars. Guth (1997a) identified *cis*-rose oxide in Gewürztraminer and 4-methyl-4-sulfanyl-pentan-2-one in Scheurebe to be the compounds with the greatest influence of the final aroma of the respective wines. In other cases, varietal compounds will not be solely responsible for the aroma of the wine, but will still have an impact on the overall impression. This impact can be beneficial and wanted, but in some cases, it can also be detrimental to the sensory impression. For example, in the case of wines produced from cultivars related to *Vitis labrusca*, where methyl anthranilate and 2-aminoacetophenone can cause a foxy smell, the presence of which is generally regarded as a fault (Rapp and Versini 1996; Jackson 2014).

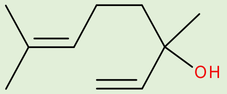
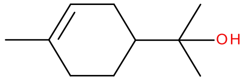
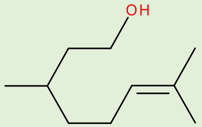
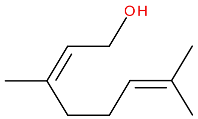
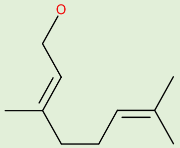
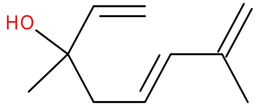
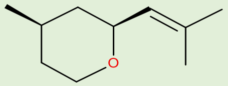
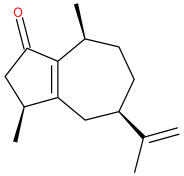
Terpenes

One group of cultivars that comes to mind when talking about strong varietal character is the family of Muscat wines. These wines are known for their floral aroma that is caused by the presence of different terpene compounds (Ferreira 2010; Mateo and Jiménez 2000). While more than 50 individual terpenes have been identified in wine (Ebeler and Thorngate 2009) only few contribute significantly to the varietal aroma. The main compounds are: linalool, α-terpineol, citronellol, nerol, geraniol and hotrienol (Ribéreau-Gayon et al. 2006; Mateo and Jiménez 2000). Table 6 summarizes the concentration of these compounds in different wines.

Table 6: Concentrations of terpenes in several wines, all concentrations in µg/L (Bakker and Clarke 2012; Ribéreau-Gayon et al. 2006; Waterhouse et al. 2016)

	Linalool	**α-Terpineol**	**Citronellol**	**Nerol**	**Geraniol**	**Hotrienol**
Scheurebe	70-370	160-260		Tr-40	Tr-30	100-240
Grauburgunder	Tr-60	Tr-180		Tr	Tr	Tr-40
Gewürztraminer	6-190	3-35	12	Tr-43	20-218	Tr-40
Morio-Muscat	160-280	240-400		Tr-30	Tr-10	80-140
Muscat of Alexandria	435	78		94	506	
Muscat of Frontignan	473	87		135	327	
Riesling	40-140	25-280	4	Tr-23	Tr-35	25-130
Sauvignon Blanc	17	9	2	5	5	25
Müller-Thurgau	100-190	100-210		Tr	Tr-0.01	
Chardonnay	100	500				40-80
Airén	500	250				
Viura	100	1,000				
Muscadelle	50	12	3	4	16	
Albariño	80	37		97	58	127
Sensory threshold in model wine	**25-50**	**250-500**	**18-100**	**400**	**30-130**	**110**

Table 7: Terpenes in wine and some of their properties (Darriet and Pons 2017; Marais 1983; Waterhouse et al. 2016; Bakker and Clarke 2012)

Name	Odor description	Typical concentration [µg/L]	Sensory Threshold in model wine [µg/L]	Structure
Linalool	Floral, citrus, coriander seed	ND-290	25	
α-Terpineol	Lilac	ND-400	250-500	
Citronellol	Rose, lemongrass	1-50	18-100	
Nerol	Floral, green	ND-360	400-500	
Geraniol	Rose	ND-290	30-130	
Hotrienol	Linden, rose	3-240	110	
***cis* Rose oxide**	Floral, green	Tr-2.2	0.2	
(-)-Rotundone	pepper	0.05-0.6	0.008-0.016	

Chemically, terpene compounds have a carbon skeleton made up of two or more isoprene units (Figure 58). Depending on the amount, the resulting compound are monoterpenes (two units), sesquiterpenes (three units), diterpenes (four units) or triterpenes (six units). Most of the compounds associated with the floral aroma of Muscat wines are monoterpene compounds, as those are the most likely to be odor active (Jackson 2014; Ribéreau-Gayon et al. 2006).

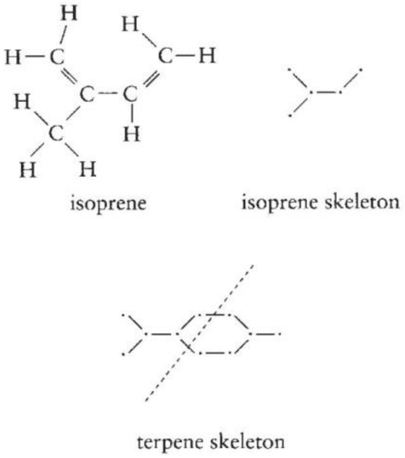


Figure 58: Isoprene and terpene structure compared (Jackson 2014, p. 390)

Linalool is one of the main monoterpene compounds that are responsible for the characteristic aroma of Muscat wines. Its impact is enhanced by the presence of other terpenols (Ferreira 2010). Guth (1997b) found that *cis* rose oxide, another monoterpene, is one of the main influential aroma compounds in Gewürztraminer. Its perception is also enhanced by the presence of other terpenols (Ferreira 2010). One example for a sesquiterpene with great influence on the varietal character of some wines is rotundone. This compound has been identified in pepper and is responsible for the spiciness in wines like Shiraz, but has also been found in Grüner Veltliner (Wood et al. 2008; Mattivi et al. 2011; Nauer et al. 2018).

Terpene compounds are present in grapes in different forms, including a free form but also as odorless glycosylated and polyhydroxylated precursors. In most cases, the glycosylated odorless form is more abundant in the juice than free terpenes (Waterhouse et al. 2016; Mateo and Jiménez 2000). Therefore, the choice of yeast can have an impact on the final concentration of some terpene compounds, including citronellol, geraniol and nerol in the wine (Styger et al. 2011; Loscos et al. 2007; Dugelay et al. 2016). Other important factors for the composition and concentration of terpenes in wine are the pH, as they are prone to acidic hydrolysis, as well as skin contact time and the enzymatic load. Further, their susceptibility to oxidation results in changes of the composition over time (Waterhouse et al. 2016).

One of the main reasons for the loss of Muscat characteristic is the decline of terpenes with low sensory thresholds in wines when those are stored for an extended period of time. This is due to the higher sensory thresholds of the terpene oxides, which can be more than ten times higher as mentioned in 5.2.1 (Jackson 2014). The concentration of geraniol and nerol will decrease relatively quickly over the first years of bottle storage, while linalool is more stable. Still, over time it will be converted to α-terpineol and other compounds (Marais et al. 1992; Darriet and Pons 2017).

Methoxypyrazines

Another group of compounds that are strongly connected to the varietal aroma of some wines are methoxypyrazines. These compounds consist of a nitrogen heterocycle with alkylated sidechains. Two of the main representatives are 2-isobutyl-3-methoxypyrazine (IBMP) and 2-isopropyl-3-methoxypyrazine (IPMP). Some of the first to identify IBMP in wine were Bayonove et al. (1975), who found it contributed to the green bell pepper aroma of Cabernet Sauvignon. Allen et al. (1991) identified IBMP and IPMP to be an integral part of the aroma of Sauvignon Blanc. In addition, these compounds have been found in wines of several other varieties, including Cabernet Franc, Merlot, Carménère and Verdejo, as well as some others below threshold concentrations (Darriet and Pons 2017).

Buttery et al. (1969) determined the sensory threshold for IBMP in water at 2 ng/L, which seems to align well with the threshold in white wine (Allen et al. 1991). In red wine, the threshold concentration was determined to be higher with 15 ng/L (Roujou de Boubée et al. 2000). With concentrations in some Caménère wines reaching concentrations of up to 160 ng/L (Belancic and Agosin 2007) and up to 40 ng/L in Sauvignon Blanc, it can have a large impact on the final aroma of a wine (Darriet and Pons 2017). While IPMP has been identified in wine at concentrations close to those of IBMP (Allen et al. 1995), it is more often associated with the presence of multicolored Asian ladybeetles during the processing of grapes. It is considered undesirable and will therefore be discussed in 6.7 (Pickering et al. 2008a; Botezatu and Pickering 2010).

As a primary aroma compound, IBMP can be found in grapes as well as in the juice of different cultivars. When looking at whole grape bunches, IBMP is mostly located in the stems (>50%). In the berries, the highest amount can be found in the skin (67%) followed by the seeds (32%) and only 1% of the IBMP is found in the flesh (Roujou de Boubée et al. 2002). Prolonged skin contact time can therefore increase the amount of IBMP in the final wine (Maggu et al. 2007). Another influence factor for the concentration of IBMP is the origin of the grapes due to different climatic conditions as briefly mentioned in 3.1.2. In Sauvignon Blanc wines for example, cooler growing condition generally result in wines with greener, more vegetal characteristics associated with the presence of methoxypyrazines (Lacey et al. 1991). Wines from warmer growing regions however exhibit more tropical aromas related to varietal sulfur compounds (Green et al. 2011).

Thiols

Until the end of the last century, most sulfur compound research focused on the negative impact of some compounds like hydrogen sulfide, dimethyl sulfide and others. This somewhat changed when Darriet et al. (1995) identified 4-methyl-4-sulfanyl-pentan-2-one (4MSP) as a potential impact odorant in Sauvignon Blanc wines using *p*-hydroxymercuribenzoic acid as a derivatization agent. 4MSP was later identified as one of the compounds in box tree and broom aroma (Tominaga and Dubourdieu 1997). In the following years, this compound was detected in wines from other varieties, including Scheurebe, where it is responsible for the characteristic black currant aroma (Guth 1997b). Subsequently two more volatile thiols, 3-sulfanylhexan-1-ol (3SH) and 3-sulfanyl-hexylacetate (3SHA), were identified in Sauvignon Blanc (Tominaga et al. 1998). In addition to Sauvignon Blanc, these compounds have been found in other white wines, including Riesling, Gewürztraminer, Semillon as well as some red wines like Merlot and Cabernet Sauvignon (Bouchilloux et al. 1998; Tominaga et al. 2000a). They are also found in fruits corresponding to some of their odor descriptors like grapefruit (Lin et al. 2002; Jabalpurwala et al. 2010), different varieties of passion fruit (Engel 1999; Engel and Tressl 1991; Schoenauer and Schieberle 2019) as well as passion fruit juice (Tominaga and Dubourdieu 2000).

Grapes and juice do not contain volatile thiols, but odorless precursors in the form of cysteine-bound conjugates of 4MSP and 3SH (Swiegers and Pretorius 2007). During fermentation they are released by non-hydrolytic cleavage with the enzyme carbon-sulfur lyase and 3SH is partially transformed to 3SHA by esterification with alcohol acyltransferase (Swiegers et al. 2006; Ugliano and Henschke 2009). Only a small fraction, between 1.6% and 3.2%, of the present Cys-3SH is actually released (Dubourdieu et al. 2006; Murat et al. 2001b). Also the fermentation conditions have been shown to have an impact, but the results of several studies gave inconsistent results (Swiegers and Pretorius 2007). Due to all of this, the final concentration and composition of the thiols largely depends on the yeast strain(s) used during fermentation (Swiegers et al. 2009; Dubourdieu et al. 2006; Murat et al. 2001a).

Another influence factor are the growing conditions. This is of particular interest in Sauvignon Blanc wines that exhibit different characteristics, depending on their origin. Thiols are more abundant in wines from warmer climates, unlike methoxypyrazines, which are more abundant for wines from grapes grown in cooler climates (Green et al. 2011; Lacey et al. 1991).

Like terpenes and other fruity aromas, thiols are prone to degradation during storage. This is particularly true for 3SHA. In one study by Coetzee et al. (2016), its concentrations declined by 30% over the course of seven months of storage at 15°C. When oxygen was present, this increased to about 75%. This echoes the findings of Herbst-Johnstone et al. (2011), who found that 3SHA concentrations dropped to 31± 7% of the initial value. They also found that 3SH concentrations decrease by 39± 9% after one year of storage after an initial increase, which occurs due to the hydrolysis of 3SHA.

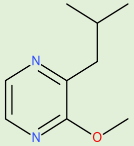
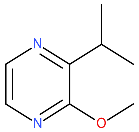
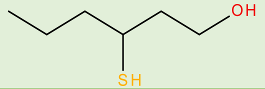
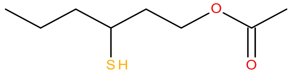
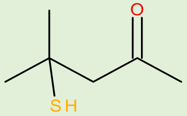
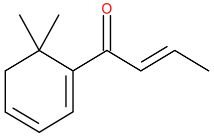
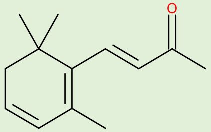
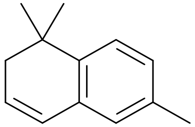
C_{13}-Norisoprenoid Derivatives

The last group of aroma compounds discussed in this section are derivates of the oxidative degeneration of carotenoids. The structure of these compounds generally consists of 13 carbon atoms; they are therefore also referred to as C_{13}-norisoprenoids. Some of the most important representatives are β-ionone, β-damascenone and 1,1,6-trimethyl-1,2-dihydronaphtalene (TDN). They are important contributors to the aroma of wines from several different cultivars, including Semillon, Sauvignon Blanc, Chardonnay as well as Merlot, Pinot noir and Cabernet Sauvignon (Mendes-Pinto 2009; Darriet and Pons 2017; Pineau et al. 2007).

While all of the compounds are present in a wide range of wines, TDN is of particular interest as it is responsible for the characteristic kerosene note in aged Riesling (Simpson and Miller 1983). TDN is also present in other wines, including Cabernet Franc, Sauvignon Blanc and Pinot Grigio, but its concentrations are significantly higher in Riesling wines. Sacks et al. (2012) found a 5-fold higher concentration in Riesling wines from New York State, compared to others from the same area. One of the main factors influencing the final concentration of TDN in wine is the grapes' exposure to sunlight during maturation, with higher exposure resulting in higher concentrations in the juice where it is mostly present as polyol precursors glycoconjugates (Gerdes et al. 2002). Other factors are aging, temperature and duration as well as exposure to oxygen (Black et al. 2012).

High concentrations of TDN in young Riesling wines from growing areas with warmer climates can be considered a defect, while it is considered to contribute to the bouquet of bottle aged Riesling (Ross et al. 2014; Winterhalter and Gök 2012).

Table 8: Other varietal compound an some of their properties (Darriet and Pons 2017; Waterhouse et al. 2016; Pickering et al. 2007)

Name	Odor description	Typical concentration [µg/L]	Sensory Threshold in model wine [µg/L]	Structure
2-Isobutyl-3-methoxypyrazine	Green bell pepper	0.0005-0.16	0.001	
2-Isopropyl-3-methoxypyrazine	Pea, asparagus	0.1-10	0.0003-0.002	
3-Sulfanylhexan-1-ol	Passion fruit, grapefruit	0.1-10	0.06	
3-Sulfanyl-hexylacetate	Boxwood, passion fruit	ND-1	0.004	
4-Methyl-4-sulfanylpentan-2-one	Boxwood, broom	ND-0.12	0.0008	
β-Damascenone	Applesauce, rose	0.1-2.5	0.06	
β-Ionone	Violet	ND-2.4	0.8	
1,1,6-Trimethyl-1,2-dihydronaphtalene	Kerosene	ND-30	1	

6. Defects in wine

For most people, wine is a luxury item which is expected to fulfill certain requirements. One very important condition is the absence of defects and imperfections (Rapp et al. 1992). The definition of what constitutes as a defect in wine can be complicated due to the difference in perception and sensitivity of consumers. In some cases, a wine that one person finds unacceptable can still be enjoyable to another (Jackson 2009).

Still, the presence of some chemical compounds in wine will reduce its quality. Sometimes faults can be very clearly defined, while in other cases it can be a matter of opinion and a compound's presence may even contribute to the complexity of a wine in lower concentrations. Goode (2018) argues that the impact a compound has on the overall impression of a wine depends on the context, the wine and the consumer. Some characteristics valued in aged red wine will feel out of place in a young white wine or even another red wine produced from a different variety. This can complicate the discussion about faults even further.

In addition to their own negative impact on the overall impression of a wine, the presence of some compounds like TCA and Brett characteristics can dull and mask the perception of fresh and fruity aromas within the wine (Licker et al. 1998; Jackson 2009).

Most defects in wine are caused by compounds with sensory thresholds in the ng/L or even pg/L range. As a result, their presence rarely poses a health risk for consumers (Ridgway et al. 2010). It can, however, have an economic impact on the producer. Cork taint alone is estimated to be responsible for an annual loss of several billion dollars worldwide (Prescott et al. 2005; Cravero 2020; Butzke et al. 1998) and 700 million Euro in Europe alone (Walker and Leins 2014). In addition to the loss due to the returned product, the presence of faults in wine also leads to a loss of confidence in the brand. Consumers are therefore less likely to buy their wine again or recommend it to others resulting in loss of future economic gains (Kim 2016; Ridgway et al. 2010; Jarrell and Peltzman 1985).

The presence of defects can be an indicator for problems that occurred during the production and contamination. Over the years, the number of faulty wines has decreased significantly due to the greater understanding of the origins of certain defects. Strategies to avoid their development during the process have been implemented as better technology and more systematic awareness have become common place (Bakker and Clarke 2012).

Defects can roughly be divided into two subgroups based on their origin: Off-flavors, which occur at some point during the winemaking process, for example due to bacterial spoilage or wrong storage conditions, and taints, which are accidentally added to the wine from an external source like packaging material or closures (Fontana 2016; Ridgway et al. 2010; Baigrie 2003). Taint compounds related to external contamination are usually more potent as their sensory thresholds are generally lower than those of off-flavors compounds, which occur during the winemaking process (Waterhouse et al. 2016). In addition to compounds that have a negative impact as soon as they are present in concentrations above their sensory threshold, there are others that are considered beneficial in most cases but can be experienced as faults, when they are present in high concentration. For example, high concentrations of TDN will, depending on the wine, resulted in the rejection of Riesling wines (Winterhalter and Gök 2012; Ross et al. 2014).

Other, non-aroma based defects include optical defects like turbidity or the presence of sediment like tartrate crystals or polymerized tannins. While these in most cases do not influence the taste of the wine, they can result in the rejection of wines by consumers and, therefore, should be avoided by winemakers (Jackson 2009). Hazes or cloudiness can also be indicators of other faults like microbiological spoilage. In more recent time with process control and more advanced equipment available, the occurrence of negative bacteria in wine has reduced significantly. Another optical fault is the premature browning of white wines due to the presence of oxygen (Robinson and Harding 2015; Boulton 1996).

The presence of carbonation in supposedly still wines is widely regarded as a fault. This can be due to early bottling, before dissolved CO_2 is released or as a result of in bottle fermentation (Robinson and Harding 2015; Jackson 2009).

Preferences and tolerances of consumers to certain characteristics can vary from country to country or even among different regions. This applies to color, Pokorný et al. (1998) for example found that Czech consumers generally prefer white wines with more yellow colors over those with greener tones. Other examples are listed by Robinson and Harding (2015) in the introduction to wine faults in the Oxford Companion to Wine. They say that Italians are more accepting of bitter wines while Americans tolerate higher levels of sweetness and Germans more SO_2, French consumers are less affected by higher tannin levels and British consumers accept more decrepit wines than consumers of other nationalities. Sensitivities also vary. Australians for example react more negatively to higher concentrations of mercaptans, while Americans are more likely to regard herbaceous wines as faulty. Also the context in which the wine is consumed can have an impact on the perception (Jiang et al. 2017).

Overall, it can be said that there are many factors that influence the enjoyment or rejection of a wine. The following part will focus on generally agreed upon off-flavors and taints, as these are the main concerns of winemakers and consumers. Most other faults have become less prevalent due to the developments and advances in winemaking technology.

6.1 Reductive Wines - Volatile Sulfur Compounds

Sulfur related off-odors account for about 25% of faults in commercial wines (Jastrzembski et al. 2017) and even up to 50% of rejections in tastings (Jackson 2014). While some sulfur compounds, mainly volatile thiols can have beneficial effects on the aroma of certain wines (see 5.2.2) others, mainly smaller compounds are associated with reduction defects. Table 9 lists some of the more common compounds associated with sulfur off-odors. Overall, volatile sulfur compounds can be divided into two main groups, based on their boiling point (bp). The first group have a bp below 90°C and are highly volatile (Darriet and Pons 2017; Ribéreau-Gayon et al. 2006). Some of the most prevalent compounds in this group are present in reduced wines, including hydrogen sulfide (H_2S) and ethane- and methanethiol. H_2S can impart rotten egg aromas to wines. The other two are associated with cabbage, onion and garlic smells. Another compound with high volatility is dimethylsulfide (DMS). Depending on its concentration, its contribution to wine aroma can be either beneficial or detrimental. At lower concentrations it can add a pleasant black currant aroma, at higher concentrations above threshold, however, it can impart aromas similar to boiled cabbage, corn or molasses (Landaud et al. 2008; Moreira et al. 2002; Mestres et al. 2000; Siebert et al. 2010). In a study looking at Californian wines with sulfur-related off-odors, DMS was present in more samples than other compounds. In 22% of the wines with defects, it was found above its sensory threshold. The study did not include H_2S (Park et al. 1994).

Lower volatility sulfur compounds can also negatively affect the aroma. However, due to their generally higher sensory thresholds, their impact is generally reduced. One of the more common representatives of this group of compounds is methionol (3-methythiopropan-1-ol). When present in above threshold concentrations, it can impart a smell of fermented cabbage. 2-mercaptoethanol has also been found in above threshold concentrations at which it imparts a smell reminiscent of H_2S (Darriet and Pons 2017). Another sulfur-containing compound with a bp above 90°C has recently been identified in white and rosé wines, especially when the juice was exposed to high amounts of oxygen during processing: ethyl 2-sulfanylacetate. When present in above threshold concentrations, it can impart an aroma described as baked beans or *Fritillaria meleagris* bulbs (Nikolantonaki and Darriet 2011). Some other representatives of this group (2-methyl-tetrahydro-thiophenone and thiophene) can reduce pleasant aromas but are very unlikely to contribute their own aroma (Darriet and Pons 2017). The use of non-Saccharomyces yeast strains like *Hanseniaspora uvarum* and *Hanseniaspora guilliermondii* during fermentation can also have an influence on the concentration of some heavy Volatile Sulfur Compounds (VSCs) (Moreira et al. 2008).

Some sulfur compounds can also have an influence of the perceived minerality of a wine. Some studies have found a correlation between reductive sulfur notes and minerality (Heymann et al. 2014; Ballester et al. 2013), while others did not find this to be true (Parr et al. 2015). Furthermore, other compounds have also been associated with minerality in wine. One of the main examples is benzenemethanethiol which contributes smoky and gunflint aromas (Darriet and Pons 2017; Parr et al. 2016; Tominaga et al. 2003). Depending on the wine, matchstick character and other aromas associated with minerality can be either beneficial or detrimental. While it might give depth in Sauvignon Blanc or a complex Chardonnay, it might be perceived negatively in a different context. The favorable impact of course is only given up to a certain point, as reductive compounds will be perceived as an off-flavor above a certain concentration (Goode 2018). The topic of minerality therefore remains a complicated one with potential for further investigation.

In wine, H_2S is mainly formed during fermentation when sulfur is assimilated by yeast in the sulfate reduction pathway for the formation of methionine and cysteine. These amino acids are needed for the growing yeast cells. If and how much H_2S is formed strongly depends on the type of yeast(s) used, as well as composition and health of the must. One important factor is the amount of yeast assimilable nitrogen (YAN). When sufficient amounts of nitrogen are present in the must, the formation of the amino acids can happen without major problems. If this is not the case, the deficiency can result in the build-up of H_2S in the cells and an off-flavor in the final wine (Swiegers and Pretorius 2007; Smith et al. 2015). Some yeast strains, however, have been found to produce less or no H_2S due to genetic traits (Linderholm et al. 2010; Cordente et al. 2009). While a fermentation with low H_2S yeasts has advantages, there can be drawbacks as well. The strains tend to produce higher amounts of SO_2, which can cause problems with a subsequent malolactic fermentation or off-flavors in the final wine (Goode 2018).

Due to its high reactivity, H_2S can form ethanedithiol and similar compounds by reacting with ethanol or ethyl acetate (Vermeulen et al. 2005; Swiegers et al. 2008; Rauhut 2009) or combine to form diethyl disulfide when the pH conditions allow for it (Bobet et al. 1990).

The use of elemental sulfur in the vineyard can lead to the production of H_2S through assimilation when it is sprayed too close to harvest (Goode 2018). The amount formed depends on the kind of elemental sulfur, with colloidal sulfur resulting in the highest concentrations

(Schütz and Kunkee 1977). For this to happen, however, the concentrations of elemental sulfur have to exceed 10 mg/L, which is unlikely to happen in a commercial environment (Araujo et al. 2017).

There are not many studies on the formation of VSCs other than H_2S during wine fermentation. Kinzurik et al. (2015) were among the first to investigate this subject. They found that several VSCs can be formed during fermentation, which compounds are formed and their concentration depends mainly on the yeast strain, but also on the medium (Rauhut 2009).

Additional sources of sulfur during the winemaking process are the tripeptide glutathione. It is present in most *S. cerevisiae* species where it can be responsible for up to 1% of the dry weight. Its concentration in the wine depends on the chosen yeast strain and if the wine is matured on lees. Due to its beneficial function as an antioxidant, glutathione is sometimes added to supplement the must. In addition, it can play a role in the protection of varietal thiols. However, when adding glutathione to the must this should happen with caution. When the concentration exceeds 50 mg/L, it can result in the formation of unpleasant VSCs (Rauhut 2009).

When wine is matured on lees, over time the yeast cell membranes will break down, resulting in the release of enzymes and other intracellular compounds. These can interact with the wine in beneficial ways but can also cause the formation of some VSCs (Pérez-Serradilla and Castro 2008). This is especially true when the maturation is carried out in larger vessels, as higher hydrostatic pressure encourages the production of VSCs with negative connotations. This development can be further promoted under anaerobic conditions (Jackson 2014). In smaller cooperage, maturation on lees can actually help to reduce the amount of sulfur-related off-flavors. Palacios et al. (1997) found that yeast cells were able to adsorb different VSCs. 0.05 g/L of lees were able to adsorb 200 µg/L of H_2S and a concentration of 0.15 g/L could adsorb 200 µg/L of methane- or ethanethiol.

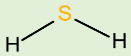
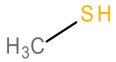
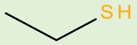
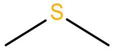
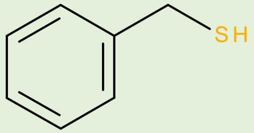
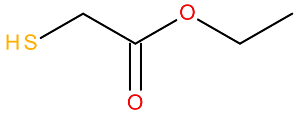
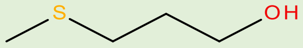
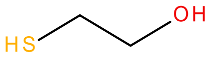
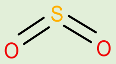
Another potential source of sulfur related off-odors is the addition of sulfur dioxide. It mainly functions as an antimicrobial agent and an antioxidant and is an essential part of the winemaking process in many wineries. Further reasons for SO_2 addition, as well as some of the drawbacks and challenges, are discussed in 4.2. An excessive amount of SO_2 present during fermentation can be converted into H_2S by yeast (Smith et al. 2015). In the bottle, excessive amounts of residual free SO_2 can result in a pungent off-odor reminiscent of match sticks. It is therefore crucial to add an adequate amount of SO_2, depending on several factors, like health of grapes, wine style and type of closure used (Henderson and Kenwood Vineyards 2009; Ribéreau-Gayon et al. 2005). Several studies have shown that yeast is also capable of transforming SO_2 to H_2S (Smith et al. 2015). An addition of SO_2 before maturation on lees can also result in higher reductive aromas in the wine. It is therefore recommended to rack the wine and remove them from the lees for about a month until the enzyme activity has decreased (Rauhut 2009).

The presence of elemental sulfur and the addition of glutathione, a sulfur containing amino acid, can result in higher H_2S concentrations in wine (Jastrzembski et al. 2017). This happens especially in low oxygen environments (Ugliano et al. 2011). Mecchi et al. (1964) show that prolonged heat exposure can facilitate the transformation of GSH to H_2S.

Another factor that can have an influence is the type of closure used during bottling. This is due to the fact that as the amount of available oxygen during storage partially depends on their respective permeability. An insufficient amount of oxygen can result in wines with reduced characteristics. Godden et al. (2001) found that after 18 months of storage there were significant differences between Sémillon wines closed with screw caps compared to other closures, like natural and artificial cork. The sensory panel found that the screw-capped wines exhibited rubbery and sulfide aromas associated with reduction. These results were confirmed in another study on Sauvignon Blanc by Ugliano et al. (2009), who additionally examined the effects of copper fining. The oxygen transfer rate of screw caps mainly depends on the type of liner used. While the most common one, “tin-Saran”, has a very low transfer rate, others, like “Seranex only” allow for a higher rate, reducing the possibility of reduction character in wine. And the higher occurrences of reductive wines have been an issue, it its most likely that this will happen less and less due to a better understanding of how to adjust for it. It has to be said that in the examination of wines rejected for reductive faults at one of the largest wine tastings over several years, the same amount had been sealed with cork as compared to screw caps (Goode 2018).

Some of the ways to avoid the formation of VSC related off-odors have been briefly touched upon already. This includes the use of low or no H_2S producing yeast strains (Linderholm et al. 2010; Cordente et al. 2009). Another option is the addition of YAN. Some of the most common forms are diammonium phosphate (DAP) or ammonium sulfate. With sufficient YAN, yeasts are able to convert excess H_2S to sulfur-containing amino acids needed for the growth of cells (Ribéreau-Gayon et al. 2006; Smith et al. 2015). Copper fining is the addition of $CuSO_4$ to reductive wine to induce the precipitation of CuS. It can also be added before bottling to prevent the development of reductive characteristics in wine during bottle storage (Smith et al. 2015). Racking before bottling can physically drive out excessive VSCs and might be chosen by winemakers, who want to avoid the addition of $CuSO_4$ (Jackson 2014).

Table 9: Off-flavor related volatile sulfur compounds and some of their properties (Bartowsky and Pretorius 2009; Mestres et al. 2000; Nikolantonaki and Darriet 2011; Smith et al. 2015; Tominaga et al. 2003; Ribéreau-Gayon et al. 2006)

Name	Odor description	Concentration in wine [µg/L]	Sensory Threshold in model wine [µg/L]	Structure
Hydrogen Sulfide[1]	rotten egg, reduced taste	nd- 370	1.1- 80	
Methanethiol[1]	cooked cabbage, purification	nd- 16	0.3-3.1	
Ethanethiol[1]	onion, rubber, fecal	nd- 50	1.1	
Dimethylsulfide[1]	cabbage, asparagus, corn	nd- 474	25	
Benzenemethanethiol[2]	struck flint, rubber	0.03-0.04	0.0003	
Ethyl 2-sulfanylacetate[2]	baked beans, *Fritillaria meleagris* bulbs	0.169- 1.56	0.2- 0.4	
Methionol[2]	potato, cooked cabbage, soup or meat like	140- 5,600	500- 1,200	
2-Mercaptoethanol[2]	poultry, farmyard, "Böckser"	nd- 400	130	
Sulfur dioxide	irritating, burning	nd- 20,000	2,000 active molecular SO_2	

[1] "light" VSCs; [2] "heavy" VSCs

6.2 Cork Taint

Cork has long been used as a closure for all sorts of vessels and bottles and it is still very common. Its properties make it a very suitable material as it seals the bottle, avoiding leakage but allowing for a minimal amount of oxygen to enter, which can help in the formation of beneficial aroma compounds during bottle aging (Pereira 2007). Figure 59 shows different quality levels of cork stoppers. In many countries cork stoppers are still regarded as a sign of high quality wine (Cravero 2020).

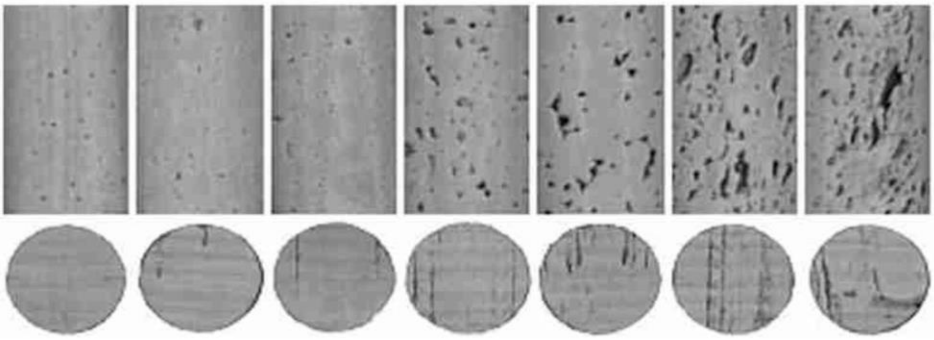


Figure 59: Different qualities of cork stoppers (Pereira 2007, p. 314)

One of the best-known defects in wine is cork taint, which is a somewhat misleading name. While the use of faulty cork stoppers can result in off-odors in wine, sound cork does not influence the wine and can even absorb some negative aromas (Jackson 2014; Capone et al. 1999). As mentioned before, taints are introduced to a medium from external sources, in this case mainly faulty cork stoppers. The musty, moldy aroma can be traced back to several different compounds, most of them haloanisoles with low sensory thresholds. Their presence in the cork stoppers is mostly due to a deterioration of halophenol-based wood preservatives during the cork ripening (Pereira 2007; Fontana 2012). One of the main sources are organochlorine insecticides, which were commonly used from the 1950s until the 1980s. While they are not used anymore, chlorine residues can still be found in the soil close to the trees (Goode 2018). Fungi and other microorganisms present on and in the cork can convert those to the respective anisoles (Figure 60). Cork taint can also be introduced in other steps of the winemaking process when it comes in contact with other contaminated materials, for example barrels or packaging material (Fontana 2016; Pereira 2007).

Estimates of how many bottles are affected by cork taint vary greatly, stretching from below 1% to about 10% (Pereira 2007; Rodríguez-Andrade et al. 2019; Silva Pereira et al. 2000). Some estimate that the yearly loss due to cork taint in wine accounts for more than $10 billion worldwide (Butzke et al. 1998). In addition to their own musty aroma, some of the compounds related to cork taint can dull pleasant fruity flavors even before being detectable (Lee and

Simpson 1993; Jackson 2014). This can lower the consumers' perception of the wine and will most probably result in them not buying the same wine anymore (Ridgway et al. 2010; Kim 2016; Jackson 2014).

There are several possibilities for contaminates to taint cork. In addition to the formation during the treatment of the cork bark, some volatiles can be transferred during improper storage and subsequently be absorbed by the wine. Another source are contaminated oak barrels and other winery equipment (Pereira 2007; Jackson 2014). Figure 60 shows the breakdown of two different halophenols (2,4,6-trichlorophenol (TCP) and pentachlorophenol) to 2,4,6-trichloroanisole (TCA), one of the main compounds responsible for cork taint in wine. This transformation is largely done by microorganisms, including different fungi species. Alvarez-Rodríguez et al. (2002) examined the capabilities of several filamentous fungi for the methylation of different halophenols. They found that out of the 14 tested species, 11 were able to increase the haloanisole (TCA) concentration significantly when provided with sub-lethal concentrations of the precursor (TCP). Out of the 14 strains, three were able to transform more than 25% of the halophenol to the respective anisole.

Another potential source is the now uncommon techniques of chlorine bleaching of cork and the washing of barrels with hypochlorite (Pereira 2007; Jackson 2014).

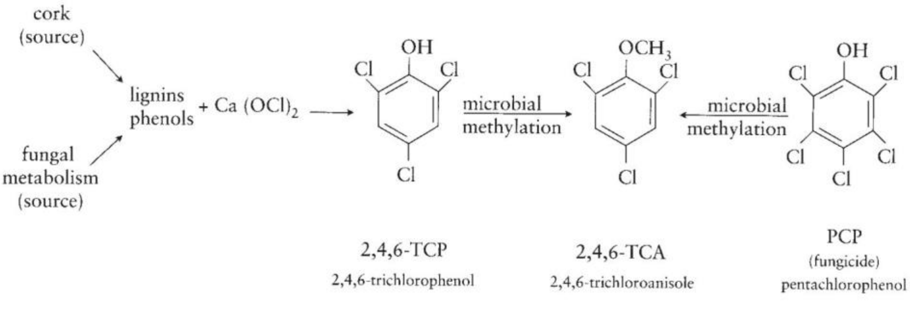


Figure 60: Formation of haloanisoles related to cork taint (Jackson 2014, p. 619)

In 1982, Buser et al. identified TCA as one of the compounds involved in cork taint. When looking at wines that exhibited a musty or moldy off-odor, different studies found that TCA could be found in a large majority (70-80%) of the tainted wines (Peña-Neira et al. 2000; Sefton and Simpson 2005). In one study, all of the tested wines contained TCA (Sefton and Simpson 2005; Rigaud et al. 1984). This and its low sensory threshold place it among the most important compounds in this context. The threshold is in a range of 0.03 to 2 ng/L in water and 0.9 to 210 ng/L in wine, largely depending on the experience of the sensory panel as well some other factors including the type of wine (Fontana 2012; Teixeira et al. 2006; Sefton and Simpson 2005; Cravero 2020; Pereira 2007). For this reason, methods for the quantification of TCA need to have high levels of selectivity and sensitivity. A review by Fontana (2012) lists several gas chromatographic methods with different detectors, including electron capture, atomic emission, olfactometry and various mass sensitive detectors. Other techniques include ion-mobility spectrometry, as well as enzyme-linked immunosorbent and bioelectric recognition assays.

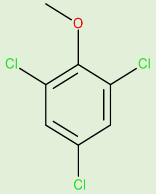
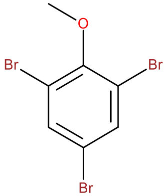
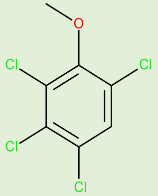
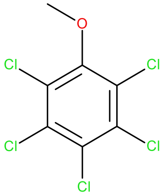
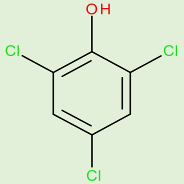
While TCA is one of the main contributors to cork taint, it is by no means the only responsible compound (Silva Pereira et al. 2000; Jackson 2014). Soleas et al. (2002) analyzed 2400 bottles of wine. Out of those, an expert sensory panel found 145 exhibited cork taint. In a majority of the tainted samples (73% white and 74% red wines), the TCA concentration was below the sensory threshold of the panel (2 ng/L) threshold. In about two thirds (60% white and 65% red wines) the concentration was even below the determined limit of detection of the analytical method used for the quantification. This only strengthens the point that other compounds contribute to cork taint.

Another important haloanisole in this context is 2,4,6-tribromoanisole (TBA). Chatonnet et al. (2004) were the first to describe it in wine as part of the musty, moldy aroma associated with cork taint. Similar to TCA, TBA has a very low sensory threshold in water, ranging from 8 to 30 pg/L (Malleret and Bruchet 2002; Saxby 1995). In wine, the threshold is higher, but with about 3.4 to 7.9 ng/L still comparatively low (Chatonnet et al. 2004). The precursor compound for TBA is, similar to TCA, most likely the respective phenolic compound. 2,4,6-tribromophenol, which is used as a wood preservative and a fire retardant, can be methylated to form TBA by *Trichoderma longibrachantum*, a common mold (Jackson 2014).

Other compounds related to cork taint are listed in Table 10.

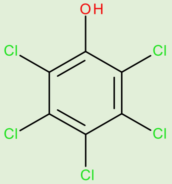
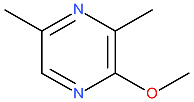
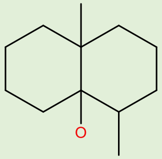
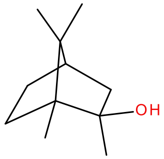
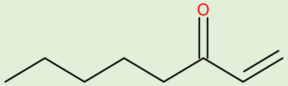
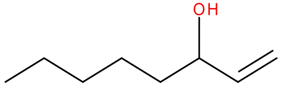
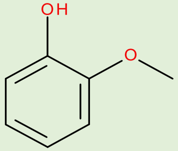
While the use of other stopper materials will in most cases eliminate the possibility of cork taint, there are methods to prevent faulty cork stoppers. Those commercially used include steam cleaning of the stoppers, as aeration and high moisture content result in a lower TCA content. This can also be done to clean granulated cork. Other techniques use supercritical CO_2, microwave treatment or enzymes that metabolize TCP without forming TCA. Coating of the stoppers with silicone membranes can also prevent the transfer of taint causing compounds to the wine (Sefton and Simpson 2005; Jackson 2014). Capone et al. (1999) also found that chloroanisoles can be absorbed by sound cork from wine.

Table 10: Compounds related to cork taint and some of their properties (Cravero 2020; Fontana 2012; Sefton and Simpson 2005; Waterhouse et al. 2016; Huang et al. 2012; Goodman 2001)

Name	Odor description	Sensory Threshold in wine [μg/L]	Structure
2,4,6- Trichloroanisole	musty, moldy, earthy	0.0014-0.21	
2,4,6-Tribromoanisole	moldy, earthy	0.0034-0.0079	
2,3,4,6- Tetrachloroanisole	moldy, dusty	0.005-0.015	
Pentachloroanisole	dusty	10	
2,4,6-Trichlorophenol	phenolic	300*	

* in water

Table 10 continued

Name	Odor description	Sensory Threshold in wine [µg/L]	Structure
Pentachlorophenol	phenolic	1,600*	
2-Methoxy-3,5-dimethylpyrazine	fungal, musty	0.002	
Geosmin	muddy, earthy	0.03-0.09	
2-Methylisoborneol	muddy, earthy	0.03-0.055	
1-Octen-3-one	mushroom	0.07	
1-Octen-3-ol	mushroom	40	
Guaiacol	smoky	20-50	

* in water

6.3 Mousy Taint

Mousiness has been an issue in wine for many years with written descriptions ranging back to the end of the 19th century. This fault is commonly described as the smell of caged mice (Künzler and Nikfardjam 2013; Lay 2003). Already in the beginning of the 20th century, the origin of the off-flavor was brought in context with a bacterial source by Müller-Thurgau and Osterwalder (1913) (referenced in Lay (2003)). The smell can only be perceived once the wine is consumed as the flavor compound only becomes active at pH values higher than those found in wine. In recent years, this fault has become more present, as natural wines gained more and more popularity (Goode 2018).

Over the years, three compounds that caused the off-flavor were identified. The first one was 2-ethyltetrahydropyridine (ETHP). It was first identified in cider in 1973 (Tucknott 1974) and a few years later in wine (Tucknott 1977). Its flavor threshold of 150 µg/L is relatively high, compared to other off-flavor causing compounds (Craig and Heresztyn 1984). This compound is practically never present in such high concentrations, even though lactic acid bacteria strains capable of producing higher concentrations were identified (Costello et al. 2001). This, and the fact that it was found in many mousy wines indicate that it can still be considered an important contributor to mousiness in wine (Snowdon et al. 2006).

The second compound identified in connection with mousiness in wine was 2-acetyl-tetrahydropyridine (ATHP) published in 1984 by Strauss and Heresztyn (referenced in Snowdon et al. (2006)). With a sensory threshold of 1.6 µg/L it is more potent than ETHP. In wine, it is usually present in the non-odor active amino form. At higher pH values it can shift to the more volatile odor-active imino form (Figure 61). It has also been suggested that ATHP can be metabolically transformed to ETHP (Grbin 1998).

Figure 61: Amino and imino form of ATHP (Snowdon et al. 2006, p. 6467)

The last compound found in connection to mousiness is 2-acetylpyrroline (APY). It was first identified in ground pearl millet (Seitz et al. 1993) and a few years later in wine by Herderich et al. (1995).

APY and ATHP have also been identified in the aroma of bread, popcorn and several other foods where they add to the cereal aroma. There it is most likely a product of the Maillard reaction. APY has additionally been found to contribute to the aroma of rice, especially in aromatic rice varieties like Jasmin and Basmati (Snowdon et al. 2006; Adams and Kimpe 2006).

One or more of these three compounds is generally found in wines with a mousy off-flavor. The source of mousiness in wine has been debated over the years but is most likely related to microbial contamination. Some of the bacteria most associated with it are different strains of lactic acid bacteria, *Brettanomyces* and some strains of acetic acid bacteria (Snowdon et al. 2006; Grbin and Henschke 2000; Costello and Henschke 2002). As mousiness is caused by microorganisms, the use of SO_2 can help prevent it. In an interview with Goode, one winemaker, who avoids the use of SO_2, estimates that 20-25% of wines can end up with a mousy taint (Goode 2018).

6.4 Volatile Phenols - Brett

This off-flavor is caused by spoilage of wine with certain *Brettanomyces* yeast strains. When maturing wine is infected with these, be it from incompletely sterilized barrels or other equipment, this can cause the formation of 4-ethylphenol and 4-ethylguaiacol. These compounds can cause aromas reminiscent of horse sweat, barnyard or leather (Suárez et al. 2007; Chatonnet et al. 1992). Opinions on the flavor of brett-tainted wines vary. Some people like the animalistic note, especially in aged red wine from regions with warmer climates (Goode 2018). In young wines, the presence of volatile phenols can, similar to TCA, reduce fruity flavors (Milheiro et al. 2019).

The most important strain in connection with volatile phenols in wine is *Brettanomyces bruxellensis*. Brettanomyces is the asexual (anamorphic) form while the sexual (telemorphic) form of the same genus is called Dekkera (van der Walt 1964; Agnolucci et al. 2017). Both variations have been found in wine and both are capable of the formation of off-flavors (Bartowsky and Pretorius 2009).

The precursor compounds for 4-ethylphenol and 4-ethylguaiacol are *p*-coumaric acid and ferulic acid respectively. Figure 62 shows their decarboxylation to the vinyl form and subsequent reduction to the ethyl form (Bartowsky and Pretorius 2009; Chatonnet et al. 1992).

As with other microbiologically related spoilage the use of SO_2 in must and wine has been the most common way to prevent Brett in wine. Winery equipment and barrels intended for wine aging need to be properly washed and sterilized (Agnolucci et al. 2017; Boulton et al. 1996b). Fabrizio et al. (2015) showed that the treatment of barrels with 60°C water for 19 minutes sufficiently reduced the yeast population. There are also suggested treatments for wines that have already been affected by *Brettanomyces*. A review by Milheiro et al. (2019) lists several methods, including different filtration and fining techniques. Due to the fact that most of them result in a loss of color and/or aroma, preventative steps should be prioritized.

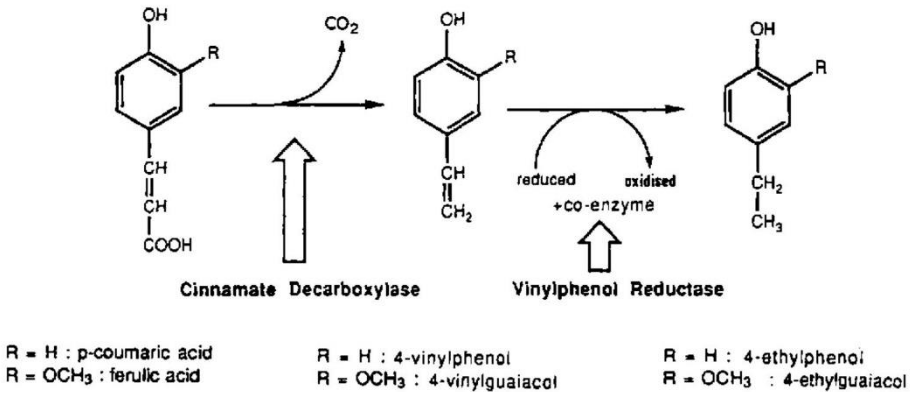


Figure 62: Formation of volatile phenols in Brettanomyces and Dekkera (Chatonnet et al. 1992, p. 176)

Contrary to wine, *B. bruxellensis* is an important strain in the production of some beer styles including lambic and other Belgian styles (Serra Colomer et al. 2019; Crauwels et al. 2015; Spitaels et al. 2014).

6.5 Volatile acidity

In wine, three types of acidity are commonly determined: total, fixed and volatile acidity (VA). The latter is made up of the acids that can be easily removed from the wine by distillation, while fixed acidity refers to the non-volatile acids. Added together they give the total acidity (Jackson 2014).

The main source of VA is acetic acid (AA), which is makes up between 90 and 96% of volatile acids in wine (Bartowsky and Pretorius 2009; Robinson and Harding 2015). Other organic acids up to a chain length of four carbon atoms (formic, propionic and butyric acid) can contribute as well but play a minor role in VA overall. This part will therefore mainly focus on AA as well as ethyl acetate (EA), which - while not an acid - does contribute to the perceived VA of a wine and is produced from AA by bacteria (Bartowsky 2009).

The sensory threshold of AA and EA of 200 mg/L and 7.5 mg/L (determined in water/ethanol (90 + 10, w/w), respectively (Guth 1997b) are relatively high compared to other off-odor causing compounds. The thresholds depend strongly on the style of wine. Corison et al. (1979) found that in white wine the threshold of AA was significantly higher than in red wine (1.13 and 0.79 g/L respectively), while the thresholds of EA were comparable (w: 0.17 and r: 0.16 g/L). In sweeter wines like ice wine, the detection threshold of AA in particular is much higher. Cliff and Pickering (2006) determined thresholds of 3.18 and 0.198 g/L for AA and EA respectively. This suggests that residual sugar can mask AA up to a certain point, which is not necessarily the case for EA (Zoecklein et al. 1999). The legal limits of VA in wine also depend on the wine. In the EU are at 1.2 g/L for red and at 1.08 g/L in white and rosé wine (European

Parliament; European Council 1/31/2019). In sound wines, the levels of AA are usually below 300 mg/L and those of EA below 50-100 mg/L (Jackson 2014).

AA can be produced by lactic acid bacteria as well as *Saccharomyces* yeasts. The main cause of excessive concentrations of AA, however, is microbiological spoilage of wine by acetic acid bacteria (AAB, Figure 63). They are capable of oxidizing ethanol to acetaldehyde and further to AA when oxygen is available (Guillamón and Mas 2009). The most important/common AAB species in wine is *Acetobacter aceti*, which is highly resistant to ethanol. It is therefore also among the species commonly used in vinegar production (Boulton et al. 1996b; Mas et al. 2014). Another product of the AAB metabolism is EA from the esterification of AA (Bartowsky 2009; Ribéreau-Gayon et al. 2006).

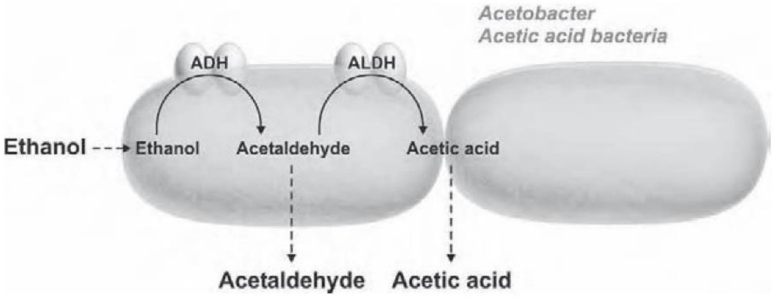
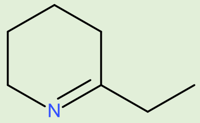
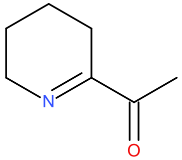
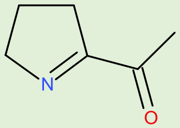
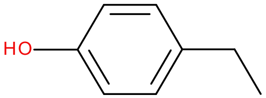
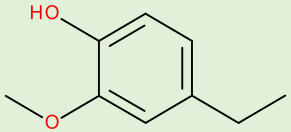
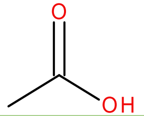
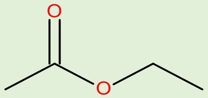


Figure 63: Formation of acetic acid by acetic acid bacteria (König et al. 2009, p. 225; Bartowsky and Pretorius 2009)

AAB are aerobic microorganisms that can survive even at relatively low oxygen levels (Drysdale and Fleet 1988, 1989). Once oxygen is introduced at some step during the winemaking process, growth can be stimulated, and spoilage may occur. AAB can infect wine at multiple points during the production but barrel maturation poses the highest potential risk factor. When the headspace over the wine is improperly topped up, oxygen can encourage the growth of AAB. Oxygen can also be introduced during other wine processing steps, such as racking and fining (Guillamón and Mas 2009; Zoecklein et al. 1999).

AAB are ubiquitous in the vinification process, starting with the grapes, especially if they are spoiled, but also on most of the winery equipment including fermentation and maturation vessels (Guillamón and Mas 2009). It is therefore essential to take preventative measures to avoid wine spoilage. Providing sufficient amounts of SO_2 and ensuring minimal oxygen ingress during maturation can help. Another important factor is the cleanliness of all equipment and vessels as the bacteria can survive on surfaces for a long time. Barrels should be treated with 80°C water for sterilization and filled with water the day before use to remove residual AA and EA. Temperature control during storage around 15°C can provide additional security, as AAB generally prefer higher temperatures (Ribéreau-Gayon et al. 2006).

Table 11: Other off-odor causing compounds identified in wine and some of their properties (Lay 2003; Darriet and Pons 2017; Snowdon et al. 2006; Bartowsky 2009; Suárez et al. 2007; Bartowsky and Pretorius 2009; Goode 2018; Jackson 2014; Guth 1997b; Cliff and Pickering 2006)

Name	Odor description	Sensory Threshold in wine [µg/L]	Structure
2-Ethyltetrahydropyridine	caged mouse	150*	
2-Acetyl-tetrahydropyridine	cracker biscuit, roast smelling, caged mouse	1.6°	
2-Acetylpyrroline	roasted, popcorn-like, caged mouse	0.1°	
4-Ethylphenol	medicinal, barnyard, phenolic, horsey	230-440	
4-Ethylguaiacol	phenolic, sweet, spicy, cloves	47-135	
Acidic acid	vinegar	200,000-3,185,000	
Ethyl acetate	nail polish remover	7,500-198,000	

* taste in water; ° smell in water

6.6 Smoke taint

Many wine-producing regions and countries, like Australia, Mediterranean Europe, California and South Africa, are prone to wild fires (Summerson et al. 2021). Some of the most recent examples are the bush fires in the 2019/2020 season in Australia and the 2020 fires in California. The first resulted in about ~1% of Australia's vineyard area being destroyed and around 4% of the total harvest being exposed to smoke. Some winemakers lost a majority of their harvest and others gave up on their 2020 vintage altogether. The estimated loss is around 40 million Australia Dollars (Claughton et al. 2020; Saladino 2020; Henly 2020). In the summer of 2020, about fifteen thousand km^2 of the vineyard area in California were destroyed and around 8% of the harvest was affected by smoke taint. Overall, experts estimate that it could result in losses of up to 3.7 billion US dollars (Mobley 2021; Saladino 2021; Gray 2020). Climate scientists predict that, in most areas of the world, wildland fires will only become more abundant and stronger over the coming years due to climate change (Flannigan et al. 2009; Krstic et al. 2015). This will most likely result in smoke taint becoming an even bigger problem than it is at the moment.

Figure 64: Burnt vines after the 2019/2020 bush fires in Australia (Claughton et al. 2020)

Wines made from grapes that have been subjected to smoke can exhibit unpleasant ashy, burnt wood and smoked aromas. One Australian winemaker describes it as "burnt salami served on an ashtray" (Saladino 2021). When wine is produced from grapes that have been subjected to smoke, the taint will cover pleasant fruity notes (Kennison et al. 2009). The main compounds associated with this taint are different volatile phenols, including guaiacol, 4-methylguaiacol and cresols (Parker et al. 2012). Table 12 lists several of the compounds and some of their properties. Which compounds are present in the smoke depends on the vegetation as the volatile phenols are decomposition products of the subunits of lignin. Their composition varies from plant to plant based on age, growing conditions and species (Kostyra and Baryłko-Pikielna 2006; Summerson et al. 2021; Krstic et al. 2015; Simoneit 2002). Several studies also showed that grapes are able to glycosylated volatile phenols. These glycosylated forms can function as precursor compounds during the winemaking process and result in increased levels of smoke taint (Hayasaka et al. 2010a; Hayasaka et al. 2010b; Parker et al. 2012).

To prevent smoke taint in wine, grapes can be analyzed for volatile phenols and potential precursor substances. If and how much smoke taint the final wine will contain is, however, complicated to predict as it depends on a multitude of factors, including the exposure, winemaking techniques and grape variety. This knowledge can help winemakers, when working with smoke affected grapes, to minimize the final taint (Ristic et al. 2016; Ristic et al. 2011; Ristic et al. 2017; Fudge et al. 2012; Kennison et al. 2009). Krstic et al. (2015) and Summerson et al. (2021) list different mitigation and remediation techniques in their reviews on the subject. They include selective harvesting, the selection of special yeast strains and several fining agents and reverse osmosis treatment of tainted wines.

6.7 Excessive Greenness and Ladybug Taint

Several different compounds can cause a wine to be excessively green, with aromas reminiscent of cut grass and herbaceous notes. One possibility is the inclusion of unripe grapes or leaves during harvesting, which can result in a high concentration of C6 compounds, fittingly called “green leave volatiles”, like hexenal and hexanol. They are generally associated with the green character attributed to mechanical harvesting and unripe berries (Herraiz et al. 1990; Gómez et al. 1995). Some of the C6 compounds, especially aldehydes and alcohols, can potentially be degraded during fermentation by yeast, therefore reducing their influence on the final wine (Jackson 2014; Joslin and Ough 1978). The best way to avoid high concentrations of C6 compounds is to harvest ripe grapes (García et al. 2003) and the exclusion of material other than grapes. With newer, more advanced harvesters and post-harvest processing this can be achieved even for mechanically harvested grapes (Fink 2017; Parenti et al. 2015).

Another compound that is specifically associated with ladybug taint is 2-isopropyl-3-methoxypyrazine (IPMP). Methoxypyrazines have already been discussed in the section about varietal aroma compounds as IPMP, alongside 2-isobutyl-3-methoxypyrazine, can be present in grapes up to a certain amount (Allen et al. 1991; Allen et al. 1995; Lacey et al. 1991).

Anecdotal evidence connected an atypical aroma to the presence of large numbers of ladybugs (up to 50 per bunch) (Pickering et al. 2004; Ejbich 2003; Koch 2003). Al Abassi et al. (1998)

found that Multicolored Asian Lady Beetle (MABL) use IPMP as pheromone to alter signal for potential predators. It is part of the beetles' hemolymph and can be released when the MALB is stressed or crushed. They were introduced as a biocontrol agent against aphids and other insects in the US and Canada (Botezatu and Pickering 2010) and have also been identified in multiple European countries, including wine producers like Spain, Portugal and Italy (Brown et al. 2008). The adult beetles are known to aggregate in vineyards on grape clusters in autumn, especially when they include damaged berries (Koch et al. 2004; Koch 2003). Pickering et al. (2004) were among the first to investigate whether the presence of MALB during grape processing can have an influence of the finished wine. They found that already one beetle per liter of juice can result in a sensory difference when tested by a trained sensory panel. The same group found that one beetle per liter of juice can result in IPMP concentrations of around 10 ng/L. This is well above the sensory threshold, which was determined at 0.32 ng/L for certain white wines and around 2 ng/L for red wines (Pickering et al. 2007). This only increased with larger amounts; the addition of ten beetles per liter resulted in concentrations above 30 ng/L (Pickering et al. 2005). More recently, other ladybug species, like the in Europe more common 7 spotted form, were also found to cause the taint (Botezatu et al. 2013).

Figure 65: The multicolored Asian lady beetle, photo by R.C. Venette, University of Minnesota (Koch 2003, p. 1)

The best way to prevent ladybug taint in wine is the removal of the beetles before crushing. This can be done by soaking affected grape bunches in water. The beetles will float and can be removed from the surface of the water. Other post-harvest treatment are the use of shaker tables or manual removal of the bugs. Further treatments options include the use of special yeast strains, different fining agents and closures (Botezatu and Pickering 2010; Pickering et al. 2006; Pickering et al. 2010; Pickering et al. 2008b)

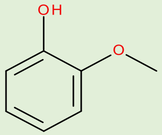
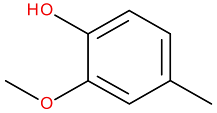
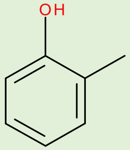
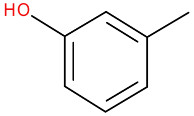
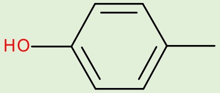
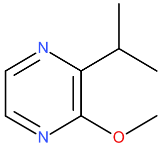
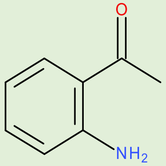
6.8 Untypical Aging Off-Flavor (UTA)

Starting in the late 1980s, German wine producers found their white wines to have an undesirable off-flavor. Descriptions of affected wines ranged from wet cloth, fusel alcohol and hybrid note to flowery. It also resulted in the loss of varietal aroma. The fault was given the name untypical aging off-flavor (UTA) (Hoenicke 2002; Hoenicke et al. 2002b; Henick-Kling et al.). Rapp et al. (1993) found that one of the main compounds differentiating wines with this off-flavor were high concentrations of 2-aminoacetopheone (AAP). This compound had also been identified in non-vinifera species (discussed in 3.1.1, Rapp and Versini 1996). In this case, the wines had been produced from *V. vinifera* cultivars, especially in years with high amounts of sun and low rainfall. It was therefore concluded that the production of AAP was most likely due to drought stress of the vine. Later, it was also connected to nitrogen deficiency (Henick-Kling et al.). Another possible contributor to UTA is the presence of skatole. This, however, is still an object of investigation (Schneider 2014). UTA appears in young white wines within a year after fermentation (Darriet and Pons 2017).

Studies found that the phytohormone indole-3-acetic acid (IAA) functions as a precursor for AAP (Christoph et al. 1998; Hoenicke et al. 2002b). For this process to occur, a sulfuration of the wine, which results in the formation of superoxide radicals, is needed (Hoenicke et al. 2002a). Another factor in the formation of AAP is the ripeness of the grapes, as more IAA is formed during the fermentation of unripe berries. Some non-Saccharomyces yeasts can additionally influence the formation of AAP (Hoenicke 2002; Sponholz and Hühn 1996). Photooxidation of the amino acid tryptophan also resulted in the formation of AAP (Horlacher and Schwack 2014).

To avoid the formation of UTA in dry years, it is advisable to ensure sufficient amounts of water and nutrients and a later harvest date. The latter ensures a high antioxidative capacity, which reduces the chances of UTA formation. During the vinification process, the use of single culture yeasts and controlled fermentation temperatures can provide additional security, by reducing the formation of IAA (Hoenicke 2002). The only post fermentation treatment that was shown to have an effect on UTA was the addition of ascorbic acid during maturation as it can prevent the formation of AAP (Schneider 2014).

Table 12: Off-flavor causing compounds and some of their properties (Summerson et al. 2021; Parker et al. 2012; Ojeda et al. 2002; Darriet and Pons 2017; Pickering et al. 2007; Hoenicke et al. 2002b)

Name	Odor description	Sensory Threshold in wine [µg/L]	Structure
Guaiacol	smoky, woody, petroleum-like, musty, phenolic	20-95	
4-Methylguaiacol	smoky, toasted, phenolic, ash, vanilla-like, sharp	65	
***o*-Cresol**	smoky, woody, dusty, pungent, petroleum-like	31-62	
***m*-Cresol**	smoky, petroleum-like, woody, musty	20-68	
***p*-Cresol**	phenolic, animal, tar, smoky, medicinal	10-64	
2-Isopropyl-3-methoxypyrazine	pea, green, asparagus, earthy	0.0003-0.002	
2-Aminoacetophenone	mothball, acaia blossom, floor polish, well wool	0.7-1	

7. Analytical Background

Wine aroma depends on many factors, some of which have been touched upon in the previous chapters. It is a very complex topic and has been a subject of scientific research for decades, if not centuries. Over time, many analytical tools have been developed for the qualitative and quantitative determination of compounds that are important in the context of wine. The tools and their applications have been refined and adapted in order to answer more and more precise questions surrounding the topic. In the following chapter some of the techniques used in the practical part will be discussed briefly.

7.1 Sample Preparation Techniques

As one of the first steps in the analytical process it is important to adequately prepare a sample for subsequent measurements. The choice of sample preparation technique depends on several factors, including the properties of the analyte(s), the scientific question and the intended analytical technique (Ebeler 2001; Guasch and Busto 2000). In addition, it is important to provide the sample in a form that can be introduced into the analytical instrument without damaging or contaminating it, for example by clogging parts of the instrument. It is therefore essential to remove potentially interfering substances and matrix constituents. Sample preparation techniques are also used to pre-concentrate certain analytes, especially when their initial concentration might be below the analytical threshold of the chosen method (Costa Freitas et al. 2012; Polásková et al. 2008).

Solvent based techniques have traditionally been used in wine aroma analysis. There are, however, some drawbacks when working with solvents. Most solvent based extraction methods are labor intensive and might need several steps. Some of the used solvents can also be harmful to the environment or the user (Polásková et al. 2008). Over the last years, techniques have been developed that minimize the amount of solvent (liquid–liquid microextraction) used or that use less or non-toxic solvents. Some of the more common solvents include diethyl ether, dichloromethane, *n*-pentane and *n*-hexane as well as Freon-11, or mixtures of two or more solvents. Which solvents and in which ratio they are used, depends on the analyte (Costa Freitas et al. 2012; Villiers et al. 2012).

Solid phase extraction (SPE) was first created to be used alongside or as an alternative to solvent based extraction methods. In this technique, the sample is applied to a sorbent where the analyte is concentrated and subsequently extracted with a selected elution solvent (Figure 66). The choice of sorbent and elution solvent depends on the analyte (Żwir-Ferenc and Biziuk; Poole 2003; Berrueta et al. 1995). It can also be used after derivatization for example in the analysis of varietal thiols (Herbst-Johnstone et al. 2013).

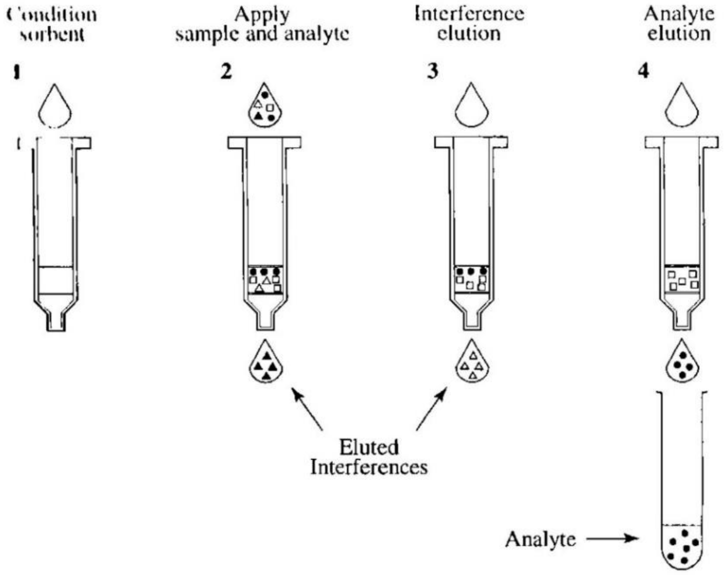


Figure 66: The steps of solid phase extraction (Thurman and Mills 1998, p. 3)

A different possibility for sample preparation are solvent free methods. In these methods the volatile compounds in the headspace above the sample are analyzed. There are several possibilities, like using a syringe to extract some of the sample headspace and inject it into an instrument or purge and trap methods. There, the headspace is purged using an inert gas and the compounds are trapped and pre-concentrated (Polásková et al. 2008; Costa Freitas et al. 2012).

In the 1990s, a new solvent free sample preparation technique, based on the principal of SPE, was introduced: solid phase microextraction (Arthur and Pawliszyn 1990).

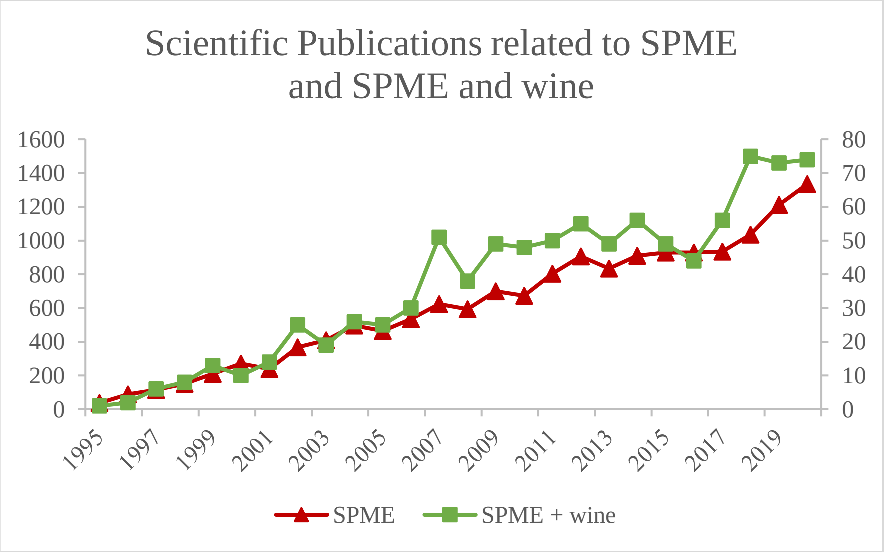


Figure 67: Number of scientific publications covering SPME (▲) and SPME and wine (■) (1995-2020)

7.1.1 Solid Phase Microextraction (SPME)

Over the last years, this has become a very common technique to analyze the volatile compounds in wine. Figure 67 shows the increasing trend in the number of publications dealing with SPME (and SPME and wine in particular) that can be found on the Scopus database. Overall, there are more than 900 out of which about 25 are review articles.

For SPME, a fused silica fiber is inserted into the headspace of the sample or the sample itself. The fiber can be coated with different materials, depending on the sample and the analyte compounds, which ad- or absorb onto the fiber. The fiber is subsequently inserted into the heated inlet of a gas chromatograph (GC), where the compounds are desorbed and enter the instrument (Figure 68). SPME can also be used in combination with liquid chromatography.

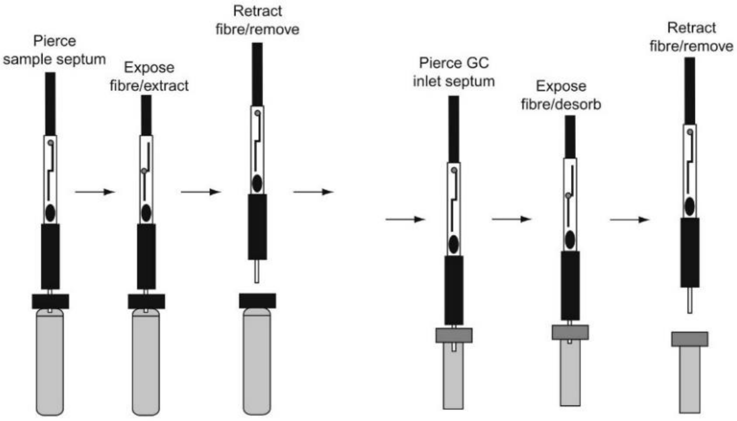


Figure 68: Operating principle of SPME (Shirey 2011)

The two operation modes for SPME are direct immersion and headspace SPME (Figure 70). In the first case, the fiber is immersed directly in the sample and the analyte compounds ab- or adsorb onto the fiber from the liquid itself. The mode more commonly used in aroma analytics is headspace (HS) SPME. Here, the fiber is inserted into the headspace above a sample in a closed vial. Agitation and heating of the sample can help to release the analyte compounds from the sample. In this variation only the volatile compounds ab- or adsorb onto the fiber, which results in a lower amount of background. It can be used with all kinds of samples (Marín-San Román et al. 2020; Kataoka et al. 2000).

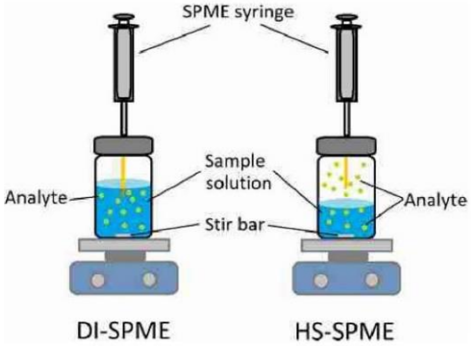


Figure 69: SPME operation methods direct immersion and headspace (Sereshti et al. 2020)

When developing an SPME method, several factors have to be optimized including the conditions, like agitation and temperature of the sample during the exposure of the fiber. In addition, the volume of sample and headspace can be adjusted, and the coating material of the fiber can be changed. Some of the most common coating materials are listed in Table 13. For the release of some compounds the addition of salt and an adjustment of the pH can be beneficial (Marín-San Román et al. 2020; Azzi-Achkouty et al. 2017).

Table 13: Types of coatings for SPME fibers (PDMS: Polydimethylsiloxane, DVB: Polydivinylbenzene, PA: Polyacrylate) (Shirey 2011)

Type of coating	Extraction Mechanism	Polarity
7 µm PDMS	Absorbent	Non-polar
30 µm PDMS	Absorbent	Non-polar
100 µm PDMS	Absorbent	Non-polar
85 µm PA	Absorbent	Polar
60µm PEG (Carbowax)	Absorbent	Polar
15 µm Carbopack Z-PDMS	Adsorbent	Bipolar
65 µm PDMS-DVB	Adsorbent	Bipolar
55 µm/30 µm DVB/Carboxen-PDMS	Adsorbent	Bipolar
85 µm Carboxen-PDMS	Adsorbent	Bipolar

During the exposure of the fiber a pre-concentration of analyte compounds on the fiber occurs. An equilibrium between sample and headspace is established in the equilibration time before the fiber is exposed to the headspace. When the fiber is exposed, the compounds ab- or adsorb and are, therefore, removed from the headspace, allowing for a re-establishment of the equilibrium between sample and headspace. As a result, the analyte compounds reach higher concentrations on the fiber than they would by using traditional HS sampling techniques, when the extraction is optimized (Rocha et al. 2001; Marín-San Román et al. 2020; Costa Freitas et al. 2012; Zhang et al. 1994; Zhang and Pawliszyn 1993).

Compared to other sample preparation techniques, SPME has several advantages over some of the previously mentioned techniques. It is a relatively quick and simple technique that does not require organic solvents. It can be used with a wide range of sample types and volumes. One major advantage is its possibility of automatization, which allows for a high sample throughput and repeatability (Marín-San Román et al. 2020; Bojko et al. 2012; Azzi-Achkouty et al. 2017).

While traditional SPME offers many advantages, there are also some drawbacks that have to be taken into account. The main disadvantages are the high fragility and the limited sorptive capacity of the fibers. One way of dealing with these problems was the development of stir bar sorptive extraction, where a coated stir bar is used for the extraction and pre-concentration of analyte compounds. While offering higher sensitivity, this technique requires special systems for the injection of the samples into the analytical instrument (Marín-San Román et al. 2020; Costa Freitas et al. 2012). Another method aiming for the resolution of the drawbacks of conventional SPME methods are PAL SPME Arrows. Compared to conventional SPME fibers, the coating is applied onto a stainless-steel rod with a solid metal tip (Figure 70). In addition, they offer a larger sorption phase, which allows for higher sensitivity. This system, usually only requires minimal changes in the instrumental setup (Kremser et al. 2016; Herrington et al. 2020).

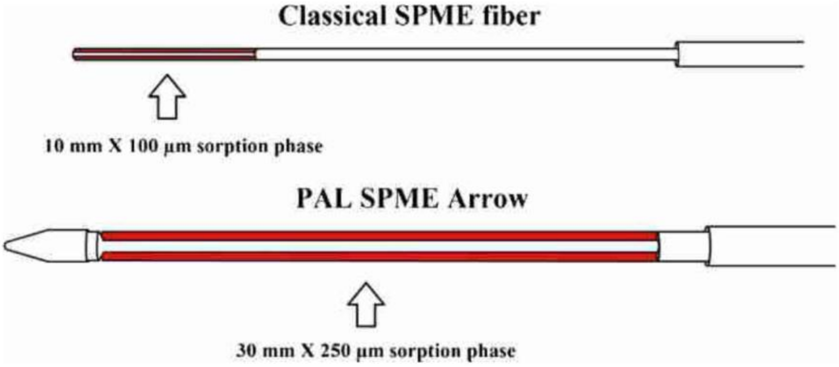


Figure 70: Conventional SPME fiber compared to PAL SPME Arrow (Kremser et al. 2016, p. 944)

7.2 Gas Chromatography (GC)

The term chromatography was first used by Russian botanist Michael Tswett at the beginning of the 20th century. He used a column made from calcium carbonate to separate a mixture of plant pigments by washing the column with carbon sulfide. The name is derived from the Greek and can be translated as “color writing”. Chromatography is a group of methods which use two distinct phases to separate a mixture: a stationary phase and a mobile phase which permeates through the stationary phase. There are two main categories based on the mobile phase: liquid chromatography, which uses different solvents and GC where the mobile phase is a gas (Miller 2003; Bartle and Myers 2002).

Today, GC is the most common analytical technique for the analysis of volatile compounds. It was first developed in the 1950s and is generally attributed to James and Martin (1952) who used it to separate volatile fatty acids. Since then, the technique has been advanced greatly, even though the basic workings remained the same (Bartle and Myers 2002).

Figure 71 shows the setup of a basic GC-system. The sample is injected at a heated injector (4) from where it reaches the analytical column (6) which resides in an oven (5). Using an inert gas, usually helium, nitrogen or hydrogen, the sample compounds are separated based on their affinity to the column material and/or their boiling point. Once they exit the column, they are registered by a detector (7), which is connected to a data system (8).

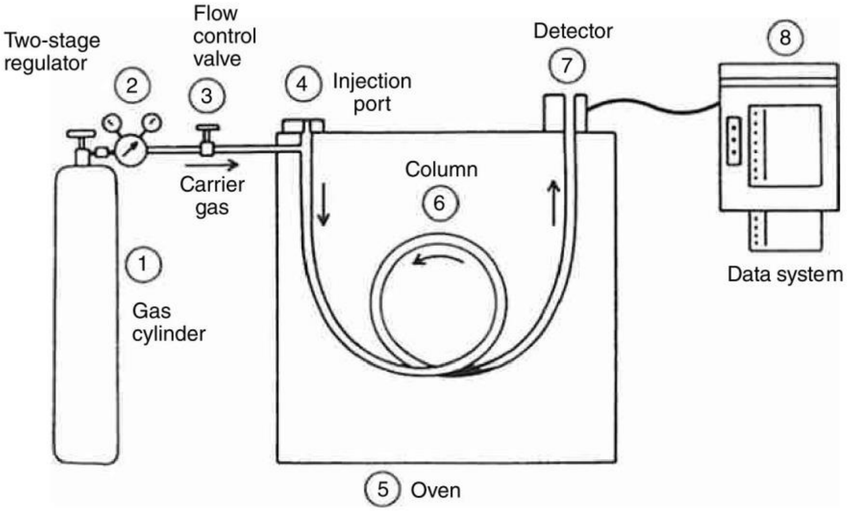


Figure 71: Standard GC System (McNair et al. 2019, p. 13)

As McNair et al. (2019, p. 12) put it: "The column is the heart of any gas chromatograph; it is where the separation occurs." In 1959, the first capillary columns were introduced. Until then, packed columns were used. Today, approximately 90% of all GC applications use capillary columns, the other 10% are packed columns (McNair et al. 2019).

There are three main types of capillary columns: the wall-coated open tubular (WCOT) as well as support-coated and porous layer OT. The first is by far the most common type. Commercially available columns are usually between 10 and 60 meters long and come in a range of different inner diameters and film thicknesses. The polarity of a column is based on the composition of the stationary phase and is vital to the separation (Poole 2012; McNair et al. 2019; Sparkman et al. 2011).

In order to archive separation, the second choice, after the column, is if and which temperature programming should be used. Figure 72 shows two separations of the same mixture of *n*-alkanes with two different temperature options. The first chromatogram (a) is the result of an isothermal separation, where the temperature stays constant over the whole course of the run. For the second (b) the oven temperature is changed based on a set program over the course of the run. The first can work for simple mixtures, where only a few compounds with rather similar properties have to be separated. As soon as the mixture gets more complex and the individual compounds differ in their properties, the use of temperature programming is almost unavoidable. This program can also include phases of staying on a constant temperature. The rate at which the temperature increases, if and when to keep the temperature constant as well as initial and final temperature can be adjusted to fit the specific scientific question and sample mixture. Overall, using a temperature program is the more common choice (Sparkman et al. 2011; McNair et al. 2019).

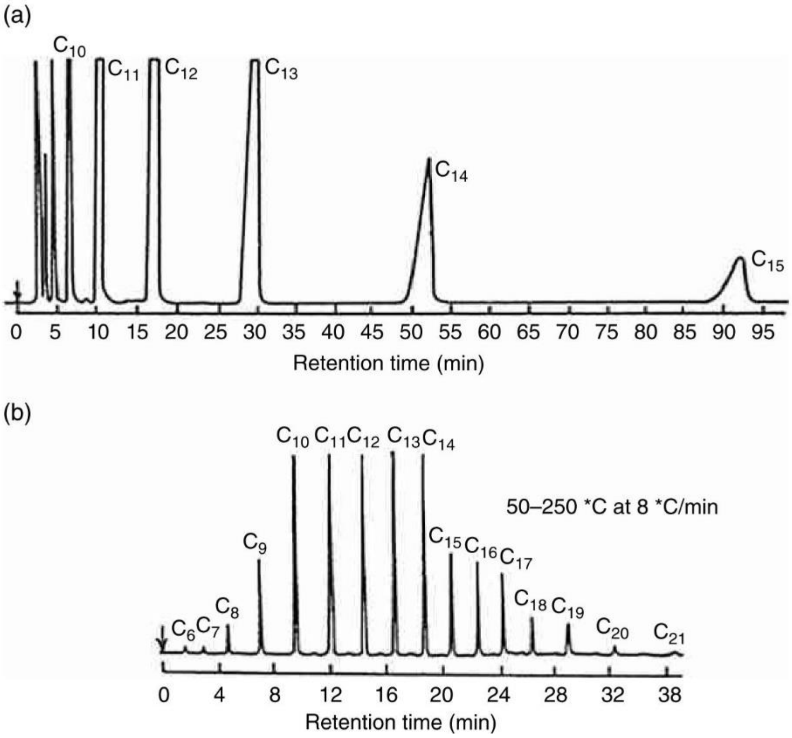
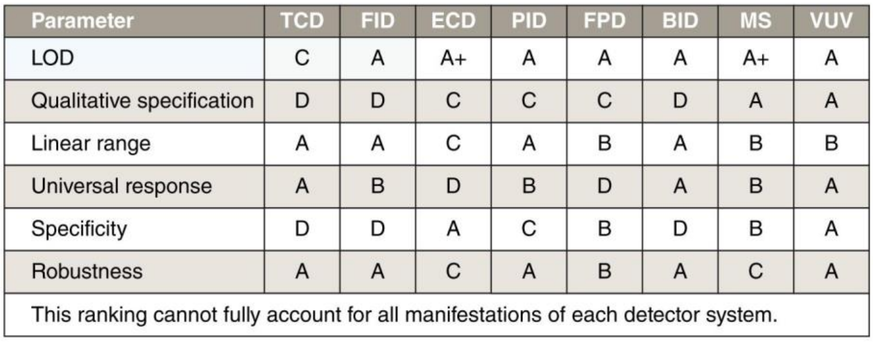


Figure 72: Isothermal and temperature programmed GC runs of the same mixture of n-alkanes (McNair et al. 2019, p. 88)

After the compounds have been separated on the GC column, they have to be recorded. This is the function of the detector. Different detectors offer different information, based on their operating principle. Some of the most common ones include the flame ionization detectors (FID) and mass sensitive detectors (MS) (McNair et al. 2019; Poole 2012). Figure 73 shows a table of some of the available detectors graded based on their abilities. Some of the detectors used in the following practical part will be discussed more in detail in 7.3.



Parameter	TCD	FID	ECD	PID	FPD	BID	MS	VUV
LOD	C	A	A+	A	A	A	A+	A
Qualitative specification	D	D	C	C	C	D	A	A
Linear range	A	A	C	A	B	A	B	B
Universal response	A	B	D	B	D	A	B	A
Specificity	D	D	A	C	B	D	B	A
Robustness	A	A	C	A	B	A	C	A
This ranking cannot fully account for all manifestations of each detector system.								

Figure 73: Grades for different detector systems used in combination with GC (McNair et al. 2019, p. 49)
TCD: Thermal conductivity detector; ECD: Electron capture detector; PID: Photoionization detector;
FPD: Flame photometric detector; BID: barrier discharge ionization detector; VUV: vacuum ultraviolet spectroscopy

7.2.1 Multidimensional GC Techniques (MDGC)

While GC is a high-resolution technique, it reaches its limits when a sample contains compounds that behave very similar on the selected column. This can result in two or more compounds leaving the column or co-eluting at the same time. To resolve this problem multidimensional GC techniques can be used. These systems use more than one column that differ from each other in polarity. Compounds that have the same retention behavior on, for example, a non-polar column, can usually be separated on a polar column.

There are two main operating principles in MDGC: Heartcut GC and Comprehensive GC. The first uses two full length columns. After a separation on the first column, one or more fractions can be chosen, which instead of reaching the detector are applied onto a second column with a different polarity from the first one. The peak(s) will not appear in the chromatogram of the first-dimension column as it has been "cut" from it, giving this technique the name "heart cutting" (Figure 74A). The second option is comprehensive GCxGC (Figure 74B), where not just single fractions, but the whole eluate from the first column is separated on two columns. In this technique, the second column is significantly shorter than the column of the 1st dimension, usually between 0.5 and 2 meters to allow for a fast separation (Dallüge et al. 2003). The two columns are connected with a press-fit connector and an interface called a modulator, which allows the transfer of short effluent fractions. Most systems are based on thermal modulation, where effluent fractions are collected by concentrating them using a set of cold jets. Those fractions are then rapidly thawed by a set of hot jets, releasing them onto the second column. The fraction size depends on the modulation period, which usually is only several seconds long. Over this time, the effluent is collected and sent onto the second column in narrow pulses. The fractions are then separated on the second column, after which they reach the detector. This allows for a full two dimensional separation of the sample and a deep insight into its composition (Poole 2012; McNair et al. 2019).

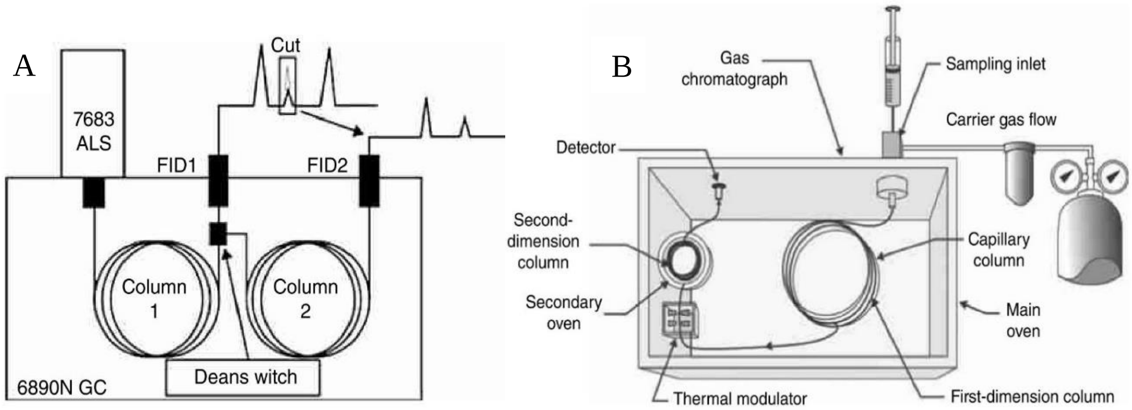


Figure 74: Schematic setup of "heart cut GC" (A) (McNair et al. 2019, p. 202) and comprehensive GCxGC (B) (McNair et al. 2019, p. 204)

7.3 Detectors

To record the compounds eluting after separation on the GC column, different detectors can be used. The choice of detector, as all things in analytical chemistry, is based on the scientific question, but also on the instruments one has available. Sensitivity as well as selectivity, the ability to detect the compounds of interest, play a role. Figure 75 shows the linear range of some of the most commonly used detector in combination with GC.

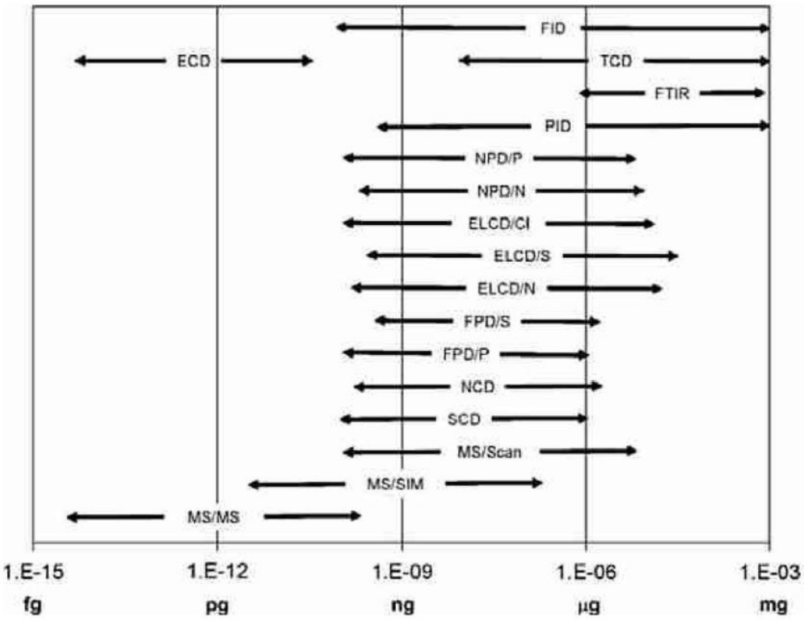


Figure 75: Operating range of some GC detectors (Poole 2012, p. 313)

7.3.1 Flame Ionization Detector (FID)

The most wide spread detector is the FID, which was specifically invented for the use with a GC. It is considered a fairly universal detector as it responds to all organic compounds that can be burned in a hydrogen-oxygen flame. It is rather cheap, easy to use and robust. Another feature that makes it a very versatile detector is its wide linear range, spanning up to seven orders of magnitude (McNair et al. 2019; Poole 2012).

In this detector, the effluent exiting the column is mixed with hydrogen and then burned in a small oxyhydrogen flame. This creates ions which are collected to form a small electrical current that generates a signal that is recorded. The FID works with organic compounds that can be burnt in its flame and the signals are proportional to the amount of carbon that reaches the detector. The concentration of a compound is relative to the peak height, allowing for relatively easy quantification (McNair et al. 2019; Poole 2012).

Identification of compounds can be more complex, but can done using the retention index of a peak. This is a system based on the retention time of a compound in relation to those of a series of *n*-alkanes first suggested by Kováts (1958).

7.3.2 Mass Spectrometry

The most important technique applied for the practical part of this work was GC coupled to different mass selective detectors. One advantage of MS techniques, in comparison to for example FID, is that they offer easier identification of the investigated compounds. This has made them a standard technique when investigating unknown samples in an untargeted approach. Looking at the operating range of some MS based detectors depicted in Figure 75, one can see that they are also very sensitive, in some cases reaching down into the ppt (ng/L) or even ppq (pg/L) range. This makes them very capable also for targeted analysis.

Mass spectrometry itself has its origins in the late 19th century and is based on the works of John Joseph Thomson and several others. The combination with GC methods followed shortly after their invention in the middle of the 20th century. The techniques became more advanced over time, resulting in the sophisticated instruments of today (Grayson 2016; Griffiths 1997; Maher et al. 2015; Sparkman et al. 2011).

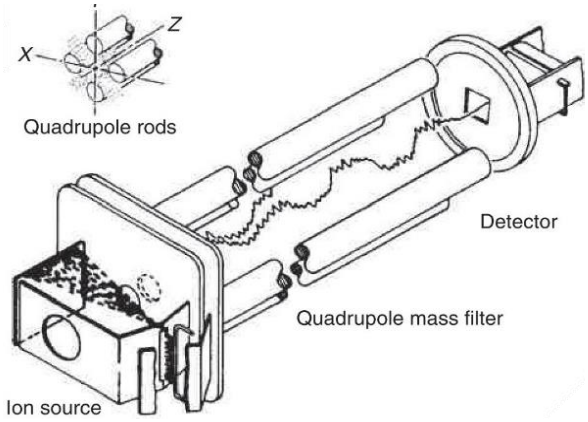


Figure 76: Quadrupole mass filter (Sparkman et al. 2011, p. 114)

MS detectors rely on the analysis of compounds based on the mass-to-charge ratio (m/z) of the compound or fragments of it. The first step is ionization that can be induced in several different ways. The most common one of which is electron impact ionization (~90%), which results in the fragmentation of the analyte compounds. This allows an identification of the compounds based on its characteristic fragmentation pattern, which can be matched to different databases. Other ionization techniques are chemical or field ionization, as well as electron capture negative ionization (Sparkman et al. 2011). After the ionization, and possible fragmentation, of the analyte compounds, they reach the m/z analyzer. There, they are separated based on their m/z value. The most commonly used analyzer is a quadrupole mass filter, which uses different types of currents (direct and alternating) to separate the ionized molecules according to their m/z values in a radio frequency electric field. It consists of two sets of metal rods to which different types of currents are applied, creating an alternating field (Figure 76). Only ions with specific m/z values can pass through while others are “filtered” out. The fragment ions are then detected, for example by a photomultiplier. The detector successively records mass spectra at a constant rate. The mass spectra collected over the course of one run can be displayed as total ion current (TIC) chromatogram (Sparkman et al. 2011).

MS instruments can be operated in different modes. One of them is the detection of full mass spectra, this is called full scan mode. This mode allows the identification of analytes based on their mass spectra, which as mentioned before are characteristic for each compound. Here, the recorded m/z values are alternated in regular intervals, called scan rate. This rate is based on the dwell time, the time one specific m/z value is observed, and the scan range, the range of observed m/z values (Hoffmann and Stroobant 2008). The scan rate has to be adjusted, so that one peak contains at least five data points, which are needed for its definition (Sparkman et al. 2011; Hübschmann 2015; Hoffmann and Stroobant 2008). In general, scan mode is usually used in untargeted analysis for the identification of compounds in an unknown mixture or when lower sensitivity is required. Figure 77A shows how mass spectra are acquired in full scan mode from start to end of the mass range.

Another mode that can be used, when the analyte compounds are known and higher sensitivity is required, is selected ion monitoring (SIM). In this targeted approach, the operator chooses selected m/z values (usually between three and ten), which are observed instead of a full range. This reduces the background noise and allows for higher scan rates or longer dwell times. As the selected m/z values are characteristic for the target compounds, this mode is also very selective. However, some m/z values can be present in mass spectra of multiple compounds or a group of compounds. Therefore, the settings have to be chosen carefully and might need to be optimized (Gross 2017; Hübschmann 2015). Figure 77B shows how mass spectra are recorded in SIM mode with three selected ions.

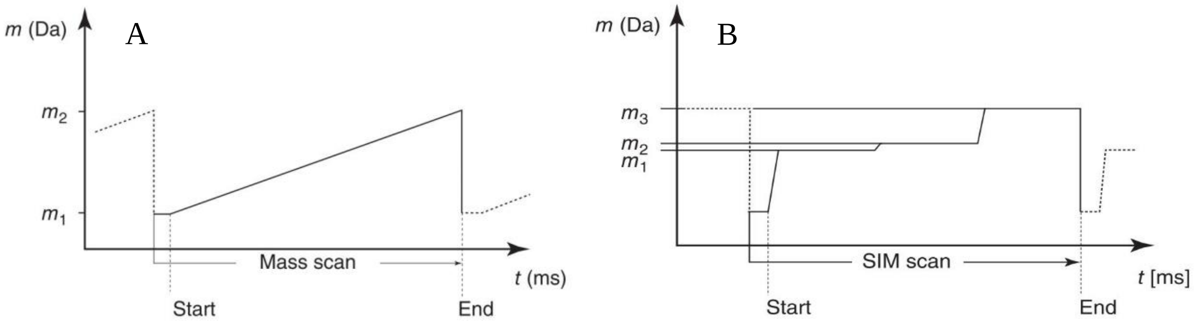


Figure 77: Different operation modes of single quadrupole mass analyzers: full scan mode (Hübschmann 2015, p. 285) and selected ion monitoring (Hübschmann 2015, p. 287)

For analysis that require higher sensitivity and selectivity than can be achieved in SIM mode, one possibility is the use of tandem mass spectrometry (GC-MS/MS). The use of this technique allows the acquisition of data in multiple reaction monitoring (MRM). For this, ions of selected m/z values are separated in one quadrupole (Q1) captured and fragmented further in a collision cell (Q2). In a second mass analyzer (Q3), selected fragment ions are then filtered out and detected (Gross 2017; Hoffmann and Stroobant 2008; Hübschmann 2015; Sparkman et al. 2011). The setup of a GC-MS/MS system is depicted in Figure 78.

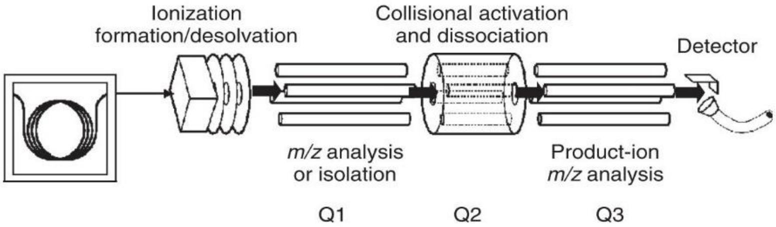


Figure 78: Schematic setup of a tandem mass analyzer (Sparkman et al. 2011, p. 143)

GC-MS/MS can be used to analyse compounds that are present at ppt or even ppq levels in a complex mixture of compounds, due to the high sensitivity and selectivity of MRM mode (Poole 2012). Figure 79 show an example of detecting contamination in a fuel oil sample. In TIC chromatogram recorded in scan mode (A) the compounds of interest are almost undetectable. For this example, two transitions (characteristic fragmentation of a selected fragment) were chosen, which makes the detection of the contaminant compound possible. For the selection of the most suitable transitions, product ion scans for different fragments are carried out at different collision energies. Out of those the most suitable transitions are chosen (Hoffmann and Stroobant 2008).

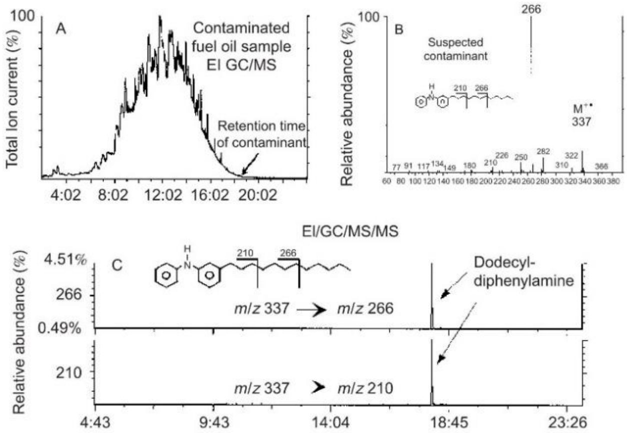


Figure 79: Example for an application of tandem mass spectrometry for increased selectivity (Hoffmann and Stroobant 2008, p. 231)

In the practical part of this work, GC-MS/MS was used for the quantification of aroma compounds with sensory thresholds in the low ng/L range, including several methoxypyrazines and varietal thiols, as well as some compounds responsible for off odors in wine, like 2,4,6-trichloroanisole.

Practical Part

8. Materials and Methods

8.1 Materials and Chemicals

8.1.1 Chemicals

The chemicals and solvents used in the different experiments are listed in Table 14. This includes the name, CAS-number, their purity as well as the manufacturer. The last column states the experiment(s) in which they have been used.

Table 14: Chemicals used; methods: a…aroma profiles, b…methoxypyrazines, c…thiols, d…TCA, e…ethylester

Compound	CAS Number	Purity	Manufacturer	Used in
Methanol (MeOH)	67-56-1	≥ 99.9%	VWR Chemicals, Randor, PA, USA	a, b, c, d, e
Ethanol (EtOH)	64-17-5	≥ 99.9%	CHEM-LAB NV, Zedelgem, Belgium	b, c, d, e
Ethyl acetate (EA)	141-78-6	≥ 99.9%	Carl Roth Gmbh & Co. KG, Karlsruhe, Germany	c
3-Heptanol	589-82-2	98%	Sigma-Aldrich, St. Louis, MO, USA	a
Methyl nonanoate	1731-84-6	97%	Sigma-Aldrich, St. Louis, MO, USA	a
Hexyl butyrate	2639-63-6	98%	Tokyo Chemical Industry, Tokyo, Japan	e
Tartaric acid	133-37-9	≥99.5%	Merck KGaA, Darmstadt, Germany	b, c, d, e
2-isopropyl-3-methoxypyrazine (IPMP)	25773-40-4	97%	Sigma-Aldrich, St. Louis, MO, USA	b
d3 2-isopropyl-3-methoxypyrazine	588732-60-9	100µg/ml in Methanol	Toronto Research Chemicals, Toronto, ON, Canada	b
2-Isobutyl-3-methoxypyrazine (IBMP)	24683-00-9	99%	Sigma-Aldrich, St. Louis, MO, USA	b
d3 2-Isobutyl-3-methoxypyrazine	588732-63-2	100µg/ml in Methanol	Toronto Research Chemicals, Toronto, ON, Canada	b
3-Sulfanylhexan-1-ol (3SH)	51755-83-0	98%	Acros Organics B.V.B.A., Fair Lawn, NJ, USA	c
d10 3- Sulfanylhexan-1-ol			University of Zagreb, Zagreb, Croatia	c
3-Sulfanylhexyl acetate (3SHA)	136954-20-6	98%	Apollo Scientific, Stockport, UK	c
d5 3- Sulfanylhexyl l acetate			University of Zagreb, Zagreb, Croatia	c
2,4,6-Trichloranisol (TCA)	87-40-1	99%	Sigma-Aldrich, St. Louis, MO, USA	d
d5 2,4,6-Trichloranisol	352439-08-8	99.5%	Dr. Ehrenstorfer GmbH, Augsburg, Germany	d
2,4,6-Tribromanisol (TBA)	607-99-8	99%	Sigma-Aldrich, St. Louis, MO, USA	d
d5 2,4,6-Tribromoanisole	1219795-33-1	99.5%	C/D/N Isotopes, Montreal, QC, Canada	d
Ethylpropriolate (ETP)	623-47-2	99%	Sigma-Aldrich, St. Louis, MO, USA	c
Sodium hydroxide (NaOH)	1310-73-2	98%	Carl Roth Gmbh & Co. KG, Karlsruhe, Germany	c
Sodium sulfate (Na_2SO_4)	7757-82-6	≥99.0%	Sigma-Aldrich, St. Louis, MO, USA	c

8.1.2 Materials

The following table lists the materials and some devices used in one or more of the methods employed over the course of the practical work conducted as part of this thesis.

Table 15: Materials and devices used; methods: a…aroma profiles, b…methoxypyrazines, c…thiols, d…TCA, e…ethylester

Material or device	Manufacturer	Used in
20 mL glass headspace vials equipped with silicone/PTFE septa (N20; 8 mm)	Machery-Nagel GmbH & Co. KG, Düren, Germany	a, b, d, e
Fisherbrand™ Magnetic Stir Bar, Glass-Encased	Fisher Scientific GmbH, Schwerte, Germany	a, b, d, e
0.2-2 mL Manual Macropipette	SOCOREX, Ecublens, Switzerland	a, b, c, d, e
Pasteur pipettes, glass, unplugged with 150 mm length	Fisher Scientific GmbH, Schwerte, Germany	a, b, c, d, e
MICROMANN™ Positive-Displacement Pipet 1-10µL	Gilson, Middleton, WI, USA	a, e
1-10µL Tips for positive displacement pipet	Gilson, Middleton, WI, USA	a, e
BLAUBRAND® Micropipettes 1 µL-200 µL	BRAND GmbH & Co. KG, Wertheim, Germany	a, b, c, d, e
2 cm stable flex SPME fibers, DVB/CAR/PDMS, 50/30 µm	Supelco, Bellefonte, PA, USA	a, e
1.1. mm DVB/Carbon Wide Range/PDMS Smart SPME Arrow, 120µm phase thickness	CTC Analytics AG, Zwingen, Switzerland	b, d
PAL3-SyS-002861 10µL Syringe, Ø=6.6 mm; Length=57 mm; Point=cone; Scale 54.1 mm	CTC Analytics AG, Zwingen, Switzerland	c
100 mL Screw cap bottles	SIMAX (Czech Republic)	c
Screw cap tube, 50 mL, (LxØ): 114 x 28 mm, PP, with print and red HD-PE cap	Sarstedt AG & Co. KG, Nümbrecht, Germany	c
Centrifuge Rotofix 32	Andreas Hettich GmbH & Co.KG, Tuttlingen, Germany	c
M2-D PRO ARGOlab MAGNETICAL STIRRER-Digital Control	ARGO LAB, Capri, Italy	
Varian Vac Elut 20 Manifold for SPE cartridges	Agilent Technologies, Santa Clara, CA, USA	c
SPE cartridges Supelclean™ ENVI™-18; bed wt. 1 g, volume 6 mL	Supelco, Bellefonte, PA, USA	c
Fisherbrand™ PTFE Magnetic Stir Bar	Fisher Scientific GmbH, Schwerte, Germany	c
Automatic solvent evaporator, TurboVap® II	Biotage, Uppsala, Sweden	c
Evaporation tubes with a total volume of 50 mL and a 0.5 mL end-point	Biotage, Uppsala, Sweden	c
1.5 mL glass vials with screw cap equipped with silicone/PTFE septa (N9; with hole; 1 mm)	Machery-Nagel GmbH & Co. KG, Düren, Germany	c
Micro inserts, 15 mm tip; for wide opening; 0.2 mL/ 6×31 mm; clear	Machery-Nagel GmbH & Co. KG, Düren, Germany	c

8.1.3 Analytical Instruments

The following table lists the analytical instruments used during the practical work conducted as part of this thesis.

Table 16: Analytical instruments used; methods: a…aroma profiles, b…methoxypyrazines, c…thiols, d…TCA, e…ethylester

Analytical Instrument	Manufacturer	Used in
Agilent Technologies 7890 MS 5975c VL MSD with PAL CTC Analytics autosampler	Agilent Technologies, Santa Clara, CA, USA	a
Shimadzu GC-2010 Plus; GCMS-QP 2020 with PAL CTC Analytics autosampler	Shimadzu, Kyoto, Japan	a
Shimadzu GC-2010 Plus; TQ8050 with AOC 6000 PAL CTC Analytics autosampler	Shimadzu, Kyoto, Japan	b, c, d
Shimadzu GC-2010 Plus; GCMS-QP 2010 Ultra with PAL CTC Analytics autosampler	Shimadzu, Kyoto, Japan	e

8.2 Riesling – Wurzelwerk

This data was presented at the 2017 Euroanalyis in Stockholm as a poster presentation.

The goal of a project called ‘Wurzelwerk’ was to find out if terroir and the vintners’ style could turn grapes from three regions fermented in three cellars into nine different wines. Three wine makers exchanged Riesling grapes from three different growing areas, which had been harvested on the same day under similar conditions (Moser 2014). The grapes were fermented using spontaneous fermentation in stainless steel tanks. The grapes originated in three well known Riesling areas of which two are located in Germany and one in Austria. The growing locations were Langenlois (Kamptal) in Austria, Nackenheim (Rheinhessen) and Oberemmel (Saar) in Germany. This project offered the possibility to determine the effect of the two important factors in winemaking: region of origin and vinification style. The wines were analyzed for their inorganic composition using ICP-OES and ICP-MS and for their aroma profiles using HS-SPME-GC/MS.

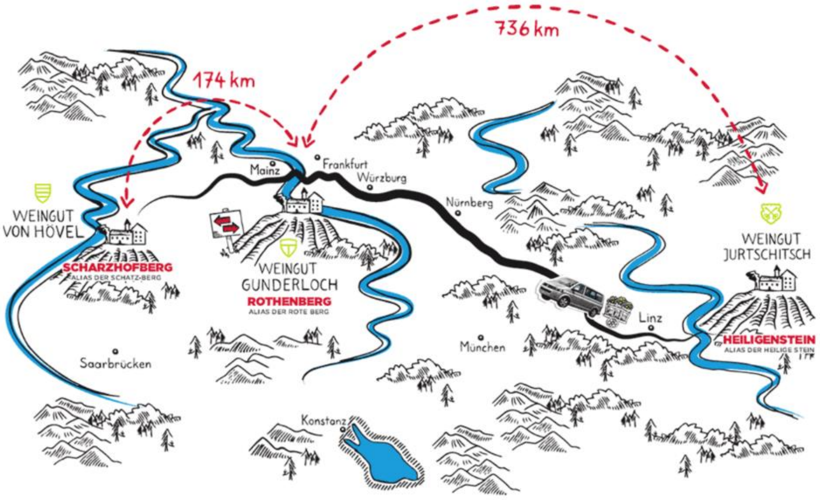


Figure 80: Graphic representation of the project (wurzelwerk.org)

8.2.1 Wine Samples

The wine samples for this project were purchased as a set of nine wines in 0.5 L glass bottles from the vintage 2012.

Table 17: Wines analyzed as part of the Wurzelwerk project

Wine	Winemaker	Origin of grapes
1	Von Hövel	Scharzhofberg (GER)
2	Gunderloch	
3	Jurtschitsch	
4	Von Hövel	Rothenberg (GER)
5	Gunderloch	
6	Jurtschitsch	
7	Von Hövel	Heiligenstein (AUT)
8	Gunderloch	
9	Jurtschitsch	

8.2.2 Materials and Methods

For the analysis of the inorganic composition, 19 elements were quantified in each of the wines. This included eleven mass elements, namely Al, B, Ca, Fe, K, Mg, Mn, Na, P, S, Zn. Additionally, the concentration of trace elements (Ti, Ni, Sn) including three isotopes of Pb (206, 207 and 208) was determined. The measurements were conducted using ICP-OES for the first group and ICP-MS for the trace elements. These measurements were carried out by the group for elemental analysis at the Institute for Analytical Chemistry and Food Chemistry of the TU Graz.

The aroma profiles of the volatile organic compounds of the wines were conducted using HS-SPME with GC-MS. The materials and chemicals used are listed in 8.1 and the instrument settings in Table 18.

Table 18: Instrument settings and method parameters for the acquisition of the aroma profiles on Agilent GC 7890

Sample	1 mL of wine in a 20 mL HS-vial flushed with N_2	**Temperature Program**	30 °C (1 min) @5 °C/min to 240 °C and at 20 °C/min to 290 °C (1 min)
Instrument	Agilent GC 7890	**Constant Flow**	35 cm/sec
SPME fiber	2 cm stable flex 50/30 µm DVB/Carboxen/PDMS	**Carrier Gas**	He; 151 kPa
Enrichment	30 minutes, 40 °C	**Ionization**	Electron ionization
Injector Temperature	270 °C	**Detector voltage**	relative to tune (2.0 kV)
Split	Splitless	**MS mode**	Scan
Column	HP5-MS UI 30 m*0.25 mm*1 µm	**m/z range**	35-350 5.19 scans/sec

The data was acquired using Agilent ChemStation version B.04.03 (Agilent Technologies, Santa Clara, CA, USA).

The results were compared using principal component analysis (PCA) using TheUnscrambler® v9.7, CAMO Software AS. For the PCA duplicate measurements were used. The data of the results of the elemental analysis and the data from the aroma analysis were compared separately and together. The results were grouped by grape origin and labelled after the winemakers. Clusters were assigned depending on the closeness of the results in the final diagrams.

8.3 Analysis of 2,4,6-Trichloroanisole

There are estimates that between 1 and 10% of wines are affected by cork related wine spoilage, which causes losses exceeding 10 billion US dollars for the wine industry annually (Pereira 2007; Rodríguez-Andrade et al. 2019; Silva Pereira et al. 2000; Butzke et al. 1998). Due to the low sensory threshold of the compounds connected to the taint, there is a need for very sensitive analytical methods to allow the quantification in the low ng/L range. One of the main compounds of cork taint is 2,4,6-trichloroanisole (TCA), which has a sensory threshold reported to be as low as 30 pg/L in water and below 1 ng/L in wine (Teixeira et al. 2006; Callejón et al. 2016).

8.3.1 Method Optimization

Parts of this work was presented at the 2018 Lebensmittelchemiker Tage as a poster presentation.

One way to ensure a selective and sensitive quantification of TCA is the use of GC with tandem mass spectrometry in multiple reaction monitoring mode (GC-MS/MS-MRM). Headspace solid phase microextraction (HS-SPME) sampling allows for solvent free extraction for the analysis of volatile compounds. As part of this thesis several different SPME methods and materials were examined to find the most suitable one for this task, otherwise the same sample preparation, instrument settings and method parameters were used.

Neutral wine without detectable faults (Grüner Veltliner, Winvino, screw capped, 2015, 11.5%v/v) was spiked with 5 ng/L of TCA and a deuterated analogue (d5-TCA), as well as another haloanisole sometimes related to cork taint, 2,4,6-Tribromoanisole (TBA) and its deuterated counterpart (d5-TBA). This wine was used in all optimization experiments.

1 mL of wine was transferred to a 20 mL glass headspace vial with a glass coated magnetic stirrer and sealed. All measurements were carried out in duplicate.

The first parameter to be optimized was the used SPME tool. Table 19 lists all SPME fibers and SPME Arrows examined.

Table 19: List of Arrow and SPME fibers used in the comparison experiment

Fiber material	Type	Manufacturer	Diameter [mm]	Coating thickness [µm]	Length [mm]
CWR/PDMS	Arrow	CTC	1.1	120	20
CWR/PDMS	SPME	CTC		95	10
DVB/CWR/PDMS	Arrow	CTC	1.1	120	20
DVB/Carboxen/PDMS	SPME	Supelco			20
DVB/PDMS	Arrow	CTC	1.1	120	20
DVB/PDMS	SPME	CTC		105	10
PDMS	Arrow	CTC	1.1	100	20
PDMS	SPME	CTC		100	10
Acrylate	Arrow	CTC	1.1	100	20
Acrylate	SPME	CTC		85	10

In the subsequent experiments the chosen fibers were the CTC SPME Arrow DVB/CWR/PDMS 1.1 mm and the 2 cm stable flex Supelco SPME DVB/Carboxen/PDMS 50/30.

The impact of the following enrichment times and temperatures was investigated:

Temperature:	Time:
- 40°C	- 20 min
- 60°C	- 40 min
- 80°C	

Table 20 lists the final method.

Table 20: Instrument settings and method parameters for the quantification of TCA

Instrument	Shimadzu TQ8050	**Constant Flow**		40 cm/sec
Sample	1 mL of wine in a 20 mL HS vial with 5 ng/L IS	**Carrier Gas**		He; 66 kPa
SPME Arrow	DVB/CWR/PDMS 1.1mm	**Interface Temperature**		270 °C
Enrichment	20 minutes, 80 °C	**Ion Source Temperature**		200 °C
Column Oven	GC-2010	**Detector Voltage**		1.7/1.8 kV
Injector Temperature	270 °C	**Solvent Cut time**		7 min
Column	RestekRXi-5ms 30m x 25 mm x 25 µm	**Acquisition Mode**		MRM
Sampling Time	1 minute	**Event time**		0.150 sec
Split	Splitless	**Quadrupole Resolution**		Q1: high; Q3: high
Temperature Program	40°C (1 min) @10°C/min to 200 °C and at 25°C/min to 310 °C (1 min)	**Transitions (Collision Energy)**	TCA	210.00>195.00(13.00); 212.00>197.00(15.00)
			d5-TCA	215.00>196.90(13.00); 217.00>198.90(13.00)
			TBA	345.80>330.80(17.00); 343.70>328.70(17.00)
			d5-TBA	350.80>332.70(17.00); 348.70>330.70(17.00)

The optimized method was then used to examine a set of sparkling wines from different origins. In addition to the TCA concentration, several other parameters were determined as described in the following chapter.

8.4 Analysis of Sparkling Wine Samples

This work was presented at the 32nd International Symposium on Chromatography 2018.

8.4.1 Wines Samples

14 samples of sparkling wine from three European countries were purchased. All originated from areas known for their sparkling wine production, namely the Champagne region and the Loire valley in France, Catalonia in Spain and Franciacorta in Italy. All wines are listed in Table 21.

Table 21: List of sparkling wines analyzed

Name	Winemaker	Country	Region	City
Brut Zéro	Chapin&Landis	France	Loire Valley	Saumur
Le Grande Saumur	Chapin&Landis	France	Loire Valley	Saumur
Montargull	Miquel Pons	Spain	Catalonia	Alt Penedés
Gran Reserva Vintage Cava	Miquel Pons	Spain	Catalonia	Alt Penedés
Cava Reserva Brut Natur	Miquel Pons	Spain	Catalonia	Alt Penedés
Cava Brut Natur	Miquel Pons	Spain	Catalonia	Alt Penedés
Francesco I	Uberti	Italy	Franciacorta	Erbusco
Tenuta Villa Crespina Miolo Brut	Fratelli Muratoni SS	Italy	Franciacorta	Adro
San Christoforo Brut	San Christoforo	Italy	Franciacorta	Erbusco
Blanc de Blancs	Cavalleri	Italy	Franciacorta	Erbusco
Monopole Blue Top Brut	Heidsieck & Co	France	Champagne	Reims
Moet Imperial Brut	Moet & Chandon	France	Champagne	Épernay
Yellow Lable Brut Champagne	Veuve Cliquot	France	Champagne	Reims
Reserve Brut	Taittinger	France	Champagne	Reims

8.4.2 Materials and Methods

The wines were analyzed for their haloanisole concentration as described in Table 20 and for their volatile compounds using the method described in 8.2.2. Three ethyl esters were quantified using the method described in Table 22. Synthetic wine was made of deionized water with 12% v/v EtOH and 350 mg/L tartaric acid.

Table 22: Instrument Settings and method parameters for the quantification of ethyl esters

Sample	100µL of wine + 890µL of synthetic wine + IS (10 ng/L)	**Constant Flow**	35 cm/sec
Instrument	Shimadzu GC-2010 Plus	**Carrier Gas**	He; 86.9 kPa
SPME fiber	2 cm stable flex 50/30 µm DVB/Carboxen/PDMS	**Solvent Cut Time**	2.3 min
Enrichment	30 minutes, 40 °C	**Ionization**	Electron ionization
Injector Temperature	250 °C	**Detector voltage**	relative to tune
Split	1 to 5	**MS mode**	Scan
Column	ZB-Wax 20 m*0.18 mm*18 µm	**Event time**	0.30 sec
Temperature Program	40 °C (1 min) @7.5 °C/min to 240 °C(2 min)	**m/z range**	46-350

The data was acquired using Shimadzu GCMSsolution Workstation version 2.5 (Shimadzu, Kyoto, Japan).

Measurements of additional parameters were conducted at Shimadzu Europe by Robert Ludwig (sugars and glycerol) and Rebecca Kelting (higher alcohols and ethyl acetate). Sugar and glycerol concentrations were determined using HPLC and the concentrations of selected higher alcohols and ethyl acetate were done using GC-FID.

In addition to one dimensional data, comprehensive GCxGC measurements were conducted using the method described in Table 23.

Table 23: Settings for the comprehensive GCxGC measurements of the sparkling wine samples

Sample	100µL of wine in a 20 µL headspace vial	**Temperature Program**	35°C (3 min)@3°C/min to 170°C @20°C/min to 280°C
SPME fiber	2 cm stable flex 50/30 µm DVB/Carboxen/PDMS	**Carrier Gas**	He; 116 kPa initial pressure, constant flow
Enrichment	30 minutes, 40 °C	**Column 1**	Restek RX1MS 30 m*0.25*0.25
Injector Temperature	270 °C	**Column 2**	Restek RXi17SilMS 2 m*0.15*0.15
Split	Splitless	**Ionization**	Electron ionization
Liner	0.75 mm SPME liner	**Detector voltage**	relative to tune
Instrument	Shimadzu QP2010 Ultra with OPTC IV Injector	**MS mode**	Scan
Autosampler	AOC 5000 Autosampler	**m/z range**	35-330 amu
Modulator	ZOEX Cryo Modulator, 280°C, hot jet 350 ms, 5 modulation frequency	**Scan speed**	50 scans/sec

Data evaluation of the GCxGC-MS-Contour plots was done using GC-Image software, version 7.2 (Zoex Corporation, Houston, TX, USA). TheUnscrambler® v9.7 (CAMO Software) and MAStat 3.02 (ANALYT GmbH) were used for PCA. The final comparison included the concentration of three ethyl esters (C6, C8 and C10) as well as their respective acids (Hexanoic acid, Octanoic acid and n-Decanoic acid) and butyric acid. In addition the concentrations of several alcohols (methanol, n-propanol, isobutanol and isoamyl alcohol) and ethyl acetate and glycerol were taken into account, as well as the peak areas of several other compounds (furfural, ethyl-2-methylbutyrate, isoamyl acetate, nonanal, phenyl ethanol, ethyl phenyl acetate, TDN and damascenone).

8.5 Analysis of Sauvignon Blanc

Figure 81: Sauvignon Blanc in the experimental vineyard of the Faculty of Agriculture of the University of Zagreb

Sauvignon Blanc is one of the most important white cultivars in Styria, where it covers about 15% of the total vineyard area. Its varietal aroma depends on the growing conditions, which influence the composition of the precursor- and aroma compounds in the grapes (Green et al. 2011). This includes the concentration of two groups of character impact compounds in Sauvignon Blanc wines: methoxypyrazines and varietal thiols. Both of the groups are characterized by low sensory thresholds in the ng/L range.

In the following experiments the impact of different harvest conditions, including time of day, region of origin and others on the concentration of 2-isobutyl-3-methoxypyrazine (IBMP) in must produced from Sauvignon Blanc grapes was examined. Previous studies found that the IBMP concentration is influenced by the ripeness of the berries. (Allen et al. 1991). Therefore, additional factors (sugar content and acidity of the must), which are used as indicators of the ripeness of the berries, were considered.

The second group of character impact odorants examined during this work were thiols. Due to their high reactivity their quantification can be demanding. Based on previous work (Herbst-Johnstone et al. 2013), a method for the quantification of two of the main varietal thiols: 3-sulfanylhexanol (3SH) and 3-sulfanylhexyl acetate (3SHA) was established. This was done as part of a bilateral project with the Department of Chemistry of the Faculty of Agriculture of the University of Zagreb.

8.5.1 Impact of Different Harvest Parameters on the Concentration of IBMP

This work was presented as poster presentations at several conferences (IVAS 2019, Analytica Vietnam 2019 and ALVA Jahrestagung 2019)

IBMP is a primary aroma compound. This means that it is present in the grapes and its concentration remains largely unchanged throughout the winemaking process. In the following experiments the concentration of IBMP in Sauvignon Blanc must from different harvesting conditions was determined. In addition, several ripeness parameters were investigated.

Must Samples

The must samples listed in Table 24 and Table 25 were provided by the Department of Fruit Growing and Enology of the Agricultural Research Center Styria (VOWH). The first three samples listed in Table 26 were hand pressed from grapes harvested in three different vineyards in Croatia, the fourth was provided by the VOWH.

Table 24: Sauvignon Blanc must samples with different harvest dates and times from the same vineyard

Harvest Date	Harvest Time
13.09.2018	8:00
13.09.2018	16:00
24.09.2018	8:00
24.09.2018	16:00
02.10.2018	8:00
02.10.2018	16:00

Table 25: Sauvignon Blanc must samples with different harvest locations in the same vineyard

Vineyard direction	Location
North	Top
North	Bottom
South	Top
South	Bottom

Table 26: Sauvignon Blanc must samples with different harvest locations

Harvest Date	Vineyard Location	Country
28.08.2018	Jazbina	Croatia
30.08.2018	Daruvar	Croatia
31.08.2018	Poreč	Croatia
19.09.2018	Pößnitz, Styria	Austria

Materials and Methods

The chemicals and materials used are listed in 8.1. The quantification was done with HS-SPME using SPME Arrow and GC with tandem mass spectrometry (GC-MS/MS). The instrument parameters and method settings are listed in Table 27. For quantification, 10 pg (10 ng/L wine sample) of deuterated IBMP was used as an internal standard. Samples were measured in triplicate.

In addition, the total acidity, pH and sugar concentration of the must samples, which had been provided by VOWH, were taken into consideration.

Table 27: Instrument setting and method parameters for the quantification of IBMP

Instrument	Shimadzu TQ8050	**Sampling Time**		1 minute
Sample	1 mL of wine in a 20 mL HS vial with 10 ng/L IS	**Constant Flow**		40 cm/sec
SPME Arrow	DVB/CWR/PDMS 1.1mm	**Carrier Gas**		He; 66 kPa
Enrichment	20 minutes, 80 °C	**Split**		Splitless
Column Oven	GC-2010	**Interface Temperature**		270 °C
Injector Temperature	270 °C	**Ion Source Temperature**		200 °C
Column	ZB-5MSi 30m x 25 mm x 25 µm	**Detector Voltage**		1.8 kV
Temperature Program	40 °C (1 min) @10 °C/min to 200 °C @25 °C/min to 310 °C (1 min)	**Transitions (Collision Energy)**	IBMP	124.10>94.10(11), 124.10>81.10(7), 124.10>79.10(23)
			d3-IBMP	127.10>95.10(13), 127.10>83.10(7), 127.10>97.10(11)

The data was acquired using Shimadzu GCMSsolution Workstation version 2.5 (Shimadzu, Kyoto, Japan).

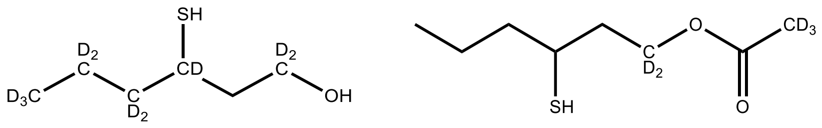
Data was analyzed using a standard least squares analysis with two categorical factors (harvest date, harvest time) for each attribute and interaction effects were considered. Student's t-test and Tukey's Honest Significant Difference (HSD) were used to detect differences ($p<0.05$). JMP 14.0.0 Pro software (SAS Institute) was used. In the vineyard harvest location experiment the statistics program SPSS Statistics Version 21 (IBM) was used.

In addition to their IBMP concentration, the composition of the volatile organic compounds (aroma profiles) of the wines from the harvest location experiment (Table 26) was investigated and compared using PCA. The aroma profiles were created using the method described in 8.2.2. The Unscrambler® v9.7 (CAMO Software) and MAStat 3.02 (ANALYT GmbH) were used for PCA.

8.5.2 Thiols

Next to methoxypyrazines, thiols are the second important group of compounds connected to the aroma of Sauvignon Blanc wines. These highly reactive sulfur compounds are responsible for tropical notes in the aroma of wines of this variety. Due to their high reactivity and relatively low sensory thresholds, their quantification can be challenging.

Many of the available methods for the analysis of thiols use organo-mercury compounds for the derivatization. However, due to their inherent toxicity and the time effort connected, alternative derivatization agents, including 2,3,4,5,6-pentafluorobenzyl bromide and ethyl propiolate (ETP), are preferable. Based on the work of Herbst-Johnstone et al. (2013) a method was developed using derivatization with ETP. The main research objective was to develop and optimize a quick and reliable method for the determination and quantification of two varietal thiols in wines, with detection limits at or near their respective sensory thresholds, based on gas chromatography coupled with tandem mass spectrometry (GC-MS/MS) with previous analyte derivatization. Following the derivatization, solid phase extraction (SPE) methods were used for separation. For quantification, stable isotope dilution analysis (SIDA) was used, as the physicochemical properties of the labelled analogues are very close to those of the analytes. The deuterated standards (Figure 82) were prepared by the Department of Agriculture of the University of Zagreb in cooperation with the Department of Organic Chemistry of the Rudjer Boskovic Institute, Zagreb.



d_{10}-3-sulfanylhexan-1-ol

d_5-3-sulfanylhexyl acetate

Figure 82: Deuterated isotopomers of 3-sulfanylhexan-1-ol (3SH) and 3-sulfanyl-hexylacetate (3SHA)

The final method was used to examine the effect of different antioxidant preparations. Antioxidants were added to the must before fermentation in order to prevent the degradation of aroma compounds in the earliest phases of winemaking.

Derivatization Procedure

During the optimization process of the derivatization, the influence of several parameters was examined (the chosen option is printed in **bold**). Tested factors included different reaction times (1h instead of **10 min**), the use of SPE cartridges from different producers (BondElut Mega-BE C18 or **Supelclean Envi-18**) and experiments with different base addition procedures (adjustment of pH after 3 min of initial stirring, second adjustment of pH). Other optimizations included the use of different bases added to adjust the pH (**2M NaOH**, 1M NaOH, Et3N, DBU, KOH) and different elution solvents (**ethyl acetate (EA)**, dichloromethane, acetone or diethyl ether (20%) in pentane).

The optimized derivatization procedure was as follows:

- Standard solutions (internal standard (IS) and standard addition solution) were added to 50 mL of wine in a 100 mL screw cap bottle (Figure 83A).
- Addition of 100 mM ETP (500 µL of ETP in 1 mL of EtOH), followed by 2 min of stirring at ~1000 rpm on a magnetic stirrer.
- Adjustment of pH to 10 with 2M NaOH, followed by 10 min of stirring (Figure 83B).
- The sample was decanted into 50 mL PP screw cap tubes and centrifuged for 10 min at 2500 rpm to remove any precipitate formed due to pH modification (Figure 83C).

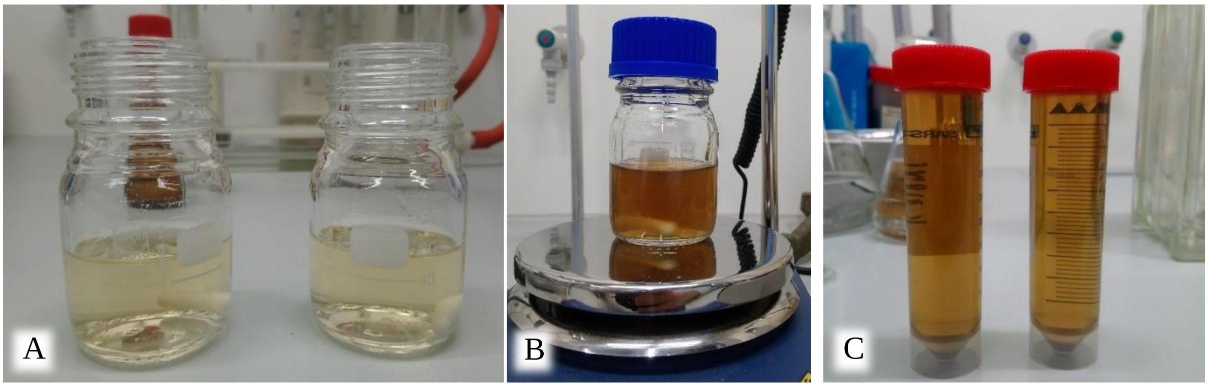


Figure 83: Procedure steps for the derivatization of thiols; wine sample in screw cap bottle after addition of standards (A), sample after pH adjustment (B), samples in PP tubes after centrifugation (C)

- SPE procedure
    - Activation and conditioning of the cartridge with 10 mL MeOH, followed by 10 mL H_2O (Figure 84A)
    - Application of the sample and washing with 5 mL H_2O (Figure 84B)
    - Drying of the cartridges for 15 min (Figure 85C)
    - Elution of the sample with 10 mL EA (Figure 85D)

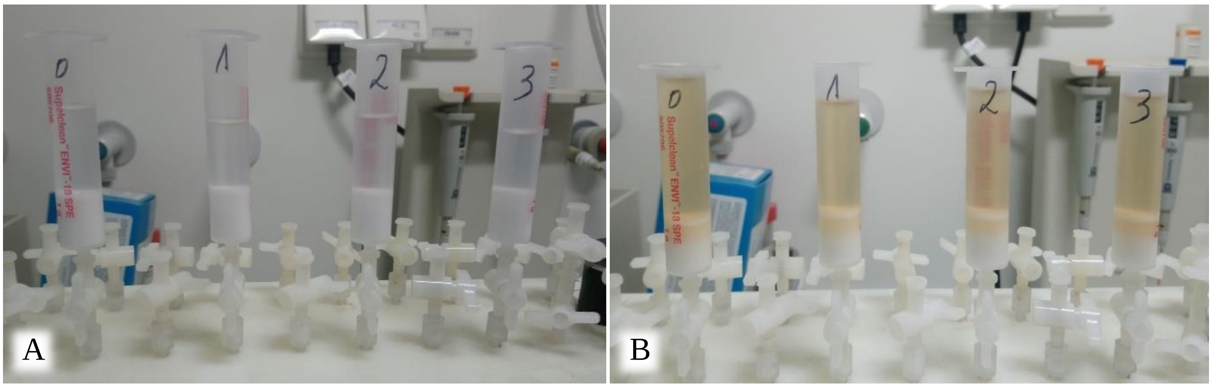


Figure 84: SPE procedure steps; activation and conditioning of the cartridges (A) and application of the sample (B)

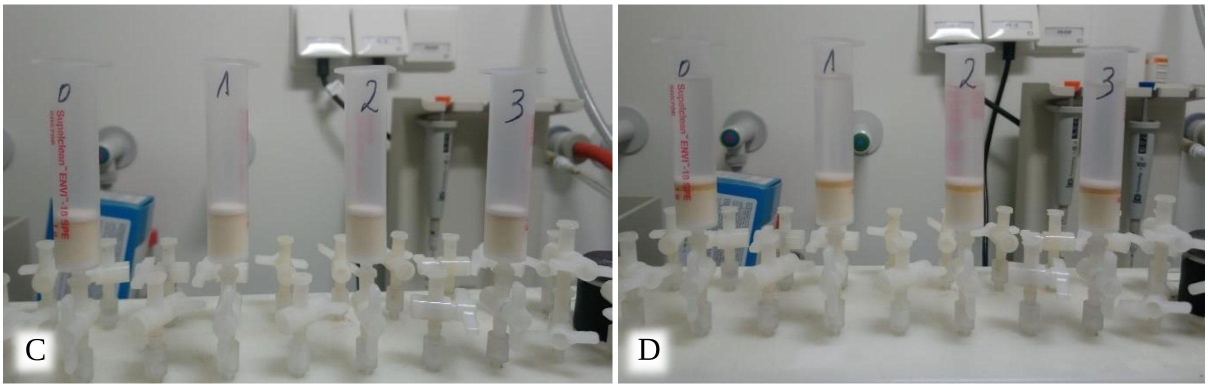


Figure 85: SPE procedure steps II; drying of the cartridges after sample application (C) and elution of the sample with EA (D)

- Drying of the eluted sample with anhydrous $NaSO_4$.
- Reduction of the sample volume to 0.5 µL with a TurboVap® II automatic solvent evaporator.
- Transfer of the sample into a 1.5 mL glass vial with screw cap equipped with 0.2 mL glass micro inserts.

Method Development

For the development of a multiple reaction monitoring (MRM) method, the mass spectra of the derivatized compounds of interest were acquired in full scan mode. For this, synthetic wine had been spiked with the deuterated and non-deuterated standards and derivatized following the previously described procedure. The method settings are listed in Table 28.

Table 28: Instrument setting and method parameters for the acquisition of full scan spectra of the derivatized thiols

Instrument	Shimadzu TQ8050	**Solvent Cut Time**	7 min
Injection Volume	1 µL	**Interface Temperature**	270 °C
Injector Temperature	220 °C	**Ion Source Temperature**	200 °C
Split	Splitless	**Ionization**	Electron ionization
Column	Restek Rxi-5ms 30m x 25 mm x 25 µm	**Detector voltage**	relative to tune -0.1 kV
Temperature Program	60 °C (1 min) @10 °C/min to 275 °C and at 25 °C/min to 310 °C (3 min)	**MS mode**	Q3 Scan
Constant Flow	40 cm/sec	**m/z range**	50-350
Carrier Gas	He; 72.8 kPa	**Event time**	0.3 sec

The data was acquired using Shimadzu GCMSsolution Workstation version 2.5 (Shimadzu, Kyoto, Japan).From the resulting mass spectra, the most prominent masses were chosen for the development of a more selective MRM method. This method was created after carrying out a product ion scan (PIS) from minute 16 to minute 17.85. The collision energy (CE) was increased in steps of 3 eV from 3 to 30 eV. Using the CE that yielded the highest peaks, the final MRM method was developed using SmartMRM (Shimadzu, Kyoto, Japan).

Table 29: Precursor ions used in the product ion scan

Compound	Precursor m/z 1	Precursor m/z 2	Precursor m/z 3
d10-3SH	242.15	223.15	197.15
3SH	232.10	214.10	187.10
d5-3SHA	279.15	246.20	234.10
3SHA	274.10	241.15	229.10

The transitions chosen for the quantification and their respective collision energies are listed in Table 30. Other instrument setting and method parameters were the same as those listed in Table 28, with the exception of the detector voltage (1.7 kV), MS mode (MRM) and event time (0.150 sec).

Table 30: Transitions chosen for the MRM for the quantification of varietal thiols

	Start time (min)	End time (min)	Quantification transition	CE [eV]	Reference transition I	CE [eV]	Reference transition II	CE [eV]
d_{10}-3SH	16.00	17.00	197.15>92.20	6.00	197.15>87.10	9.00	197.15>60.20	21.00
3SH	16.00	17.00	187.10>83.10	6.00	187.10>87.10	12.00	187.10>55.10	18.00
d_5-3SHA	17.00	17.75	234.10>85.10	12.00	279.15>85.10	21.00	234.10>148.30	6.00
3SHA	17.00	17.75	229.10>143.10	6.00	229.10>83.10	12.00	229.10>55.20	24.00

The data was acquired using Shimadzu GCMSsolution Workstation version 2.5 (Shimadzu, Kyoto, Japan).

Wine Samples

The developed method was used to examine the thiol concentration of Sauvignon Blanc wines. The must had been treated with different antioxidant mixtures before fermentation. The additives and sample descriptions are listed in Table 31.

Table 31: List of wine samples and antioxidant treatment of the wines examined for their thiol concentration

Sample	Additive	Dose
A	Sumpovin (H_2SO_3) - control	1 mL/L
B	Sumpovin (H_2SO_3)	0.5 mL/L
C	Aromax® ($K_2S_2O_5$ + ascorbic acid)	0.2 g/L
D	Aromax Super® ($K_2S_2O_5$ + ascorbic acid + proanthocyanidins + ellagitannins)	0.2 g/L
E	Aromax Gal® ($K_2S_2O_5$ + ascorbic acid + gallotannins)	0.2 g/L
F	Noxitan® ($K_2S_2O_5$ + oenological tannin)	0.1 g/L

The derivatization was carried out following the described procedure. Wine samples were spiked with deuterated standards (200 ng/L d10-3SH and 100 ng/L d5-3SHA). Standard addition was done on four levels:

1. 0 ng/L
2. 100 ng/L 3SH/ 50 ng/L 3SHA
3. 250 ng/L 3SH/ 100 ng/L 3SHA
4. 500 ng/L 3SH/ 250 ng/L 3SHA

8.6 Analysis and Characterization of Wine from Fungus Resistant Grape Varieties

Fungus-resistant grape varieties (PIWIs derived from the German word Pilzwiderstandsfähig) are the result of crossing European *Vitis vinifera* cultivars with American and/or Asian cultivars. The offspring are selected for their resistance to certain adversities, like fungal diseases or their tolerance for harsh environmental conditions. As a result, the need of plant protection agents and other treatments during cultivation can be reduced compared to conventional varieties.

Chambourcin is one of these first hybrid cultivars. In cooperation with the University of Arkansas, several wines produced from this cultivar were compared. Some of the grapes were sprayed with inactive dry yeast two times over the course of the ripening process. In addition, during fermentation, oak chips and tannins were added to a part of the samples. The finished wines were analyzed to examine the effects of the different treatments.

Compared to traditional varieties, many of these new cultivars are still relatively unknown to both, winemakers and consumers. And while the benefits in cultivation might convince winegrowers, they need assurance that wines produced from PIWI grapes will be at least comparable to those from other varieties.

The Department of Fruit Growing and Enology of the Agricultural Research Center Styria (VOWH) has a long running project focused PIWI cultivation and winemaking. As part of this work, wines from different PIWI varieties from five years were chemically analyzed to gain a deeper understanding of the resulting wines and their potential for the Styrian and Austrian wine market.

8.6.1 Determination of the Impact of Different Treatments on Chambourcin Grapes and Wine

Parts of this work (analysis of ethyl esters and impact of foliar treatment) were presented as poster presentations at IVAS 2019.

Wine Samples

The wine samples were produced by Sarah Mayfield at the University of Arkansas. During ripening several rows were treated with inactivated dry yeast (LalVigne® MATURE). This was done at approximately 5% veraison of the grapes. The treatment was repeated a second time ten days later. During fermentation, tannins and (800 mg/L) and oak chips (8 kg/t_m) were added to two batches of each foliar treatment. In addition, two batches of each foliar treatment were fermented without additions. The wines were fermented on the skins and pressed after five days. A description of the winemaking procedure in greater detail can be found in Mayfield (2020), chapter II.

Table 32: Chambourcin wine samples and treatments

Name	Foliar treatment	Additions
CA	not sprayed (control)	tannins (800 mg/L), oak chips (8 kg/t_m) (additions)
CNA	not sprayed (control)	no additions
LVA	treated with LalVigne® MATURE (sprayed)	tannins (800 mg/L), oak chips (8 kg/t_m) (additions)
LVNA	treated with LalVigne® MATURE (sprayed)	no additions

Materials and Methods

Mayfield (2020), chapter II describes the analysis of the same samples, based on one-dimensional GC, including the quantification of four ethyl esters (according to the method described in Table 22), as well as sensory evaluation of the wines.

This work focused on exploring the wines with comprehensive GCxGC-MS. Instrument settings and method parameters are listen in Table 33.

Table 33: Instrument settings and method parameters for the GCxGC-MS analysis of the Chambourcin wine samples

Sample	50 µL of wine in a 20 µL headspace vial	**Temperature Program**	20°C (3 min)@4°C/min to 220°C @20°C/min to 280°C
SPME fiber	2 cm stable flex 50/30 µm DVB/Carboxen/PDMS	**Carrier Gas**	He; 79.2 kPa initial pressure, constant flow (30 cm/sec)
Enrichment	30 minutes, 40 °C	**Column 1**	ZB1HT Inferno 30 m*0.25*0.25
Injector Temperature	270 °C	**Column 2**	Restek Rtx-200 2 m*0.15*0.15
Split	Splitless	**Ionization**	Electron ionization
Liner	0.75 mm SPME liner	**Detector voltage**	0.7 kV
Instrument	Shimadzu QP2010 Ultra with OPTC IV Injector	**MS mode**	Scan
Autosampler	AOC 5000 Autosampler	**m/z range**	35-300 amu
Modulator	ZOEX Cryo Modulator, 280°C, hot jet 350 ms, 5 modulation frequency	**Scan speed**	50 scans/sec

The data was acquired using Shimadzu GCMSsolution Workstation version 2.5 (Shimadzu, Kyoto, Japan). Data evaluation of the GCxGC-MS-Contour plots was done using ChromSquare, version 2.2SP1 Build 1456 (Chromaleont S.r.l., Messina, Italy).

8.6.2 Characterization of Styrian PIWIs

Parts of this work were presented at several conferences as talks[1] *and poster presentations*[2] *(JunganalytikerInnen Forum 2017*[1]*, Weurman Symposium 2017*[2]*, Anakon 2019*[1]*)*

This is the first time the chemical characterization of wines produced from fungus resistant grape from Styria is described over the course of several vintages.

Wine Samples

All wines were produced by members of the Department of Fruit Growing and Enology of the Agricultural Research Center Styria, who also provided all the data in this part.

The wines were produced from grapes grown in the Südsteiermark DAC region, in Leutschach an der Weinstraße (46°38’82”N; 15°30’32”), 415 – 435 meters above the Adriatic. The vineyard was oriented east to southeast and had a slope inclination of 20 - 35%.

Table 34: Parentage and other characteristics of the PIWIs

	Parentage	Clone	Rootstock
Blütenmuskateller	Severny × (Muscat Lunel × Muscat d’Alexandrie)	Klbg A 205	SO4
Bronner	Merzling [=(Seyve-Villard 5-276 x (Riesling x Ruländer)] x Gm 6494 [=Saperavi severnyi x Saint Laurent]	FR 320	Kober 5BB
Cabernet Blanc	Cabernet Sauvignon × Resistenzpartner		SO4
Chardonel	Seyval blanc x Chardonnay		SO4
Muscaris	(Merzling x (Saperawi Severni x Muskat-Ottonel) (vulgo Solaris)) x Gelber Muskateller	FR 400	Kober 125AA
Sauvignac	Sauvignon blanc x Riesling x Resistenzpartner		Kober 5BB
Solaris	Merzling x (Saperawi Severni x Muscat Ottonel)	FR 360	SO4
Souvignier gris	Cabernet Sauvignon X Bronner	FR 420	SO4
Sauvignon Soyhieres	Cabernet Sauvignon X Vitis amurensis		Teleki 5C

Table 35: Average susceptibility of cultivars to fungal diseases 2015-2019, 1=no/low susceptibility; 9=very high susceptibility

	Peronospora on the leaves OIV-452	Oidium on the leaves OIV-455	Black rot	Grape rot OIV-459
Blütenmuskateller	3.4	3.1	2.6	2.4
Bronner	2.6	1.2	1.0	2.2
Cabernet blanc	5.8	1.0	3.4	1.0
Chardonel	3.0	1.0	2.6	2.2
Muscaris	2.0	1.0	1.0	1.6
Sauvignac	4.8	1.0	1.8	1.7
Sauvignon Soyhieres	3.6	1.0	1.2	3.0
Solaris	3.3	1.0	1.0	1.8
Souvignier gris	4.0	1.0	1.8	1.0
Sauvignon blanc	7.2	1.0	1.0	1.0

Table 36: Amount of treatments of the vines by year

Year	Nr. of treatments	Agent/amount per treatment
2015	4	Reticulated sulfur 2 kg/ha Copper hydroxide 361 g/ha (=235 g pure-Cu/ha)
2016	3	Reticulated sulfur 2 kg/ha Copper hydroxide 383 g/ha (=250 g pure-Cu/ha)
2017	2	Reticulated sulfur 3 kg/ha Copper hydroxide 460 g/ha (=300 g pure-Cu/ha)
2018	3	Reticulated sulfur 3 kg/ha Copper hydroxide 421 g/ha (=275 g pure-Cu/ha)
2019	3	Reticulated sulfur 4 kg/ha Copper hydroxide 460 g/ha (=300 g pure-Cu/ha)

The grapes were harvested manually. All diseased, rotten or withered parts of the grapes were removed and the grapes were transported in 15 kg plastic crates.

All grapes were crushed and subjected to a maceration period of 6 hours at 15°C. The pressing of the must was done with an adapted hydropress (Lancman VSPX 80 presses) at a maximum pressure of 1 bar. All musts were sulfurized with 50mg/L of SO_2. The musts were clarified by simple settling over a 12-hour period at a temperature of 12°C. The residual lees content of the musts was approximately 50 NTU. The must sample volume (500 mL) for aroma analyses was deep frozen at minus 20°C immediately after degumming. The musts intended for alcoholic fermentation were heated to a starting temperature of 20°C and inoculated with reactivated pure culture yeast (Lalvin EC1118). During fermentation the temperature was constantly held at 17°C in glass containers of 34 and 54 liters, respectively, or in stainless steel containers of 100 liters capacity. Immediately after the end of fermentation, the young wines were cooled to a temperature of 10°C. After one week they were sulfurized with 60 mg/L SO_2 and filtered with clarified layers (Seitz K150, 200x200) another 7 days later. The wines were stored in glass and stainless-steel containers, respectively, until bottling. With a content of 40 mg/L free SO_2, the wines were finally bottled in 0.5 or 0.75 liter glass bottles and closed with screw caps (VinoTwist, tin-saran, BVS 30x60mm). Until further use, the bottles were stored dark in the cooled (12°C) sample storage.

Table 37: PIWI samples with vintages

	Years
Blütenmuskateller	2015, 2016, 2017, 2018, 2019
Bronner	2015, 2016, 2017, 2018, 2019
Cabernet Blanc	2015, 2016, 2018, 2019
Chardonel	2015, 2016, 2017, 2018, 2019
Muscaris	2015, 2016, 2017, 2018, 2019
Sauvignac	2015, 2016, 2017, 2018, 2019
Solaris	2015, 2016, 2017, 2018, 2019
Souvignier gris	2015, 2016, 2017, 2018, 2019
Sauvignon Soyhieres	2015, 2016, 2017, 2018, 2019

Materials and Methods

The wine samples were analyzed for the composition of their volatile compounds using the method described in Table 38. 3-Hepanol and methyl nonanoate were used as internal standards at concentrations of 100 and 200 µg/L, respectively. A mixture of n-alkanes (C8-C20) was measured using the same method for the determination of the retention index (Kováts 1958). Data was acquired using Shimadzu GCMSsolution Workstation version 2.5 (Shimadzu, Kyoto, Japan).

PARADISeVersion3.9 was used to process the raw data of the TIC and identify peaks with a mass spectra database (NIST11, National Institute of Standards and Technology) (Johnsen et al. 2017). Retention indices were calculated according to Lusebrink (2016). Concentrations were estimated using the peak areas of the internal standards. Odor activity values (OAVs) were calculated using odor thresholds listed in van Gemert (2011).

Table 38: Instrument settings and method parameters for the acquisition of the aroma profiles on Shimadzu GC-2010 Plus

Sample	1 mL of wine in a 20 mL HS-vial flushed with N_2 with 10 µl of internal standard solution	**Temperature Program**	30 °C (1 min) @5 °C/min to 230 °C and at 20 °C/min to 280 °C (1 min)
Instrument	Shimadzu GC-2010 Plus; GCMS-QP 2020	**Constant Flow**	35 cm/sec
SPME fiber	2 cm stable flex 50/30 µm DVB/Carboxen/PDMS	**Carrier Gas**	He; 43.8 kPa
Enrichment	30 minutes, 40 °C	**Ionization**	Electron ionization
Injector Temperature	270 °C	**Detector voltage**	0.85 kV
Split	Splitless	**MS mode**	Full Scan
Column	Restek Rxi5MS 30 m*0.25 mm*1 µm	**m/z range**	35-350 3.33 scans/sec

In addition, the concentration of IBMP and four ethyl esters was determined using the methods described in 8.5.1 (Table 27) and 8.4.2 (Table 22), respectively. Data was additionally analyzed using a standard least squares analysis with two categorical factors (variety and year) for each attribute and interaction effects were considered. Tukey's Honest Significant Difference (HSD) was used to detect differences ($p<0.05$). SPSS Statistics Version 21 (IBM) was used for this.

All identified compounds were categorized by class (Ester, Alcohol, Carboxylic Acid, Aldehyde, Ketone, Terpene, Naphthalene, Pyrazine) and aroma attribute (Fruity, Roasted/ caramelized, Unpleasant, Green/ fat, Floral, Chemical, Herbal/ spicy) according to identifiers listed on flavornet (Arn and Acree 1998).

The results were compared using principal component analysis (PCA) using TheUnscrambler® v9.7, CAMO Software AS.

Sensory Analysis

To assess the sensory characteristics of the PIWI wines, yearly blind tastings with panelists trained in the sensory analysis of wine were conducted and evaluated by the Department of Fruit Growing and Enology of the Agricultural Research Center Styria. The data used in this work, is reproduced with their permission. Table 39 shows the wines examined each year and the number of panelists. In 2018, two tastings were conducted.

The panelists were asked to rate the wines based on two general characteristics (overall impression and odor intensity) and their similarity to wine from conventional *V. vinifera* varieties that are common in Styria. This included Welschriesling, Pinot Blanc, Sauvignon Blanc and Muscat/ Traminer wines. In 2017 and 2018, Riesling was added to that list.

Table 39: Wines subjected to sensory Analysis

	2015	**2016**	**2017**	**2018**
Blütenmuskateller	x	x	x	
Bronner		x	x	x
Cabernet Blanc		x		x
Chardonel		x	x	x
Muscaris	x	x		
Sauvignac	x	x	x	x
Solaris	x		x	x
Souvignier gris		x		
Sauvignon Soyhieres		x	x	x
Number of panelists	11	11	14	14/8

The resulting data was compared to the results of the chemical analysis to see if there is a consensus between sensory and instrumental methods.

9. Results and Discussion

9.1 Riesling - Wurzelwerk

In this study, nine wines were analyzed that originated from a project called Wurzelwerk. It was carried out by three winemakers, two in Germany and one in Austria, who decided to exchange Riesling grapes and make them into wine using spontaneous fermentation. This resulted in nine wines from three origins from three cellars. Looking at the composition of the finished wines gives an insight into the effect viti- and vinicultural techniques have on wines, and which might have the bigger impact in this specific case.

The wines were analyzed for their inorganic composition using ICP-OES and ICP-MS and for their aroma profiles using HS-SPME-GC/MS.

The results of the ICP-OES measurements can be found in Table 52, those of the ICP-MS in Table 53. The volatile organic compounds identified in the wine samples are listed in Table 54 and Table 55 in the appendix.

The recorded data was used to perform several different PCAs. The results were grouped by grape origin and labelled after the winemakers. Clusters were assigned depending on the closeness of the results in the final diagrams. Figure 86 shows the results of the ICP-OES and MS measurements; Figure 87 using the results of the aroma analysis and Figure 88 the combined data. Table 40 shows the colors and labels used in the PCAs. The same color indicates wines of the same origin, while the labels indicate the winemakers.

Table 40: Explanation of the colors used in the PCAs

Wine	Winemaker		Origin of grapes
1	Von Hövel	VH	🟩 Scharzhofberg (GER)
2	Gunderloch	G	
3	Jurtschitsch	J	
4	Von Hövel	VH	🟥 Rothenberg (GER)
5	Gunderloch	G	
6	Jurtschitsch	J	
7	Von Hövel	VH	🟦 Heiligenstein (AUT)
8	Gunderloch	G	
9	Jurtschitsch	J	

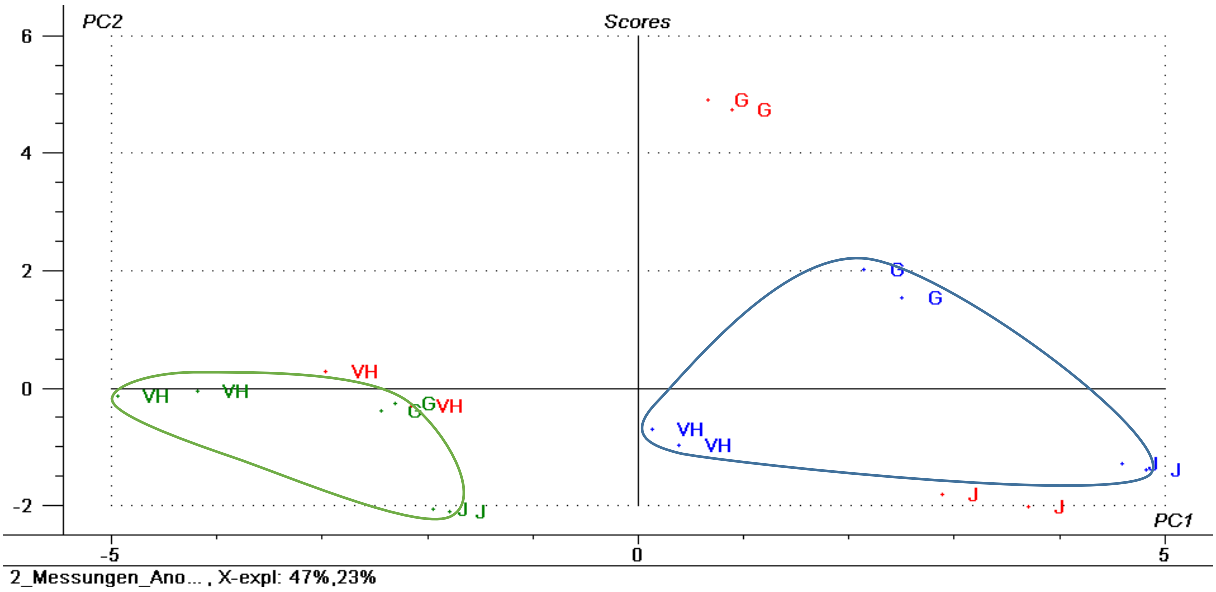


Figure 86: PCA of the results of the analysis of the inorganic composition of the Riesling wines; points with the same color have the same origin and points with the same label are produced by the same winemaker, solid lines indicate a grouping based on geographic origin

Looking at the PCA of the inorganic analysis (Figure 86) two groups were found, both based on the origin of the grapes: Scharzhofberg (green) and Heiligenstein (blue). Especially the origin Scharzhofberg seems to have an impact on the inorganic composition of the wines. Wines from the origin Rothenberg did not show similarities, indicating, that in this case the influence seems to be relatively small.

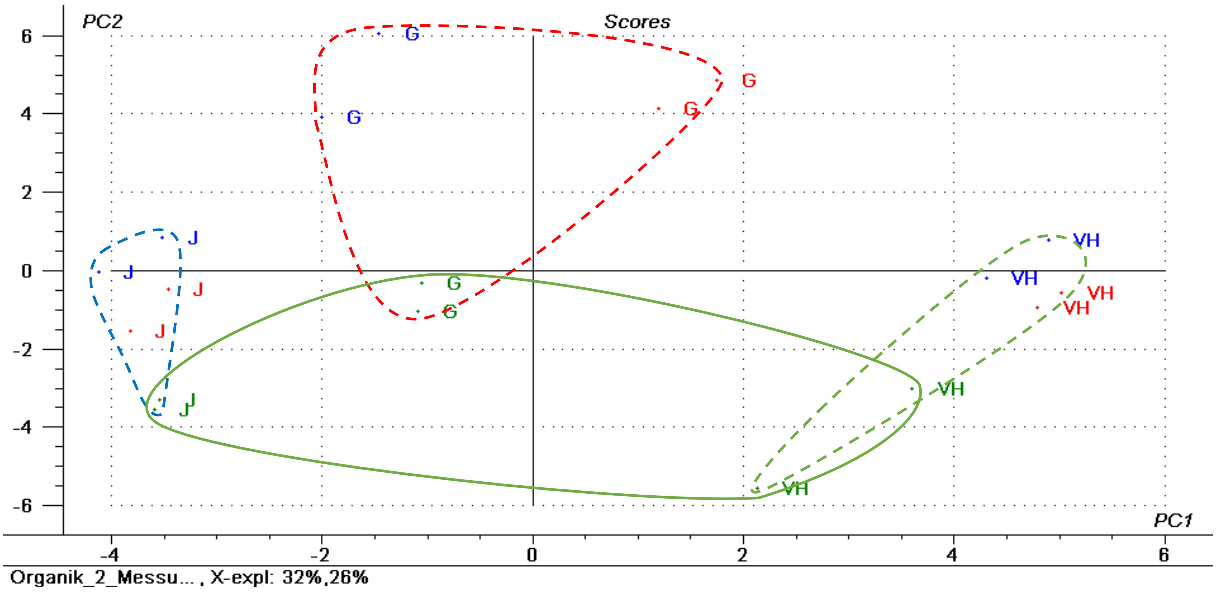


Figure 87: PCA of the volatile organic compounds composition of the Riesling wines; points with the same color have the same origin and points with the same label are produced by the same winemaker, solid lines indicate a grouping based on geographic origin, dashed lines indicate a grouping based on winemaker

When looking at the PCA using the data of the volatile composition, the grouping of the wines is mainly based on the winemaker, the only exception are the wines produced from grapes with the origin Scharzhofberg.

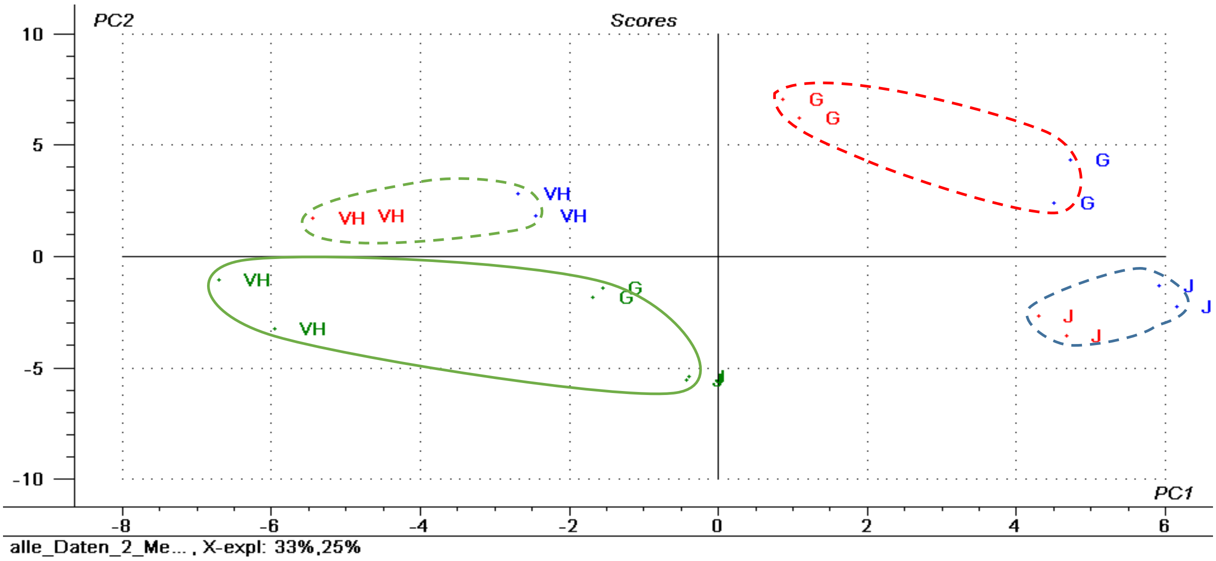


Figure 88: PCA using the combined data of organic and inorganic analysis; points with the same color have the same origin and points with the same label are produced by the same winemaker, solid lines indicate a grouping based on geographic origin, dashed lines indicate a grouping based on winemaker

Figure 88 shows the PCA of the combined data of the analysis of the inorganic and VOC composition of the wines. The only grouping of three here are the wines with the origin Scharzhofberg. In the case of the other origins, the winemaker seems to have the bigger impact on the final wine. Wines made from grapes that originated from the other two vineyards can be grouped based on the winemaker.

One of the reasons for the difference in the results of the analysis of the volatile and the inorganic composition is that many of the volatile compounds are only created or released during fermentation, while the inorganic composition of the grapes is probably less influenced by this process. The type of yeast has a large impact on the final flavor of the wine, as has been discussed in a previous chapter (4.3). In this project, the wines were fermented using spontaneous fermentation, meaning that no selected yeasts were added. As a result, the microorganisms (MOs) responsible for the fermentation will be different for each wine, based on the MOs present in the vineyards, as well as in the cellars. Over the years, many studies showed the importance of the MOs involved in fermentation on the final product, including Puertas et al. (2018), Swiegers and Pretorius (2005), Tempère et al. (2018), Thorngate (1998) and Ugliano and Henschke (2009) as well as many others. Also in this experiment, it seems that the MOs had a great influence on the composition of the wine. In a tasting conducted by a wine magazine in 2014, the wines were awarded different points ranging from 89 to 93 with no apparent connection, to neither winemaker nor origin (Moser 2014).

Overall, it seems that in this experiment the winemaker had the bigger impact on the final wine. The one exception were the wines produced from grapes with the origin Scharzhofberg. This experiment demonstrates how nature and nurture can influence the final product.

9.2 Analysis of 2,4,6-Trichloroanisole

Due to their low sensory threshold, haloanisoles, including 2,4,6-Trichloroanisole (TCA) and 2,4,6-Tribromoanisole (TBA), can have a large impact on the overall impression of a wine. Therefore, highly selective and sensitive analytical methods are needed for their detection and quantification. In this chapter the optimization of a quantification method is described. The method is then applied for the analysis of sparkling wines from different origins.

9.2.1 Method Optimization

Using neutral wine that had been spiked with TCA and TBA as well as with their deuterated counterparts, different SPME methods and fiber materials were investigated for their suitability. The results of the TBA measurements can be found in the appendix.

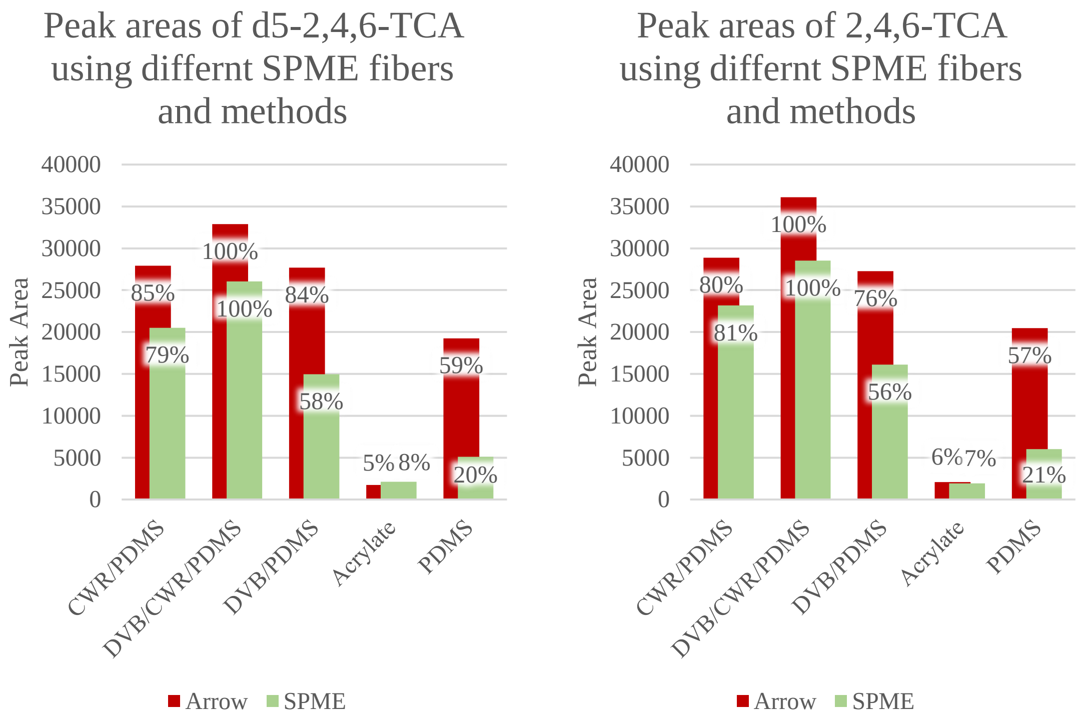


Figure 89: Results of the SPME method and fiber comparison for TCA; enrichment was done at 80°C for 20 min; data labels are the percentages compared to the largest peak area; values are the mean of two measurements

Figure 89 shows the results of the comparison of five different fiber materials and two SPME methods: conventional SPME and SPME Arrows. For both methods the largest peak areas were achieved using the polydivinylbenzene/carbon wide range/polydimethylsiloxane (DVB/CWR/PDMS) fibers. They were therefore used in the subsequent optimization steps. Overall, the use of SPME Arrow resulted in larger peak areas due to a larger surface area.

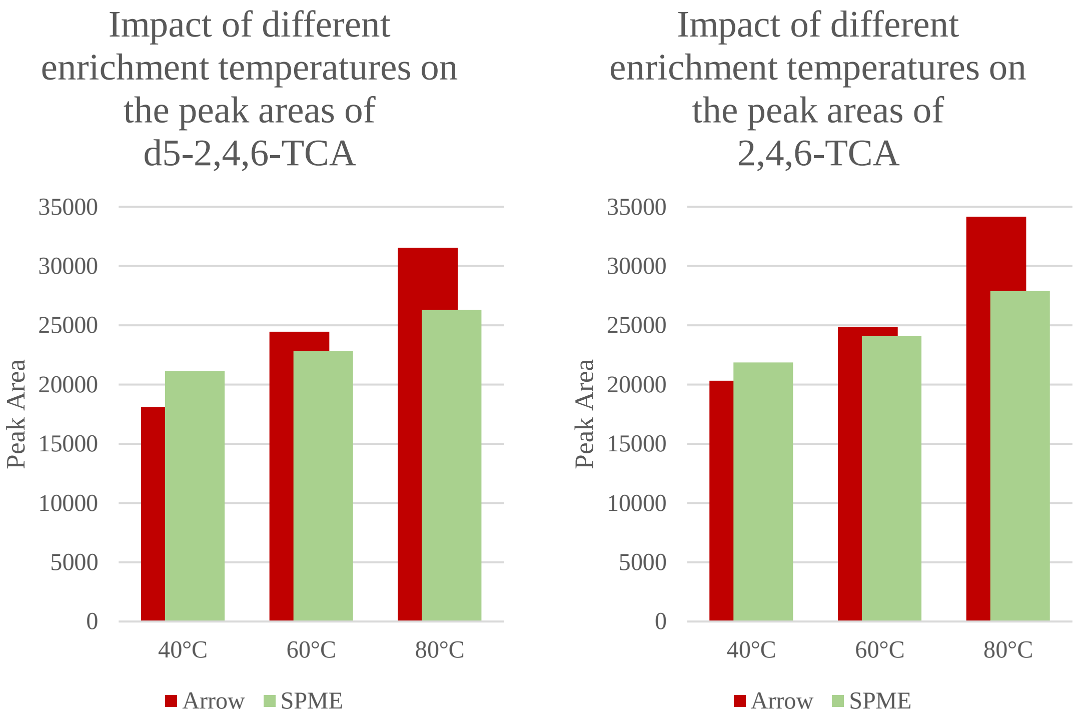


Figure 90: Results of the enrichment temperature comparison for TCA and d5-TCA using the DVB/CWR/PDMS fibers; enrichment time was 20 min; values are the mean of two measurements

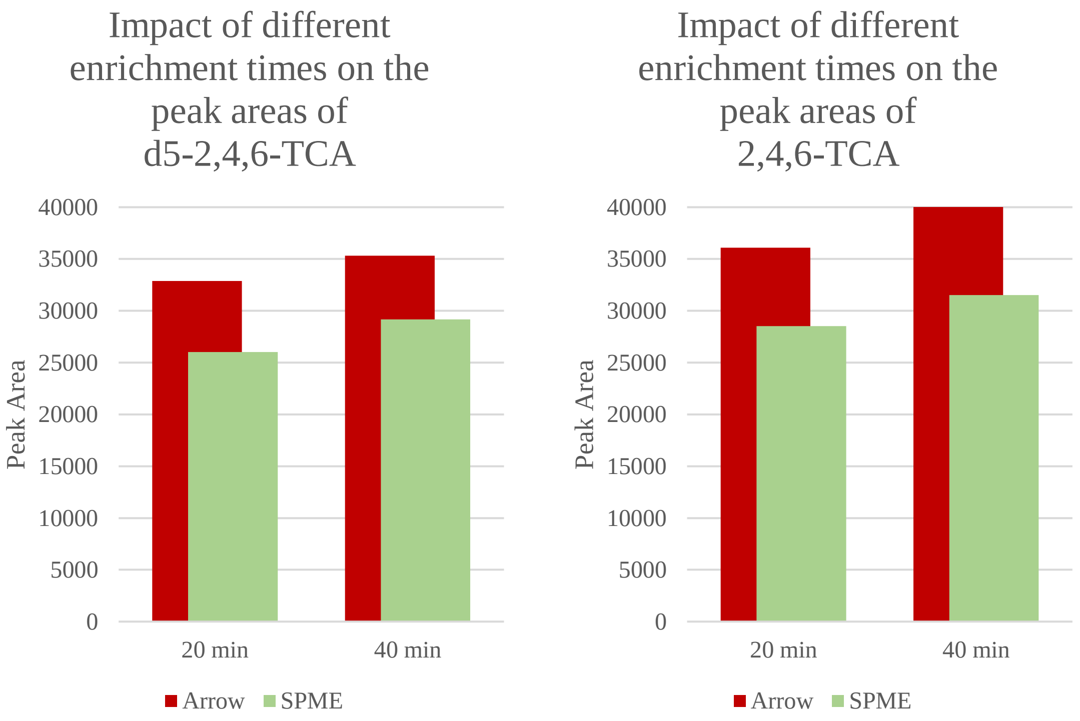


Figure 91: Results of the enrichment time comparison for TCA and d5-TCA using the DVB/CWR/PDMS fibers; enrichment temperature was 80°C; values are the mean of two measurements

Different enrichment conditions were examined to find the best suited ones for the quantification of TCA and TBA in wine samples. The results of the comparisons of three different enrichment temperatures and those of two time periods are shown in Figure 90 and Figure 91. The largest peak areas were obtained at 80°C and after 40 minutes of fiber exposition, respectively. The increase of peak area was more noticeable when using SPME Arrows. In the case of deuterated TCA, an increase of exposition temperature from 40 to 80°C resulted in a peak area increase of 24% and 74% for SPME fibers and SPME Arrows, respectively. Similar trends were observed for non-deuterated TCA (27 and 68%). The prolongation of the exposure time, on the other hand, resulted in an increase of only around 7-12% for both compounds and both methods. As doubling the exposure time did not markedly influence the peak areas and for the sake of creating a time-efficient method, 20 min were considered as sufficient.

The final method used DVB/CWR/PDMS SPME Arrows with a diameter of 1.1 mm and a fiber exposition at 80°C for 20 minutes.

9.3 Analysis of Sparkling Wine Samples

Fourteen sparkling wines from different European origins were analyzed for several parameters, including their composition of volatile compounds with one- and two-dimensional gas chromatography. The samples were compared using principal component analysis to see, if their composition can give an indication about their origin.

The samples originated in four European regions known for their sparkling wines: Champagne and the Loire Valley in France, Franciacorta in Italy, and Catalonia in Spain.

The raw data of the one-dimensional analysis was used to carry out a principal component analysis (Figure 92). A grouping based on the region of origin can be observed. To explore the differences between the wines from different origins in more detail, further analysis was carried out. The results are listed in the appendix.

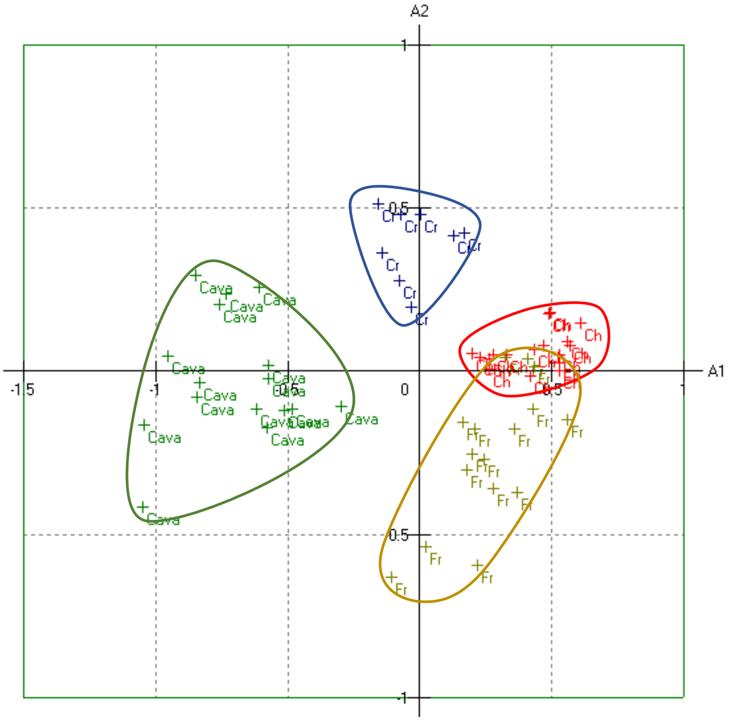


Figure 92: Statistical analysis of the raw data of the one-dimensional aroma analysis of the sparkling wine samples

The contour plots on the next page (Figure 93, Figure 94, Figure 95 and Figure 96) help to illustrate some of the differences between the wines.

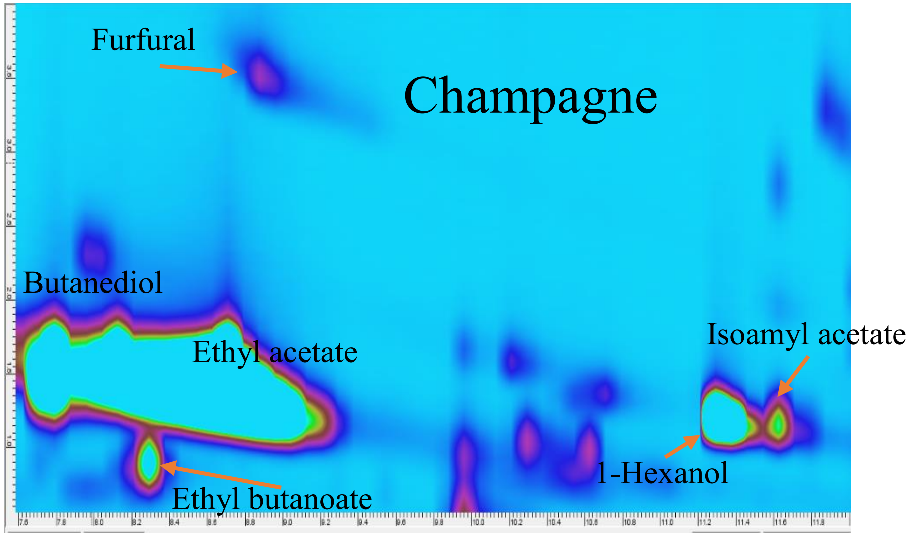


Figure 93: Contour plot of a sparkling wine from Champagne, France

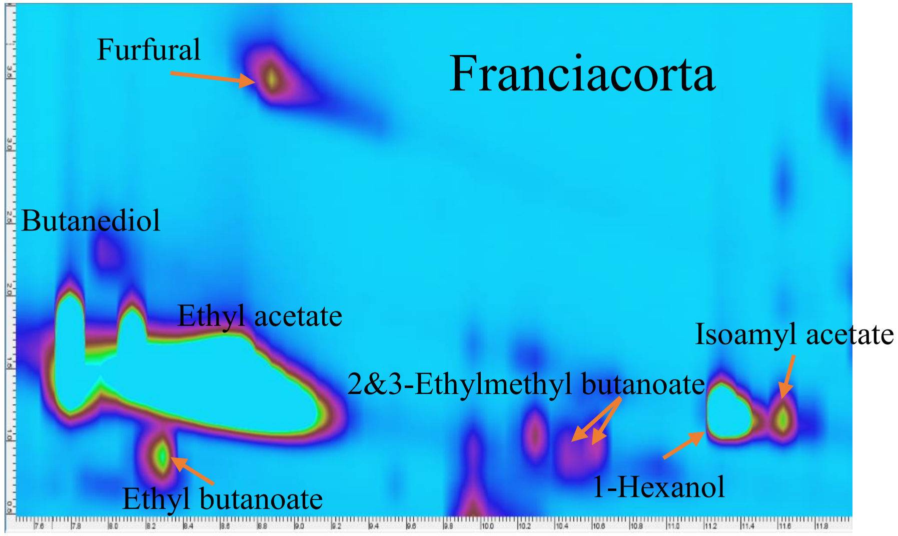


Figure 95: Contour plot of a sparkling wine from the Franciacorta region, Italy

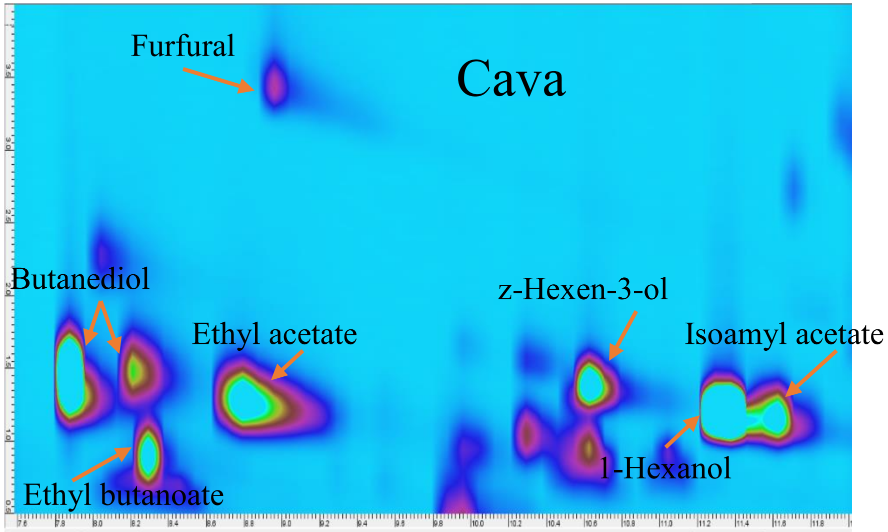


Figure 94: Contour plot of a sparkling wine from Catalonia, Spain

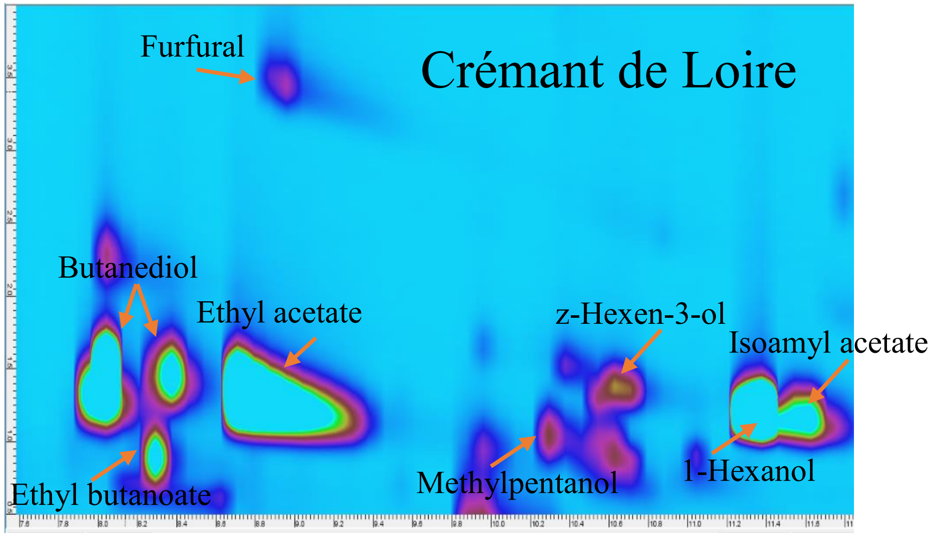


Figure 96: Contour plot of a sparkling wine from the Loire Valley, France

The results of the analysis of the volatile composition of the sparkling wines were used for a statistical comparison with PCA. The concentrations and peak areas of 21 volatile compounds were taken into account (Figure 97 and Figure 98).

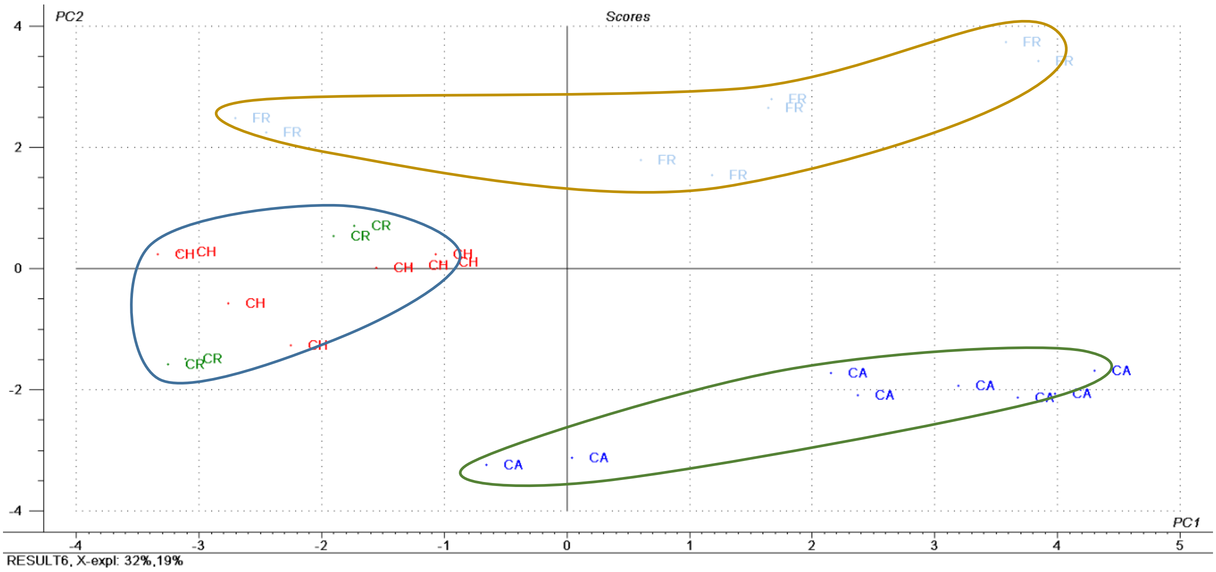


Figure 97: PCA of the volatile compounds of the sparkling wines

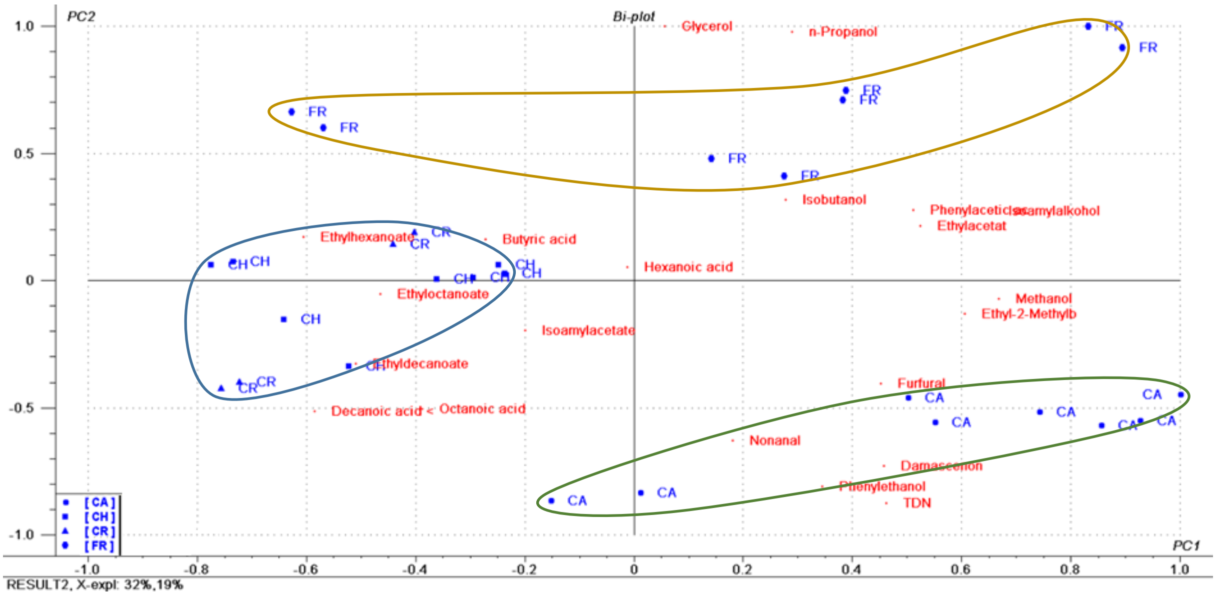


Figure 98: Scores and Loadings PCA plot of the results of the analysis of the volatile compounds

In the PCA the sparkling wines of the two regions in France could not be separated. For the other regions three of the four samples clustered closely, while one sample in each case was further away on the first principal component, but clustered on the second. Overall, a similarity based on the volatile composition of the wines was detectable for wines from different countries. A distinction between the wines originating from the two regions in France could not be made using the chosen volatile compounds. However, when looking at the PCA of the raw data, the two regions were distinguishable. Using a combination of both techniques allows the differentiation of all origins.

Besides the volatile composition, the samples were analyzed for their TCA and TBA concentration using the optimized method from 8.3. The results are listed in Table 41.

Table 41: Results of the TCA measurements of the sparkling wines

	Region	**TCA [ng/L]**
Brut Zéro	France/Loire	<0.5
Le Grande Saumur	France/Loire	<0.5
Montargull	Spain	**1.0±0.05**
Gran Reserva Vintage Cava	Spain	**1.0±0.04**
Cava Reserva Brut Natur	Spain	**0.6±0.08**
Cava Brut Natur	Spain	**1.2±0.24**
Francesco I	Italy	<0.5
Tenuta Villa Crespina Miolo Brut	Italy	**7.2±0.66**
San Christoforo Brut	Italy	<0.5
Blanc de Blancs	Italy	<0.5
Monopole Blue Top Brut	France/Champagne	<0.5
Moet Imperial Brut	France/Champagne	<0.5
Yellow Lable Brut Champagne	France/Champagne	<0.5
Reserve Brut	France/Champagne	<0.5

Five out of the 14 samples had a TCA concentration above the sensory threshold of 0.5 ng/L. This is a percentage of 36%, which is higher than the average (<10%). One of the reasons for this could be the fact that all Spanish wines were from the same winemaker. The taint could be related to a contamination in the winery or the winery equipment. However, the concentration in the Spanish wines is still relatively low and might only effect sensitive people. The highest concentration was found in a sparkling wine from the Franciacorta region in Italy.

The TBA concentration was below the sensory threshold for all tested samples.

9.4 Analysis of Sauvignon Blanc

9.4.1 Impact of Different Harvest Parameters on the Concentration of IBMP

Using HS-SPME-GC-MS/MS and a deuterated internal standard, the concentration of IBMP in Sauvignon Blanc musts from several harvest experiments was analyzed. With a sensory threshold of approximately 1 ng/L (Allen et al. 1991), even very low concentrations of IBMP can give wine a green bell pepper aroma that is characteristic for cool climate Sauvignon Blanc wines.

Impact of Harvest Date

In this experiment, Sauvignon Blanc must samples produced from grapes harvested from the same vineyard, but on three different harvest dates and at two different harvest times in each case, were compared. Table 42 lists the results of the sugar, total acidity and pH determination as well as the IBMP quantification of the must samples. The results of the measurements were compared using Student's t-test and Tukey's Honest Significant Difference. The results of the statistical tests are listed in Table 43. Any p-values below 0.05 indicate a statistically significant impact on the examined characteristics.

Table 42: Sugar concentration, total acidity, pH values and IBMP concentration of the Sauvignon Blanc musts harvested from the same vineyard on three different dates at two times of day

Harvest Date	**Harvest Time**	**Sugar [°Brix]**	**Total Acid [g/L]**	**pH**	**IBMP [ng/L]**
13.09.2018	8:00	19.9 ± 0.24	9.3 ± 0.17	2.94 ± 0.01	1.12 ± 0.10
13.09.2018	16:00	20.3 ± 0.24	9.2 ± 0.20	2.93 ± 0.03	1.13 ± 0.09
24.09.2018	8:00	21.5 ± 0.26	7.5 ± 0.23	3.13 ± 0.04	0.77 ± 0.03
24.09.2018	16:00	21.7 ± 0.30	7.5 ± 0.12	3.12 ± 0.02	0.97 ± 0.12
02.10.2018	8:00	22.5 ± 0.26	7.2 ± 0.25	3.14 ± 0.02	0.90 ± 0.09
02.10.2018	16:00	22.7 ± 0.29	7.2 ± 0.10	3.15 ± 0.01	1.01 ± 0.15

Table 43: Model effect p-values for pH, total acidity, °Brix, and IBMP concentration (ng/L) of the Sauvignon Blanc must samples harvested from the same vineyard on three different dates at two times of day

Model effect	**°Brix**	**Total acidity [g/L]**	**pH**	**IBMP [ng/L]**
Harvest date	**<0.001**	**<0.001**	**<0.001**	**0.003**
Harvest time	**0.046**	0.464	>0.999	**0.047**
Harvest date x Harvest time	0.931	0.954	0.672	0.290

The p-values in Table 43 indicate that the harvest date has a significant impact on all of the examined parameters. The mean pH, for example, increased from 2.94 to 3.14 and total acidity decreased from 9.25 to 7.22 g/L as the harvest date increased. This is in accordance with the expected development of grapes during ripening. In addition to harvest date, the harvest time had a significant impact on two of the examined parameters: sugar content and IBMP concentration. The mean values of the measurements are compared in Figure 99 and Figure 100.

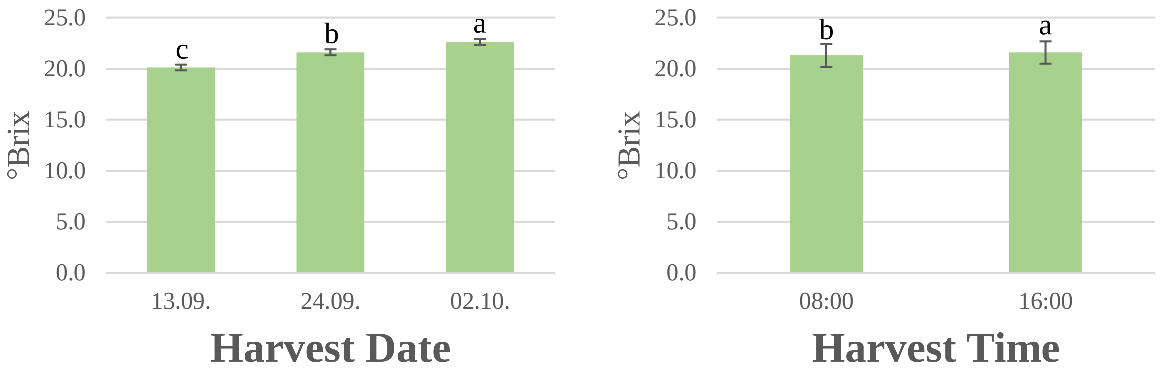


Figure 99: Harvest date and time main effect plots for °Brix; Means connected by the same letter are not significantly different according to student's t-test (p<0.05)

Figure 99 shows that °Brix increased with harvest date, as expected during grape ripening. In addition, the results show that samples harvested at 16:00 had a higher sugar content (21.6 °Brix) than those harvested at 8:00 (21.3 °Brix), across all harvest dates.

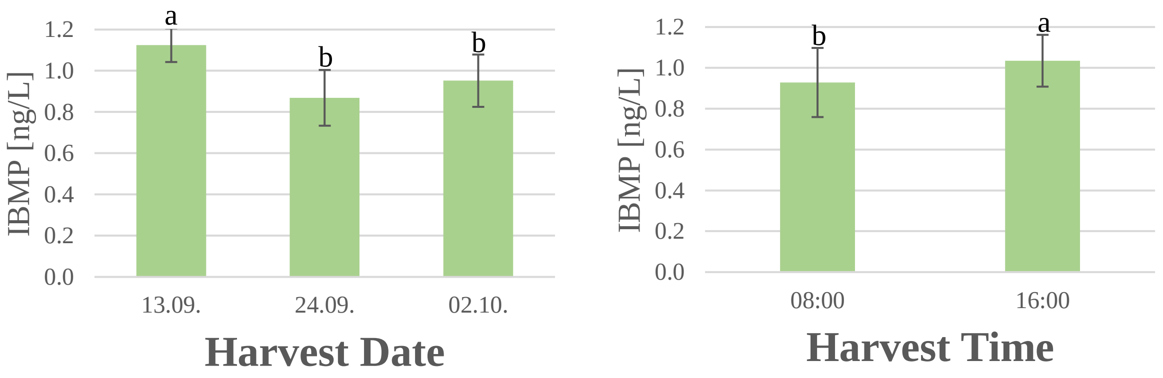


Figure 100: Harvest date and time main effect plots for IBMP concentration (ng/L); Means connected by the same letter are not significantly different according to student's t-test (p<0.05)

The IBMP concentration was highest on 13th September (1.12±0.08 ng/L), followed by 2nd October (0.95±0.13 ng/L) and 24th September (0.87±0.14 ng/L). Figure 100 shows that the IBMP concentration of the must from the first harvest date is statistically significantly different from the two others. Samples harvested at 16:00 had a higher IBMP concentration (1.04±0.13 ng/L) than those harvested at 8:00 (0.93±0.17 ng/L). This is true across all harvest dates.

The results show that there is a connection between the ripeness of the grapes and the IBMP concentration, which decreases with greater ripeness. However, the IBMP concentration of all must samples was either just above or even slightly below the sensory threshold. This means that wines produced from these grapes will most likely exhibit no or only very little of the characteristic green bell pepper aroma of cool climate Sauvignon Blanc wines.

Impact of Location in the Vineyard

The aim of this experiment was to see if the concentration of IBMP depends on the location of the grapes in the vineyard. For this, must produced from Sauvignon Blanc grapes harvested on the same day under similar conditions, but from different locations in the same vineyard was analyzed. Grapes were harvested from two different cardinal directions, north- and south-facing, from the top and the bottom of the slope. Sugar concentration, pH value and total acidity measurements were provided and are listed in Table 44. The IBMP concentration, determined in triplicate, is also listed there. Using the statistics software SPSS the results were compared to see if location, direction and/or the combination of both have a statistically significant impact on the IBMP concentration.

Table 44: Sugar concentration, total acidity, pH values and IBMP concentration of the Sauvignon Blanc musts harvested from the different location in the vineyard

Direction	**Location**	**IBMP [ng/L]**	**Sugar [°Brix]**	**pH**	**Total Acid [g/L]**
South	Top	0.65 ± 0.05	19.7	3.32	7.1
South	Bottom	0.77 ± 0.01	19.8	3.32	7.2
North	Top	0.81 ± 0.02	21.8	3.14	8.1
North	Bottom	0.97 ± 0.04	19.4	3.14	8.9

Table 45: Model effect p-values for IBMP concentration (ng/L) of the Sauvignon Blanc must samples harvested from the different location in the vineyard

Model effect	**IBMP [ng/L]**
Direction	**<0.001**
Location	**<0.001**
Direction x Location	0.258

Looking at the p-values listed in Table 45, the conclusion can be drawn that both factors, location and direction, have an impact on the IBMP concentration. The combined value, however, does not. Due to the fact that the other parameters were provided as mean without repeated measurement, their statistical significance could not be investigated.

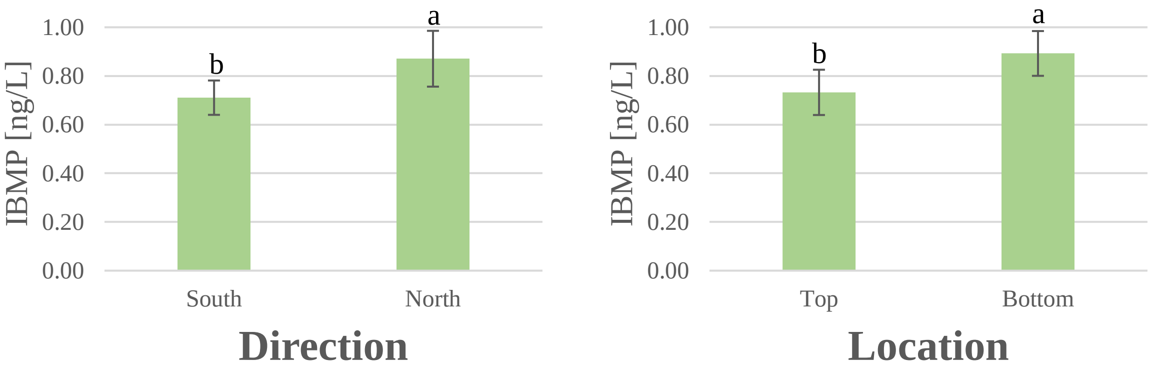


Figure 101: Direction and location main effect plots for IBMP concentration (ng/L); Means connected by the same letter are not significantly different according to student's t-test (p<0.05)

Figure 101 shows the mean IBMP concentration across the different factors. Overall, must produced from grapes harvested on the north-facing slope contained higher concentrations of IBMP (0.87 ± 0.11 ng/L) than that from the south-facing slope (0.71 ± 0.07 ng/L). The same is true for the must of grapes harvested at the bottom of the slope (0.89 ± 0.09 ng/L) compared to the one from grapes harvested at the top (0.73 ± 0.09 ng/L). The IBMP concentration in all of the examined musts was below the sensory threshold.

With the exception of the sugar concentration of the must produced from the grapes harvested at the top of the north facing slope, most parameters indicate that there is a connection between ripeness of grapes and IBMP concentration. Musts from riper grapes, which generally contain higher amounts of sugar and are less acidic, showed lower IBMP content. Grapes grown on a south facing slope are exposed to higher amounts of sunlight, the same is true for those on the top of the slope. Therefore, it is likely that those are riper than those from north-facing or the bottom of the slopes. The highest IBMP concentration was found in the must from the bottom of the north-facing grapes, which also had the highest total acidity as well as lowest sugar content and pH. In contrast to this, must from grapes grown on the top of the south-facing slope had a higher sugar content and pH as well as lower total acidity and the lowest IBMP concentration of all the samples.

These results are in accordance with those from the harvest date experiment, where later harvest dates resulted in lower IBMP concentrations.

Impact of Grape Origin

In this experiment musts produced from Sauvignon Blanc grapes harvested in August and September of 2018 from four different locations (Figure 102) were analyzed for their IBMP content and the composition of their volatile organic compounds.

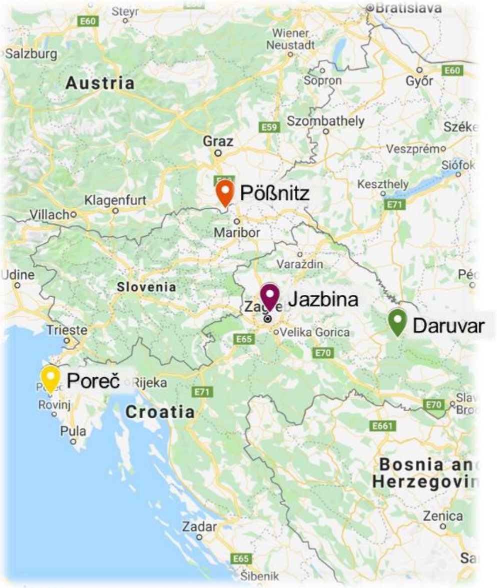


Figure 102: Harvest locations of the Sauvignon Blanc grapes used to produce the must samples

IBMP, sugar concentration and total acidity are listed in Table 46. They were determined in triplicates. In addition, aroma profiles of the wines were generated. The results were used to perform two PCAs, one based on the parameters in Table 46 and one based on their aroma profiles.

Table 46: Sugar concentration, total acidity and IBMP concentration of the Sauvignon Blanc musts harvested from four different vineyards

Location	Country	IBMP [ng/L]	Sugar [°Brix]	Total Acidity [g/L]
Pößnitz	Austria	0.36 ± 0.06	23.3 ± 0.15	7.1 ± 0.12
Jazbina	Croatia	2.33 ± 0.10	19.0 ± 0.06	7.8 ± 0.15
Daruvar	Croatia	2.06 ± 0.06	22.3 ± 0.06	6.2 ± 0.10
Poreč	Croatia	1.70 ± 0.22	23.0 ± 0.09	6.7 ± 0.06

Figure 103 shows the PCA of the three parameters (IBMP concentration, total acidity and sugar concentration). Based on those parameters, the samples from Poreč and Daruvar are similar, as they fall into the same quadrant of the diagram and are closer to each other than to the other samples. However, there is a separation between samples from different locations. While the samples from two of the locations in Croatia are closer to each other, the third is not. In this case the country of origin seems to have a minor role on the characteristics of the must.

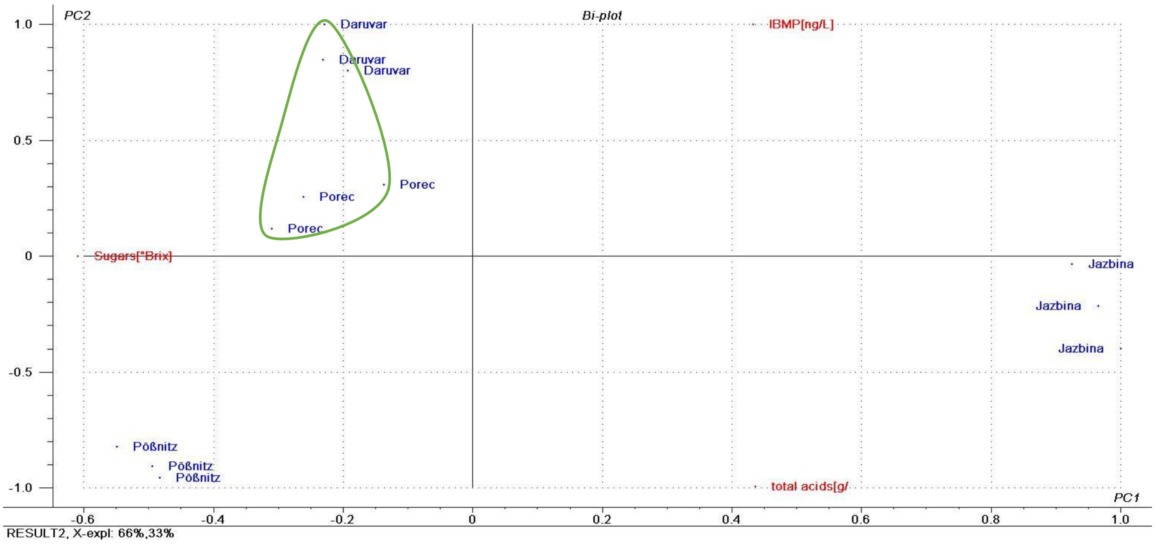


Figure 103: PCA of Sauvignon Blanc samples from four different vineyards as a function of total acidity and sugar and IBMP concentration

Figure 104 shows the PCA based on the aroma profiles of the must samples. The samples from Daruvar and Poreč are clustered together. This indicates similarities in their aroma profiles. Again, the country of origin does not lead to clustering in these samples.

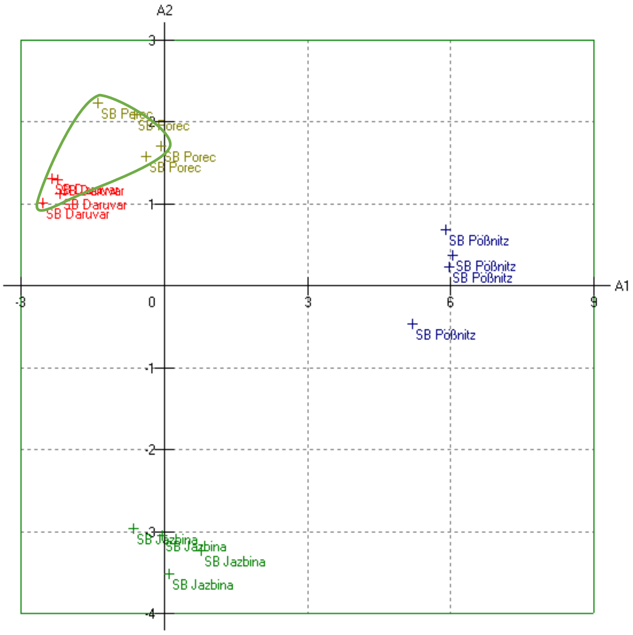


Figure 104: PCA of Sauvignon Blanc samples from four different vineyards as a function of the composition of their volatile organic compounds

The samples from Poreč and Daruvar clustered in both PCAs. This means that they show similarities in the composition of volatile compounds as well as in the concentrations of the three additional parameters. Therefore, wines made from these grapes might show similar characteristics. The largest difference between the examined must samples was the IBMP concentration, which was particularly low in the sample from Southern Styria. One of the reasons for this could be the later harvest date as the grapes were harvested almost two weeks after the others. The other ripeness indicators (total acidity and sugar concentration), however, are both at similar levels compared to those of the other samples. The IBMP concentration in all Croatian samples was higher than 1 ng/L. Therefore, wines produced from these grapes will most probably exhibit a characteristic green bell pepper aroma.

In this experiment, the characteristics of Sauvignon Blanc must samples from four different locations were analyzed. While two samples from Croatia (Poreč and Daruvar) did show similarities in their composition, these similarities are not solely based on the country of origin, as the third Croatian sample did not cluster with the other two. This can likely be explained by the similar climatic conditions of Austria and Croatia. Both countries are part of the cool climate growing areas for Sauvignon Blanc. Wines produced from grapes grown in these areas usually exhibit more green characteristics, compared to the more thiol driven tropical aromas of Sauvignon Blanc wines from warmer areas (Green et al. 2011). For a deeper understanding of similarities and differences in the composition of the musts, factors like the conditions of the vineyards, the individual soil and other agrotechnical measures can be considered.

Conclusion of IBMP Experiments

The first experiment that examined the connection between harvest date and time showed that grapes that have been harvested later had lower levels of IBMP. In the second experiment, must produced from grapes grown at sites with higher sun exposure had the lowest IBMP concentration. Furthermore, the third experiment showed that grapes that had been harvested earlier in the year had overall higher levels of IBMP.

The results of the experiments point to a correlation between the overall ripeness of the grapes and their IBMP content. Riper grapes, which are generally characterized by higher concentrations of sugar and lower levels of acidity, had lower concentrations of IBMP. This connection echoes the findings of Lacey et al. (1991), who observed a decrease of the IBMP concentration over the course of the ripening process.

As mentioned before, Styria is part of the cool climate wine growing regions. IBMP is therefore an important varietal compound in wines from this region. In most of the samples the IBMP concentration was either only slightly above the sensory threshold or below. Over the next years, Styrian winemakers could face additional challenges due to a predicted increase of the overall temperature of about 1.5 °C until 2050 (Austrian Panel on Climate Change 2014).

Despite all the challenges winemakers will face due to the changing climate, the composition of a wine will still very much depend on the conditions of each individual year, as well as the viti- and vinicultural techniques employed in its making. These differences and the resulting lack of uniformity are some of the factors that ultimately make wine such an interesting research subject.

9.4.2 Thiols

Varietal thiols are an essential part of the characteristic aroma of Sauvignon Blanc wines. Two of the varietal thiols related to the aroma of Sauvignon Blanc are sulfanylhexan-1-ol (3SH) and 3-sulfanyl-hexylacetate (3SHA). The quantification of these compounds is comparatively complex, due to their high reactivity and their low sensory threshold. One commonly used approach to deal with these issues, is derivatization of the compounds to increase their stability. In this work ethyl propiolate (ETP) was chosen as derivatization agent for thiols, based on the work of Herbst-Johnstone et al. (2013). In cooperation with the Department of Agriculture of the University of Zagreb, the derivatization procedure was optimized and a quantification method using GC-tandem mass spectrometry (GC-MS/MS) was developed. This method was then employed to quantify the compounds in six wines that had been treated with different antioxidant preparations, which had been produced for this project.

For the first step of method development mixtures of the deuterated and the non-deuterated standards respectively were derivatized in a synthetic wine matrix. The mixtures were measured in full scan mode (Figure 105).

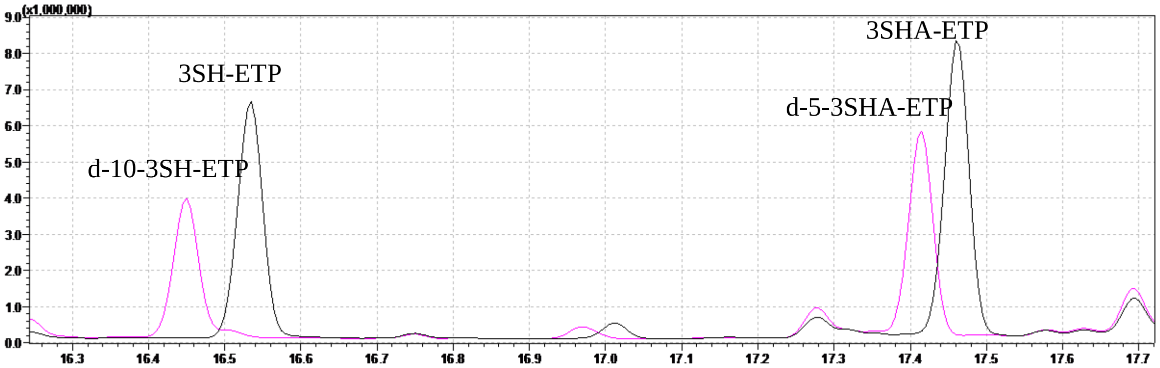


Figure 105: Chromatogram of the overlaid full scans of derivatized thiols; pink: mix of deuterated thiols (10 mg/L), black: mix of non-deuterated thiols (10 mg/L)

From these measurements, mass spectra of the derivatized thiols d10-3SH-ETP (Figure 106), 3SH-ETP (Figure 107), d5-3SHA-ETP (Figure 108) and 3SHA-ETP (Figure 109) were created. Some of the most prominent unique masses were chosen and used to acquire product ion scans and to set up a multiple reaction monitoring (MRM) method.

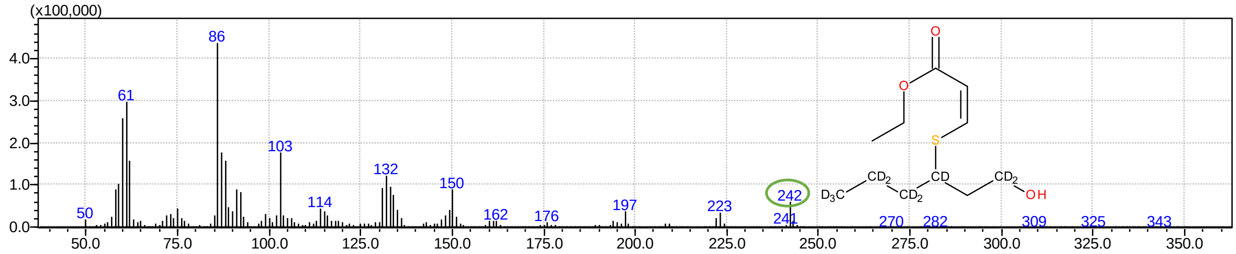


Figure 106: Mass spectrum of derivatized d10-3SH-ETP, molecule peak encircled

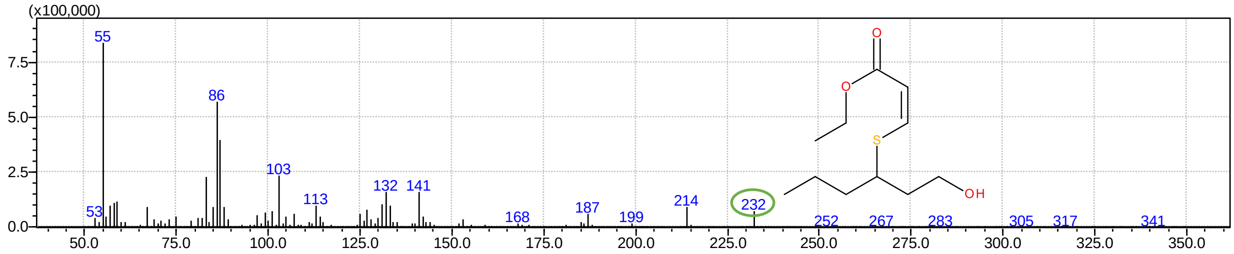


Figure 107: Mass spectrum of derivatized 3SH-ETP, molecule peak encircled

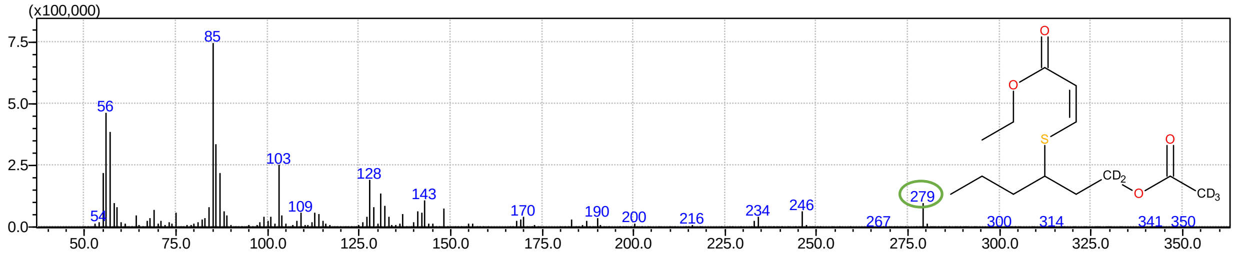


Figure 108: Mass spectrum of derivatized d5-3SHA-ETP, molecule peak encircled

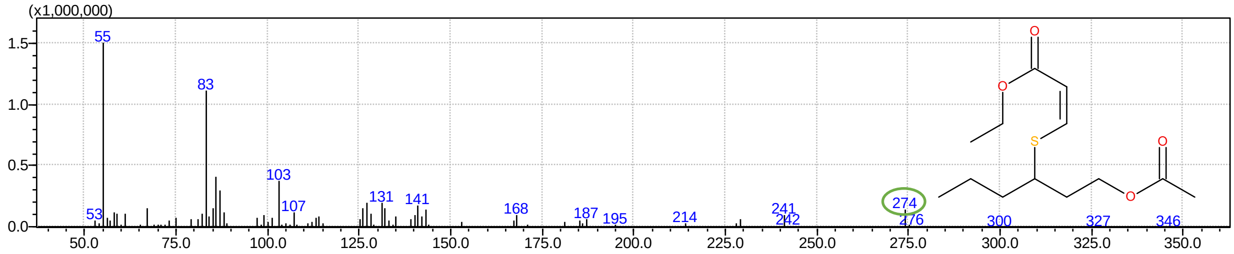


Figure 109: Mass spectrum of derivatized 3SHA-ETP, molecule peak encircled

Figure 110 and Figure 111 show the two chromatograms of the monitored transitions for the deuterated and non-deuterated thiols at the lowest and highest level of standard addition, respectively. Using an MRM method increases both, sensitivity and selectivity allowing for the observation of analytes at low concentrations.

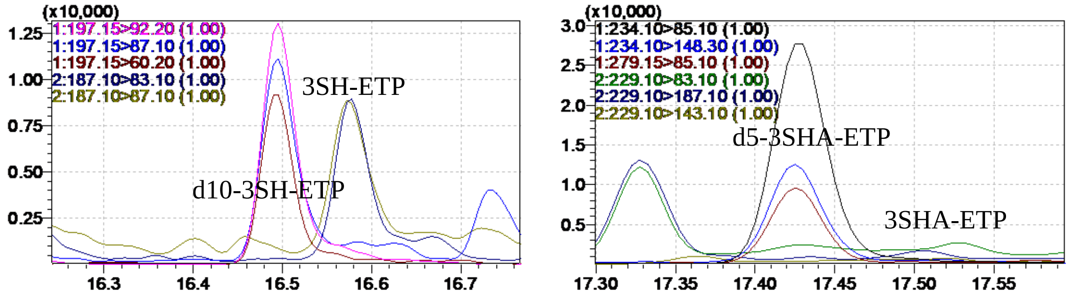


Figure 110: Chromatogram of the transitions used for identification and quantification of the derivatized thiols at the lowest standard addition level (only IS)

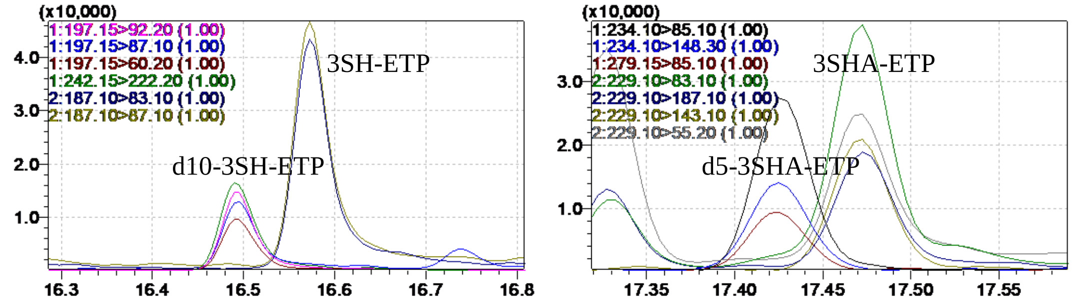


Figure 111: Chromatogram of the transitions used for identification and quantification of the derivatized thiols at the highest standard addition level (500 ng/L 3SH; 250 ng/L 3SHA)

The results of the quantification of the two varietal thiols, 3SH and 3SHA, for the six wines of interest can be found in Table 47. All wines exceeded the respective sensory thresholds of ~60 ng L-1 (3SH) and ~4 ng L-1 (3SHA).

Table 47: Results of the thiol quantification of the wines treated with different antioxidant preparations

Wine	**3SH [ng/L]**	**3SHA [ng/L]**
A	95±5	7±1
B	87±9	6±2
C	87±8	10±3
D	77±2	8±1
E	135±3	8±2
F	95±7	14±1

The concentration of varietal thiols in the experimental wine samples varied between 6 ng/L and 135 ng/L, and all the wines showed thiol presence above the respective sensory thresholds. However, this method had a quantification limit for 3SHA above its sensory threshold and further improvements need be done. The 3SH concentration was significantly higher in the wine treated with a blend of potassium metabisulfite, ascorbic acid and gallotannins and lowest for light sulfurization treatment with 5% sulfurous acid. The results are in accordance with some previous findings which emphasize the protective role of tannins and antioxidants for the preservation of varietal thiols in wine.

A relatively quick and reliable derivatization and quantification method for two of the most common varietal thiols related to Sauvignon Blanc aroma was established. It can be used to examine the effect of different treatment of Sauvignon Blanc grapes, must of wine or the impact of various microorganisms used in fermentation. When looking at multiple antioxidant treatments, we found one that resulted in a significantly increased 3SH concentration. This finding and others can help winemakers when choosing treatment options.

9.5 Analysis and Characterization of Wine from Fungus Resistant Grape Varieties

9.5.1 Determination of the Impact of Different Treatments on Chambourcin Grapes and Wine

Mayfield (2020) found that the foliar treatment impacted the final concentration of several important aroma compounds. This included a significant impact on three of the four ethyl esters examined (Figure 112). As a result, the wines produced from grapes that had been treated were found to exhibit more fruity characteristics in a GC-olfactometry experiment.

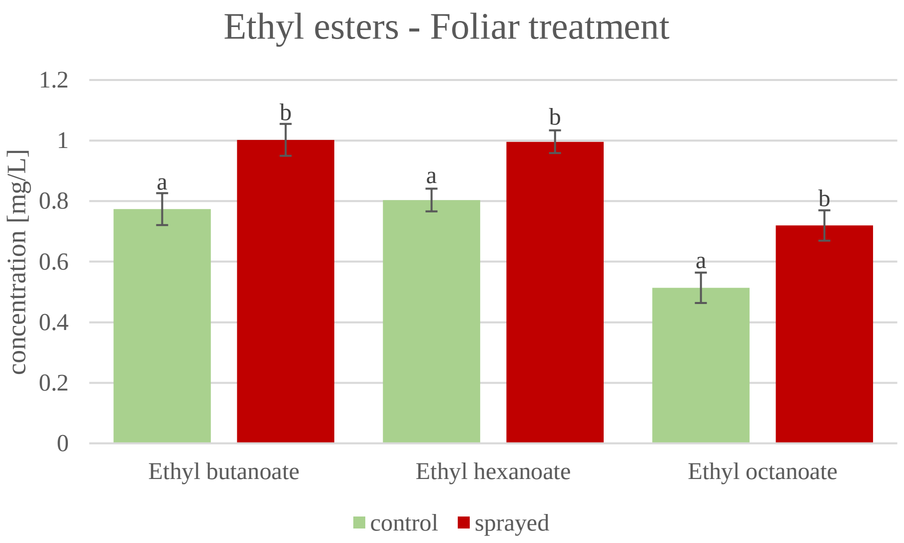


Figure 112: Impact of foliar treatment on the concentration of ethyl esters (Mayfield 2020), treatments marked with different letters are statistically significantly different, error bars represent standard error

No differences between wines which had been treated with tannins and oak chips and those that had no additions were reported.

To gain a deeper understanding of the wines and how the different treatments impact the composition of volatile organic compounds, comprehensive two dimensional GC was performed with all samples. Figure 113 shows contour plots of all four treatment combinations. While the impact of the foliar treatment is not obvious on first sight, two compound blobs are noticeable in the samples that had additions of oak chips and tannins. The two compounds were identified as *cis*- and *trans*- oak lactone. Both have been previously identified in wine matured in wooden barrels (Pollnitz et al. 1999; Aznar et al. 2001; Cerdán et al. 2002). The odor threshold in synthetic wine of the *trans* and *cis* isomers are 122 and 35 µg/L respectively (Chaves et al. 2007). Using the known concentrations of the closest ethyl ester, the concentrations were estimated at 34±5 µg/L for *trans*-oak lactone and 113±13 µg/L for *cis*-oak lactone over all wines. These concentrations are in good accordance with previously reported by Cerdán et al. (2002) values for wines aged in American oak (between 20 and 37 µg/L for *trans*-oak lactone and between 100- and 151 µg/L for *cis*-oak lactone).

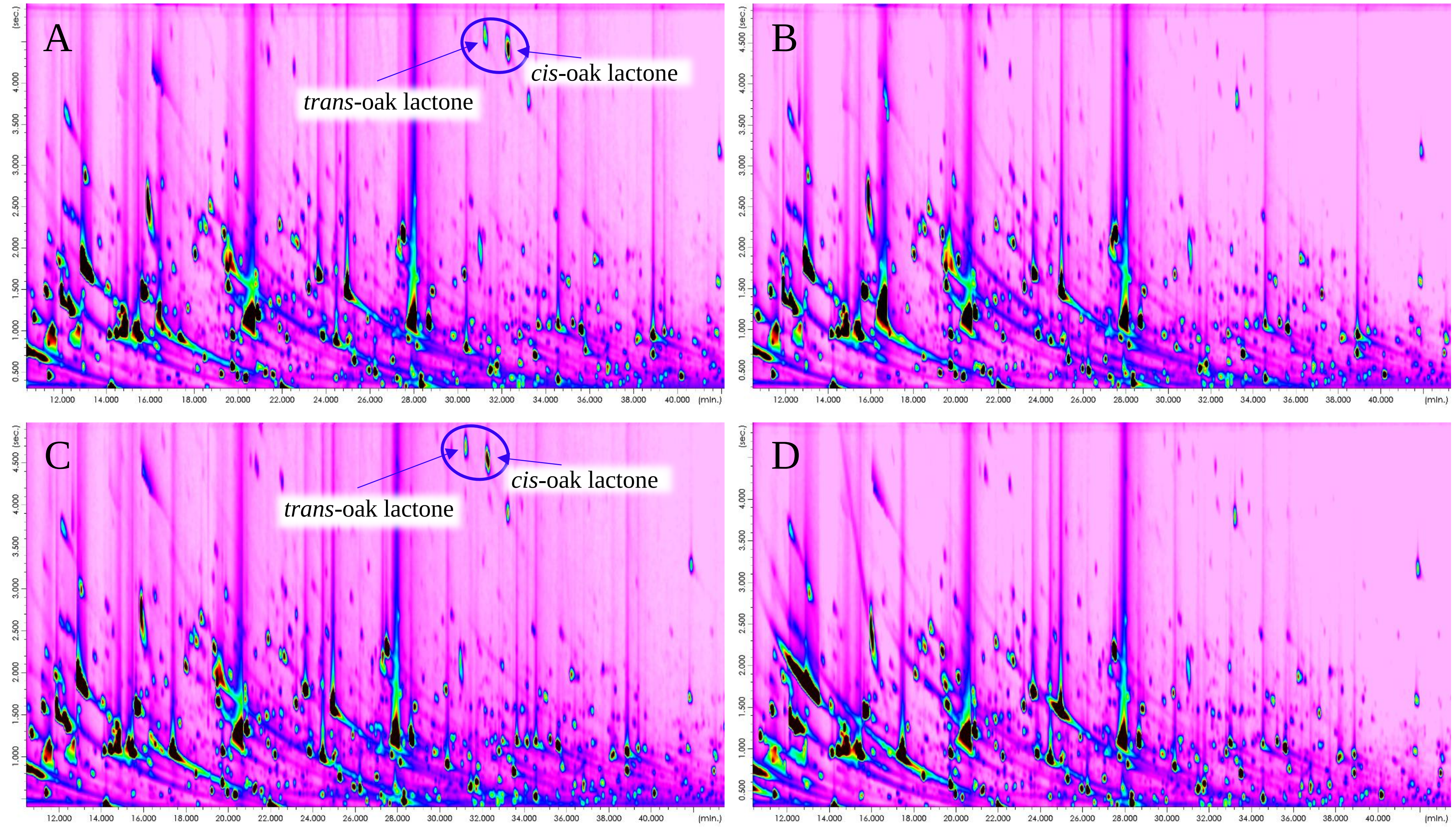


Figure 113: GCxGC contour plots of the Chambourcin wine samples, A…control with additions; B…control, no additions; C…sprayed with additions; D…sprayed, no additions; highlighted area shows two compounds unique to wines with additions

Cis-oak lactone was present in a concentration above its odor threshold. With an OAV of 3.2, it contributes to the overall aroma of the wines with additions. The concentration of *trans*-oak lactone was below its odor threshold.

Waterhouse and Towey (1994) reported the possibility to distinguish between wines aged in American and French oak based on the ratio of the oak lactone isomers. They found that in wines aged in French oak the ratio is between 1 and 1.5, compared to ratios between 5 and 8 for American oak. Figure 114 shows the mean peak areas of the two oak lactone isomers. For the analyzed Chambourcin wines, the ratio was calculated to be 3.4 (*cis*- to *trans*- oak lactone). This value indicates a higher probability of American oak chips being used.

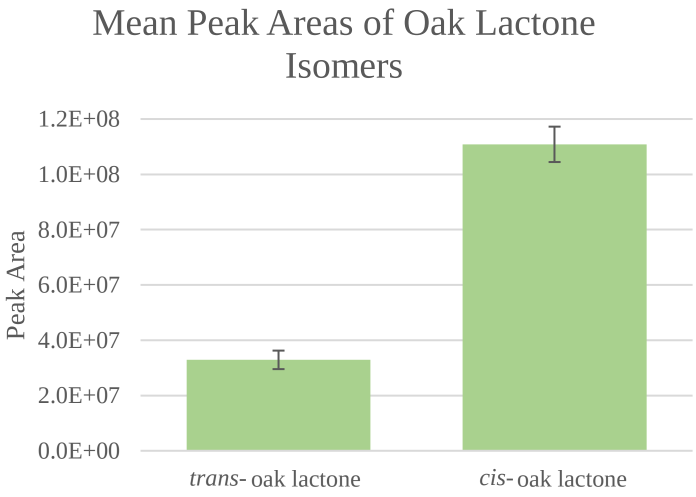


Figure 114: Mean peak areas of the two oak lactone isomers over all samples with additions, error bars represent standard deviation

Using comprehensive two-dimensional analysis to examine the wines in more detail revealed two compounds that were only found in wines treated with tannins and oak chips during fermentation. While *cis*-oak lactone was also found in the one-dimensional GC data, the *trans* isomer was not. The use of a two-dimensional technique allowed an approximate quantification of both compounds. According to the determined OAVs, the conclusion can be made that the *cis*-oak lactone has an influence on the overall aroma impression of the wines treated with oak chips.

In addition, the origin of the used oak chips could be determined. Previous work by Cerdán et al. (2002) and Waterhouse and Towey (1994) strongly suggests that the added oak chips originated from American oak.

9.5.2 Characterization of Styrian PIWIs

In this project, wines from different fungus resistant grape varieties from five consecutive years were analyzed. This included a chemical characterization using aroma profiles and the quantification of several important compounds and a sensory analysis. In the end the results of both are compared.

Aroma Profiles

Using the PARADISe software, 77 compounds were identified. Figure 115 shows an unresolved peak and the resolved peak with two identified compounds (D-Limonene and α-Pinene). The number of compounds used in the comparisons was reduced to 53 (including two internal standards) by comparing calculated retention indices to previously reported ones and other factors, including the existence of odor descriptors and sensory thresholds in relevant matrices (wine, wine matrix or water). A full list of the identified compounds can be found in the appendix (Table 59).

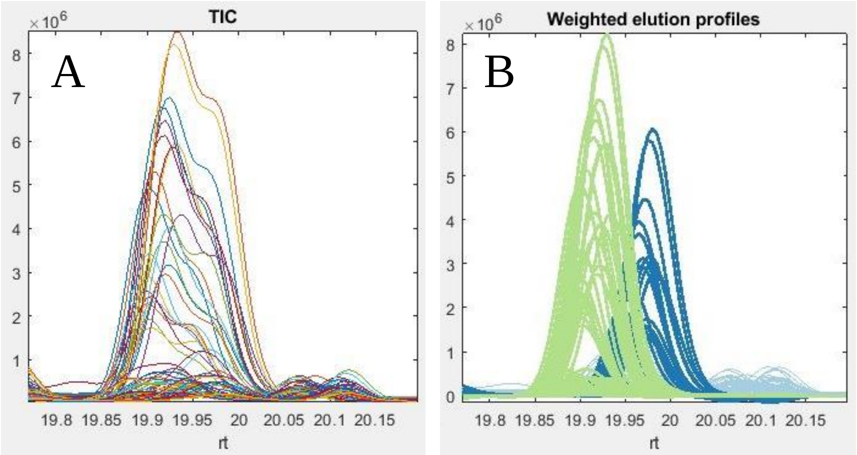


Figure 115: Images from PARADISe Version 3 of an unresolved TIC peak (A) and the weighted elution profiles (B)

The identified compounds were grouped by compound class. This included esters, alcohols, carboxylic acids, aldehydes, ketones, terpenes and naphthalene. The compounds were also sorted into groups based on their odor descriptors: fruity, roasted/ caramelized, unpleasant, green/ fat, floral, chemical and herbal/ spicy. Using the areas of the internal standards, the concentration of the compounds was estimated. These were used to calculate the odor activity values (OAVs), which were also summed up based on compound class and aroma type. Table 48 and Table 49 list the shares of each group, as well as the highest and lowest values, based on their peak areas as well as on the calculated OAVs. (The OAV of 2-isobutyl-3-methoxypyrazine was taken into account as well.)

Table 48: Share of compound group and highest and lowest values in percent by area and OAVs calculated based on 3-Heptanol and Methyl nonanoate, largest share printed in bold

	Area			**OAV based on 3-Heptanol**			**OAV based on Methyl nonanoate**		
Compound group	**Ø**	**Max**	**Min**	**Ø**	**Max**	**Min**	**Ø**	**Max**	**Min**
Ester	**62.9**	72.7	51.3	**97.2**	99.6	91.1	**99.4**	100.0	96.4
Carboxylic Acid	18.5	25.3	13.8	0.04	0.06	0.03	0.00	0.01	0.00
Alcohol	7.05	13.7	4.18	0.05	0.11	0.02	0.01	0.02	0.00
Terpene	1.33	8.59	0.10	1.54	8.37	0.13	0.19	1.09	0.02
Aldehyde	0.18	0.67	0.02	0.00	0.00	0.00	0.00	0.00	0.00
Naphthalene	0.10	0.52	0.01	0.83	4.05	0.05	0.10	0.44	0.01
Ketone	0.10	0.17	0.06	0.11	0.27	0.01	0.01	0.03	0.00
Pyrazine	n.a.	n.a.	n.a.	0.24	2.80	0.00	0.28	3.32	0.00

When looking at the areas and OAVs of the compound groups, esters are responsible for the largest share. The second and third largest groups by area are carboxylic acids and alcohols. However, due to the high sensory thresholds of these compound groups, their impact is almost negligible when it comes to the OAVs. Here, terpenes and pyrazines, which generally have lower odor thresholds than alcohols and carboxylic acids, are responsible for the second and third largest share. This distribution is not present in all wines. Some have higher concentrations of certain compound groups. For example, those with Muscat varieties (Blütenmuskateller and Muscaris) in their pedigree generally show higher concentrations of terpenes. Other examples are pyrazines in wines with Sauvignon cultivars (e.g. Cabernet Sauvignon or Sauvignon Blanc) in their linage. This is the case for Cabernet Blanc, Sauvignac and Sauvignon Soyhieres.

Table 49: Share of compound group and highest and lowest values in percent by area and OAVs calculated based on 3-Heptanol and Methyl nonanoate, largest share printed in bold

	Area			**OAV 3-Heptanol**			**OAV Methyl nonanoate**		
Aroma group	**Ø**	**Max**	**Min**	**Ø**	**Max**	**Min**	**Ø**	**Max**	**Min**
Fruity	**59.0**	65.8	49.9	**89.8**	96.9	79.5	**98.5**	99.7	94.8
Unpleasant	18.5	25.4	13.8	0.04	0.06	0.03	0.00	0.01	0.00
Green/ fat	6.19	12.1	2.33	7.71	18.5	1.42	1.22	4.92	0.15
Floral	4.82	7.06	3.22	1.08	5.32	0.17	0.13	0.69	0.02
Roasted/ caramelized	0.72	2.23	0.21	0.00	0.00	0.00	0.00	0.00	0.00
Herbal/ spicy	0.46	3.13	0.03	0.50	3.06	0.02	0.06	0.44	0.00
Chemical	0.38	1.55	0.03	0.85	4.05	0.07	0.10	0.44	0.01

Similar trends are noticeable for the aroma groups. Esters often have fruity aroma descriptors. As a result, the largest share in all three groups is taken up by the compounds of the fruity aroma group. When looking at the distribution by area, the second and third largest groups are unpleasant and green/ fat. This changes for the OAVs. Here, green/fat is still represented (second largest share), unlike unpleasant aromas. One of the reasons for this is that this aroma group is often associated with carboxylic acids, which is less consequential due to their high odor thresholds. With regard to OAVs, floral aromas are the third largest group.

A PCA based on the peak areas of all compounds (without internal standards) and sample grouping based on variety is depicted in Figure 116. The Muscaris and Blütenmuskateller wines can be differentiated from the other samples. The main reason for this is their higher concentration of terpene compounds. This can be seen in Figure 117, a scores and loadings plot of the same PCA. No clear trends can be observed for the other samples, as they are all clustered on the second principal component. To see if other trends can be observed, a second PCA was carried out without the Blütenmuskateller and Muscaris samples (Figure 118 and Figure 119).

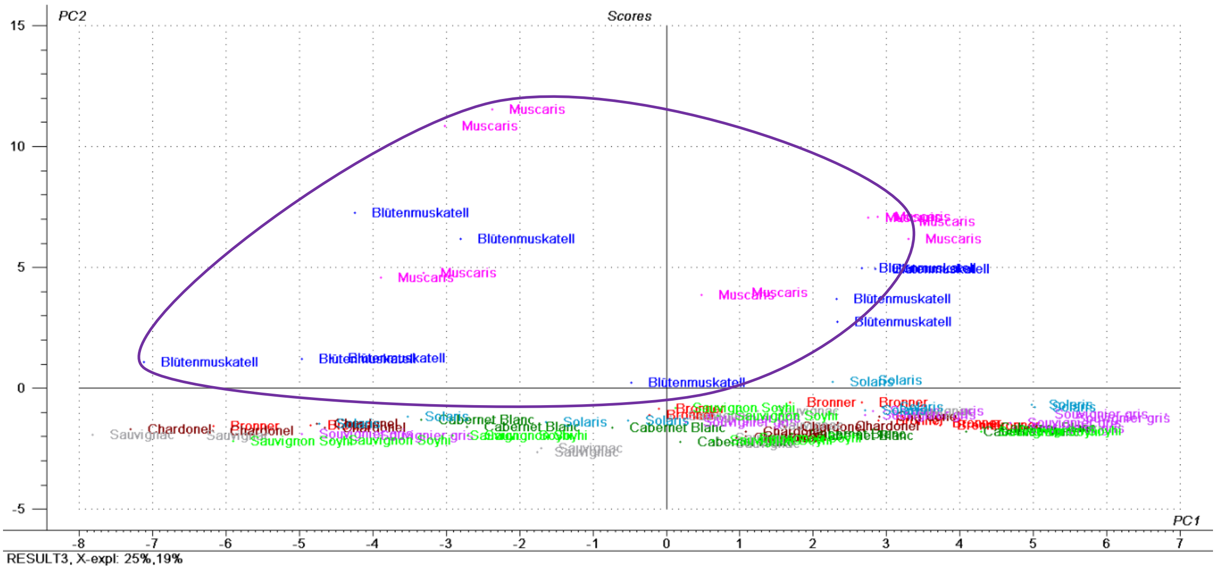


Figure 116: PCA based on peak areas all compounds, samples grouped by variety, Muscat group encircled

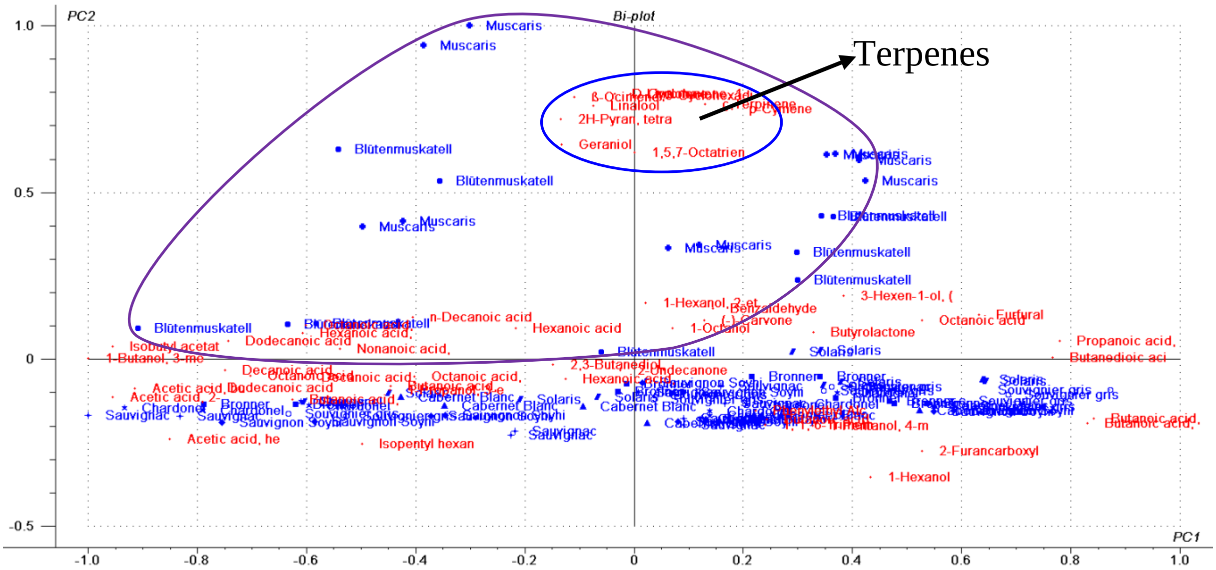


Figure 117: Scores and Loadings PCA plot based on the peak areas of all compounds, samples grouped by variety, Muscat group and terpenes encircled

The PCA of the remaining samples with a grouping based on variety does not reveal any clear trends (Figure 118). However, when the samples are grouped by year, trends are visible (Figure 119). Those are more obvious for certain years (2019 and 2018), while the other years show a certain amount of overlap.

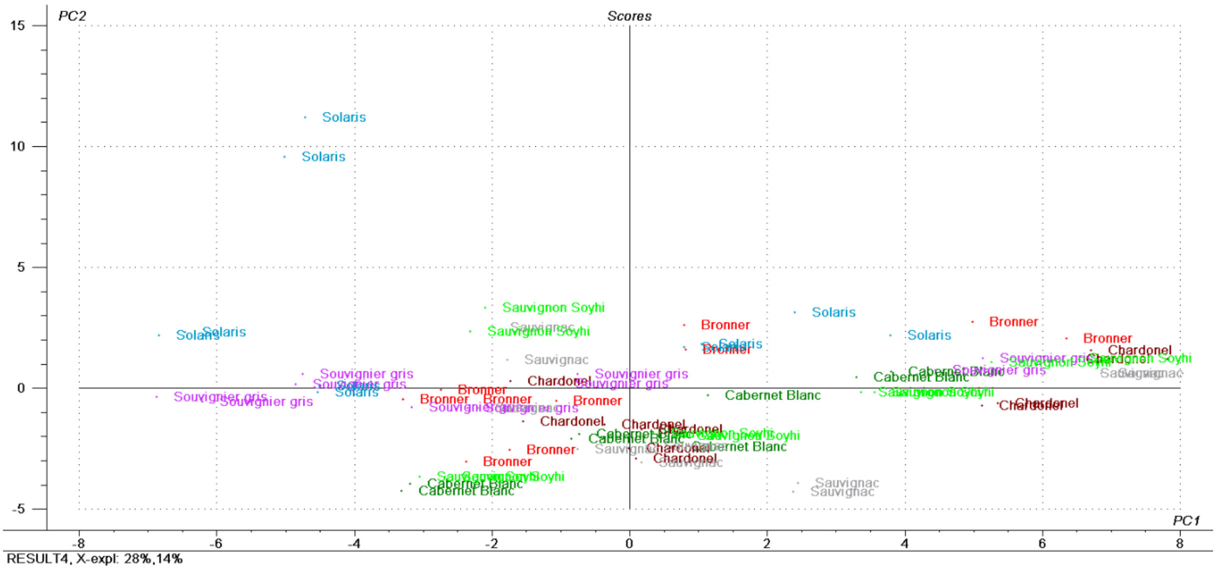


Figure 118: PCA of PIWI wines based on the peak areas of all compounds without Muscat wines, samples grouped by variety

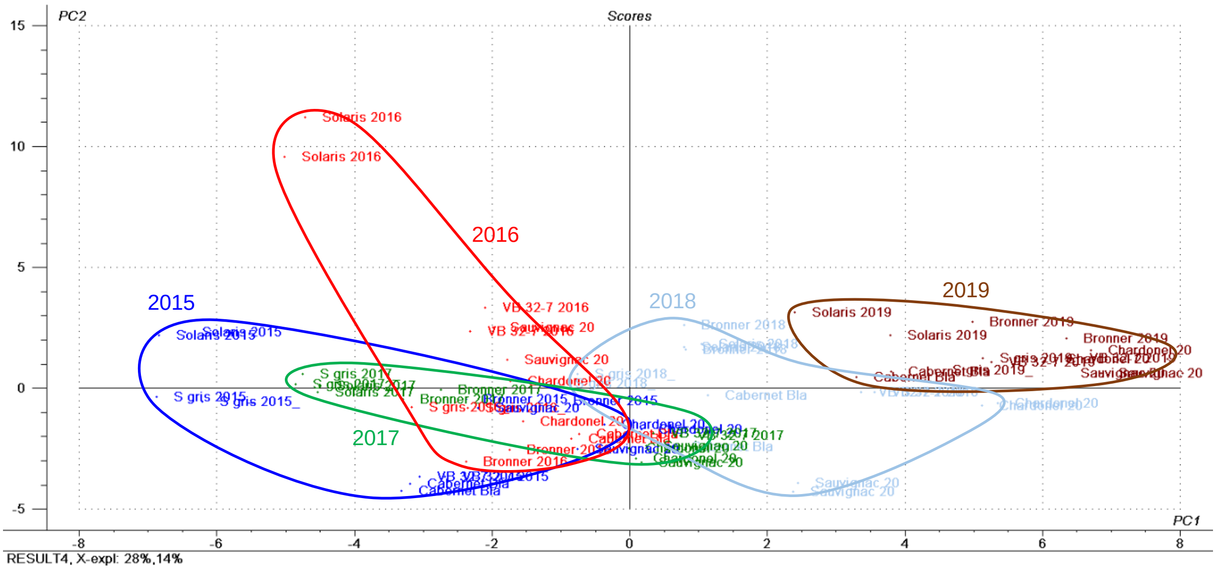


Figure 119: PCA of PIWI wines based on the peak areas of all compounds without Muscat wines, samples grouped by year, wines from the same year encircled

Based on the results of the PCA, it seems that in most cases the year has the largest impact on the overall concentration and composition of the volatile compounds analyzed in this experiment. However, in some cases, the variety can be the more important influence factor. In our case, this was true for Blütenmuskateller and Muscaris, the PIWIs with Muscat cultivars in their lineage. They had significantly higher concentrations of certain terpene compounds, compared to the other samples (Figure 120), allowing for a clear distinction independent of vintage.

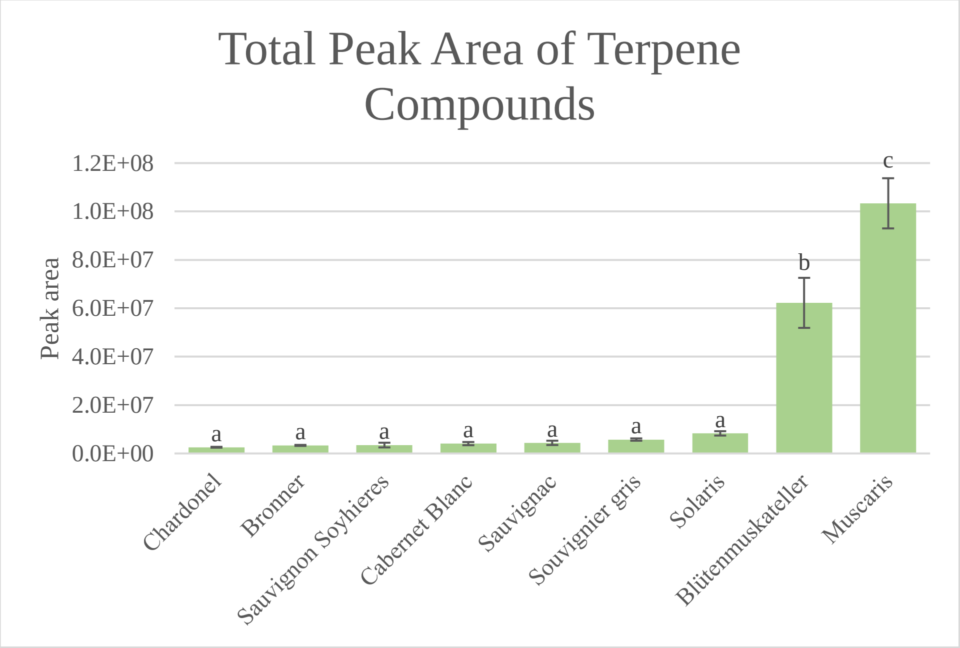


Figure 120: Statistical comparison of the total area of terpene compounds over five years by variety; Means not connected by the same letter are significantly different according to Tukey Honest Significant Difference (HSD) (p<0.05). Error bars represent standard error.

Quantification of Four Ethyl Esters and IBMP

To gain additional insight several compounds were quantified. This included the four main ethyl esters: Ethyl butanoate, hexanoate, octanoate and decanoate. As seen in the PCA experiments, esters are responsible for a majority of the aroma of a wine. It is therefore interesting to gain a deeper understanding of these compounds in the context of variety and vintage.

The other compound quantified is IBMP, a varietal compound of Sauvignon Blanc and several other varieties. While this compound can have a big impact on the overall impression of a wine due to its low sensory threshold (1 ng/L, Allen et al. 1991), it is generally present in concentrations in the ng/L range. Therefore, it is unlikely to be detectable in the aroma profile and a more sensitive method, like GC-MSMS is needed for quantification.

Table 50 lists the main and interaction effect p-values for the concentrations of the four ethyl esters and IBMP. For all compounds variety, vintage and the interaction of variety and vintage were significant with a p-value of below 0.001.

Table 50: Main and interaction effect p-values for ethyl esters and IBMP in PIWI wines

Model effect	**Ethyl butanoate [mg/L]**	**Ethyl hexanoate [mg/L]**	**Ethyl octanoate [mg/L]**	**Ethyl decanoate [mg/L]**	**Sum Esters C_4-C_{10} [mg/L]**	**IBMP [ng/L]**
Variety	**<0.001**	**<0.001**	**<0.001**	**<0.001**	**<0.001**	**<0.001**
Year	**<0.001**	**<0.001**	**<0.001**	**<0.001**	**<0.001**	**<0.001**
Variety x Year	**<0.001**	**<0.001**	**<0.001**	**<0.001**	**<0.001**	**<0.001**

The results were compared using a multivariate generalized linear model with vintage and variety as fixed factors. Tukey's Honest Significant Difference was used to detect significant differences among means (p<0.05).

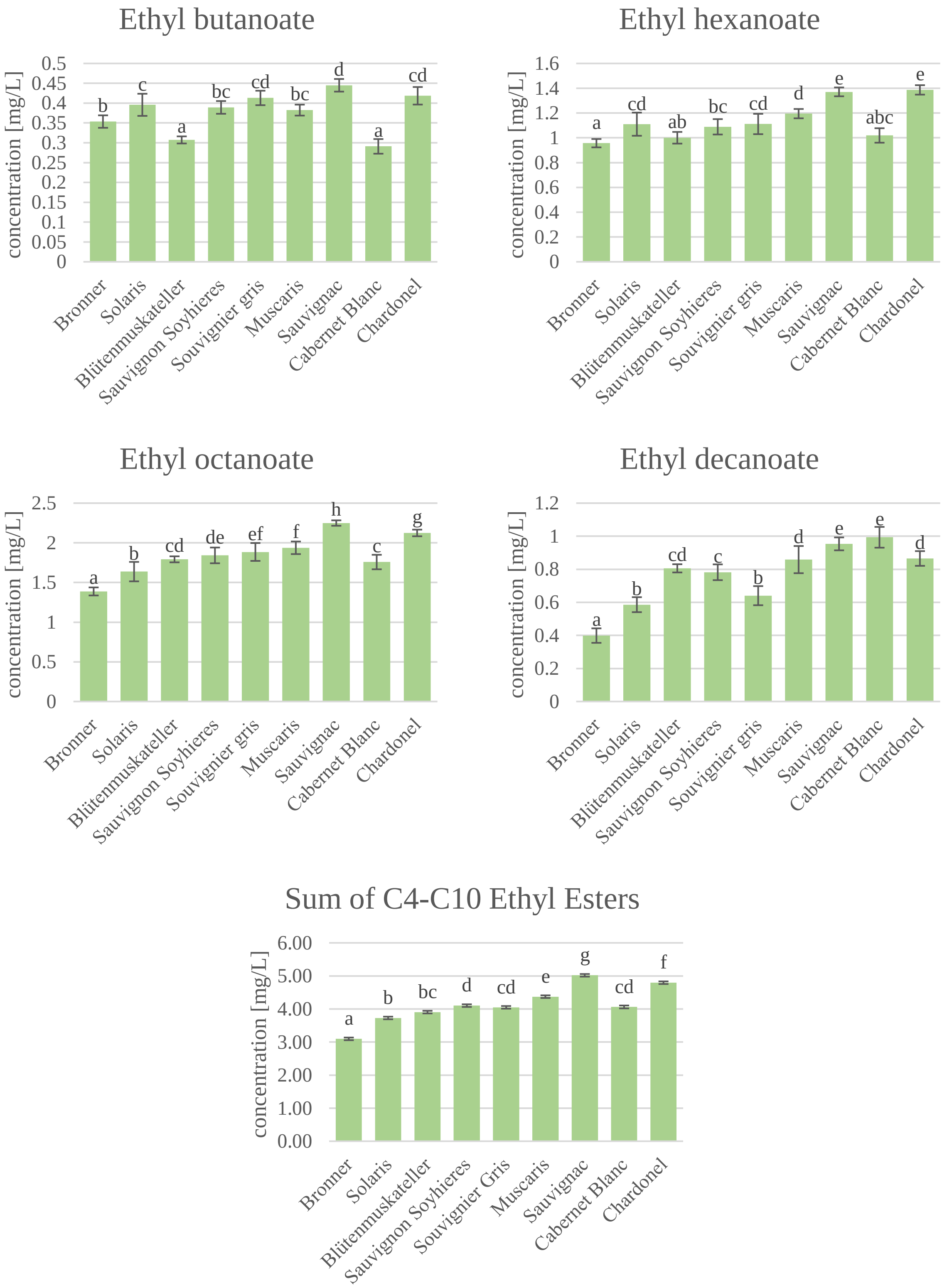


Figure 121: Statistical comparison of the concentration of ethyl esters and the sum of all four over five years by variety; Means not connected by the same letter within the same attribute are significantly different according to Tukey Honest Significant Difference (HSD) (p<0.05). Error bars represent standard error.

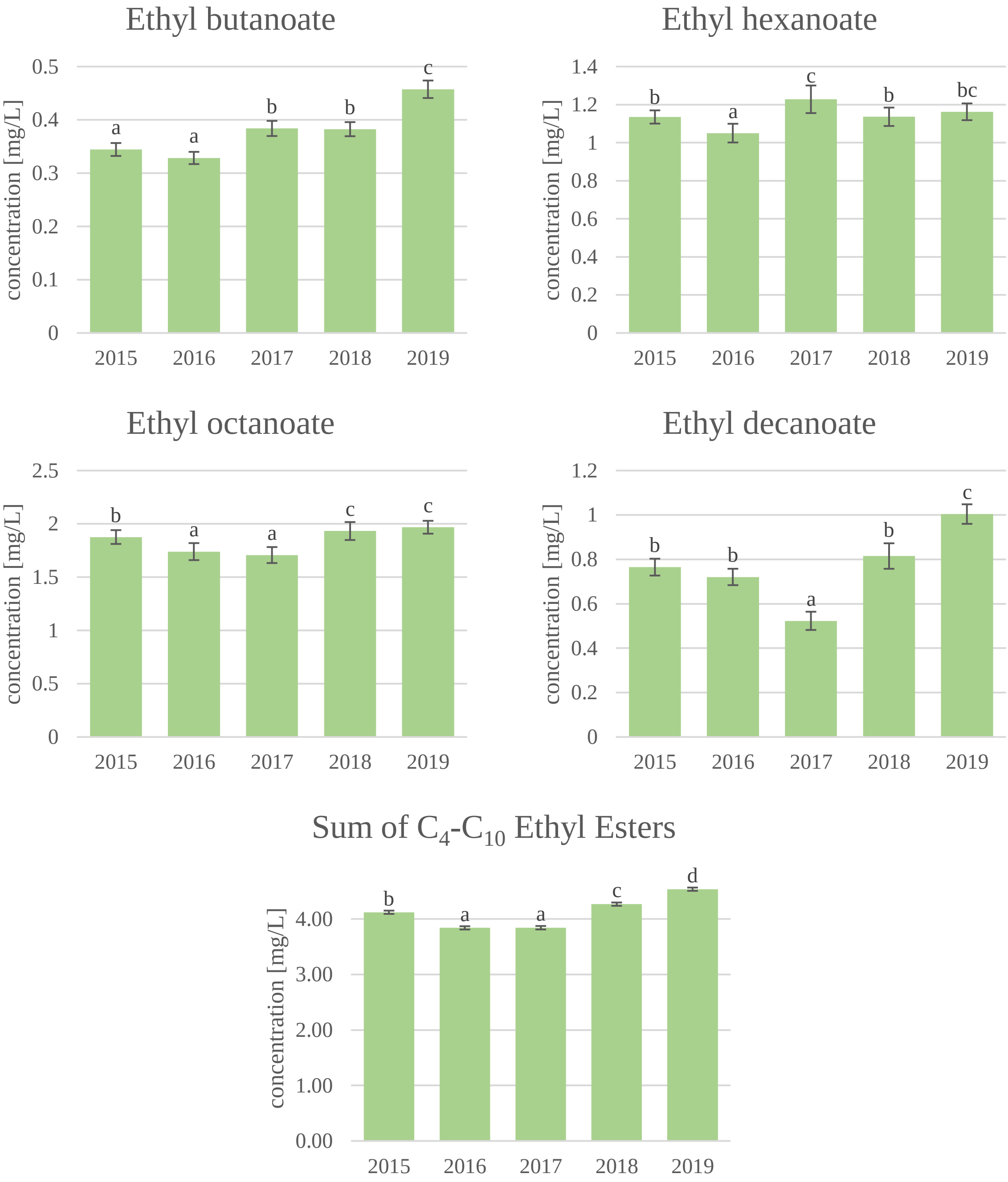


Figure 122: Statistical comparison of the concentration of ethyl esters and the sum of all four over all varieties by year; Means not connected by the same letter within the same attribute are significantly different according to Tukey Honest Significant Difference (HSD) ($p<0.05$). Error bars represent standard error.

Figure 121 and Figure 122 show the results of the statistical comparison of the ethyl ester concentrations. No clear trends emerge, neither based on variety nor for different vintage.

All wines were made using a standardized procedure and the same strain of pure culture yeast. Due to the fact that yeast and fermentation conditions have the biggest impact on the composition of secondary aroma compounds, this lack of clear differences is plausible.

Minimizing differences in the winemaking procedure allows for a better comparison of influence factors other than fermentation, like climatic conditions or variety.

Unlike the four ethyl esters, IBMP is a varietal compound and its concentration depends on factors other than fermentation. It was found in wines from five cultivars: Blütenmuskateller, Muscaris, Sauvignac, Cabernet Blanc Sauvignon and Soyhieres. Table 51 lists the mean concentrations of IBMP for those wines over five vintages. Statistical analysis was carried out in the same way as described before. The results are shown in Figure 123.

In the other four examined cultivars no IBMP was found (Bronner, Solaris, Souvignier gris and Chardonel).

*Table 51: Means of the IBMP concentration measured in the PIWIs over five years in ng/L; concentrations below sensory threshold are marked with **

	Blütenmuskateller	**Muscaris**	**Sauvignac**	**Cabernet Blanc**	**Sauvignon Soyhieres**
2015	2.0 ± 0.38	2.6 ± 0.24	3.4 ± 0.07	2.3 ± 0.04	13.6 ± 0.25
2016	2.4 ± 0.31	0.7 ± 0.09*	11.8 ± 0.46	14.1 ± 0.26	34.3 ± 0.14
2017	0.7 ± 0.27*	1.9 ± 0.33	3.7 ± 0.08	n.d.	7.5 ± 0.57
2018	1.8 ± 0.47	2.4 ± 0.49	1.4 ± 0.32	1.7 ± 0.08	13.1 ± 0.62
2019	0.5 ± 0.05*	0.7 ± 0.13*	7.6 ± 0.40	3.7 ± 0.02	12.2 ± 0.06

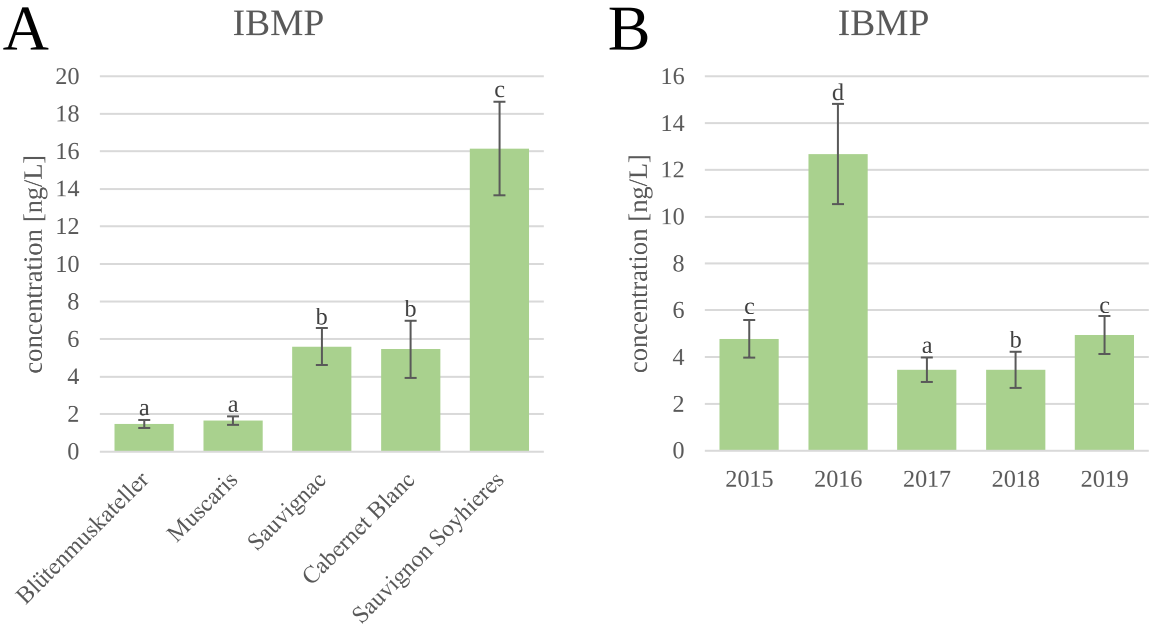


Figure 123: Statistical comparison of the concentration of IBMP over five years by variety (A) and over all varieties by year (B); Means not connected by the same letter within the same attribute are significantly different according to Tukey Honest Significant Difference (HSD) (p<0.05). Error bars represent standard error.

In the case of IBMP clear differences between varieties are detectable. The highest concentrations were found in Sauvignon Soyhieres followed by Sauvignac and Cabernet Blanc. All of these PIWIs have Sauvignon varieties in their pedigree. IBMP was also found in the two varieties with Muscat cultivars in their linage.

When looking at the concentration in context of the year, one year (2016) shows significantly higher values compared to the other 4, with more than double the mean concentration.

According to the harvest data, there is one distinctive difference between this year and the others: In 2016 a late frost occurred at the end of April with a temperature of down to -6°C, snow and hail (Weninger 2016). This resulted in 61% reduction in the overall wine production in Styria compared to 2015 (Statistik Austria 3/3/2017). If and how this is related is beyond the scope of this work.

The results demonstrate the different impacts of primary and secondary aroma compounds. The latter generally make up the majority of volatile compounds in wines. However, when the impact of fermentation on the diversity between wines is minimized by using standardized winemaking methods, primary or varietal compounds are responsible for the differences between wines from different cultivars and vintages.

Comparison of Sensory and Instrumental Results

The sensory evaluation of the PIWI wines was conducted by Wolfgang Renner (Department of Fruit Growing and Enology of the Agricultural Research Center Styria) and are reproduced here with permission.

The results of the sensory analysis were compared to a PCA of the relevant wines using the OAVs of the compound groups calculated based on the peak area of methyl nonanoate. The wines were rated based on their similarity to characteristics of traditional Styrian white wines, like Welschriesling, Pinot Blanc, Sauvignon Blanc and Muscat wines. Spider plots are used to visualize the results. In the PCAs, sample groups are classified by their similarity to a certain wine type.

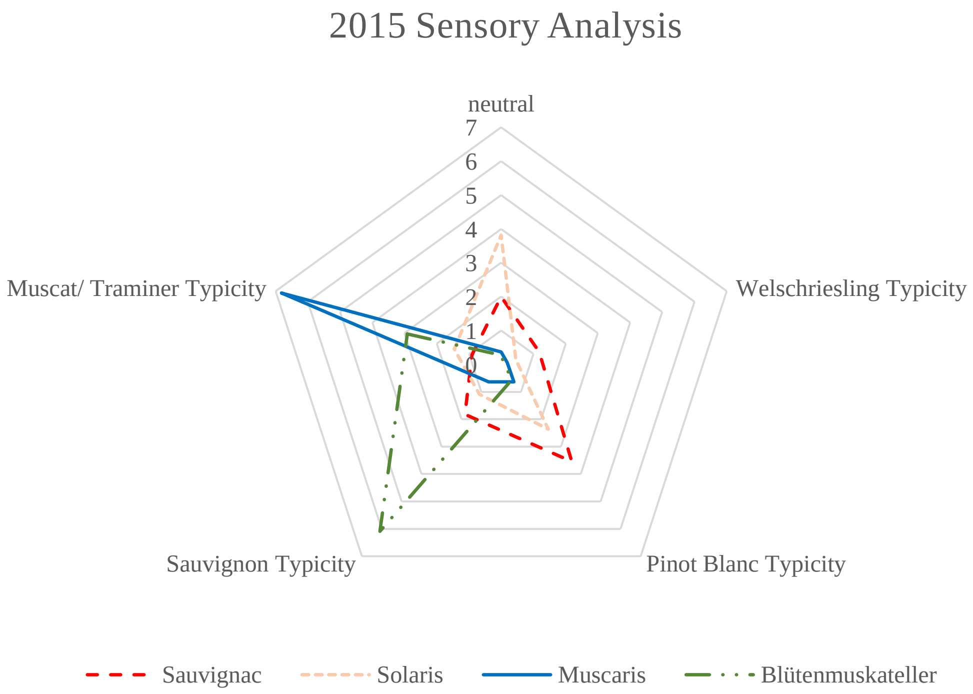


Figure 124: Spider plot of the results of the 2015 sensory analysis

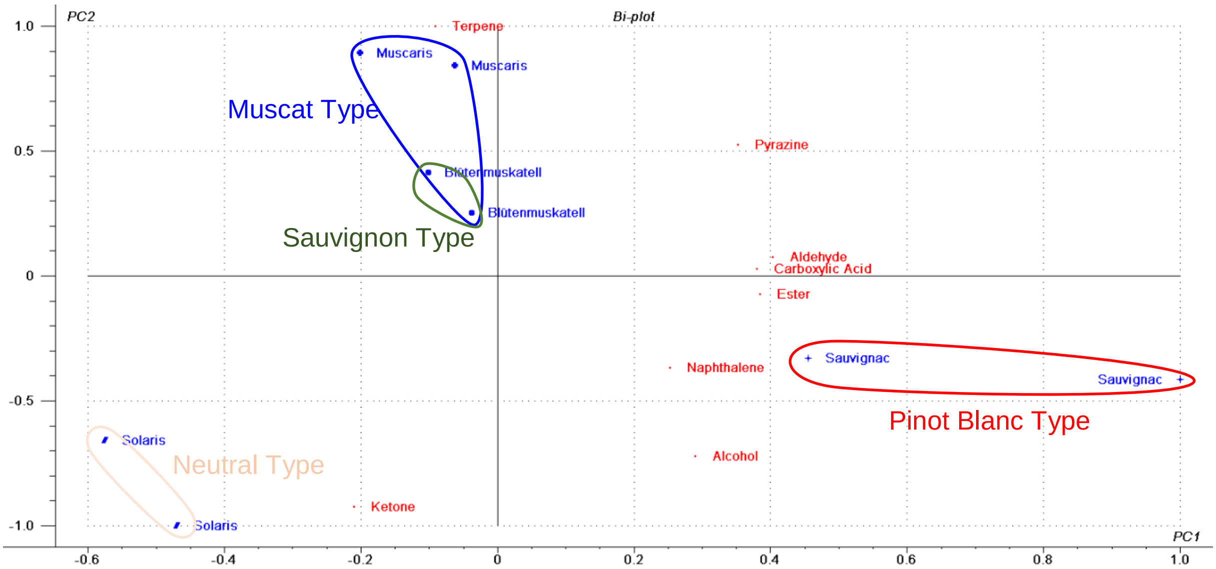


Figure 125: Scores and Loadings PCA plot of the PIWI wines included in the 2015 sensory analysis, groups based on sensory analysis encircled, based on the OAVs of the compound groups (calculated from concentrations based on methyl nonanoate)

Figure 124 shows the results of the blind tasting. Two wines were rated to exhibit similarities to Muscat and Traminer wines: Blütenmuskateller and Muscaris. Blütenmuskateller additionally exhibited Sauvignon typicity. Sauvignac wines were rated to exhibit Pinot Blanc characteristics. Similar trends could be observed in the PCA (Figure 125), with Muscaris and Blütenmuskateller clustering together in the same quadrant. This can be explained by the higher terpene concentration of these cultivars. They were separate from the other two wines.

Overall, a good correlation between the sensory and the instrumental results was found.

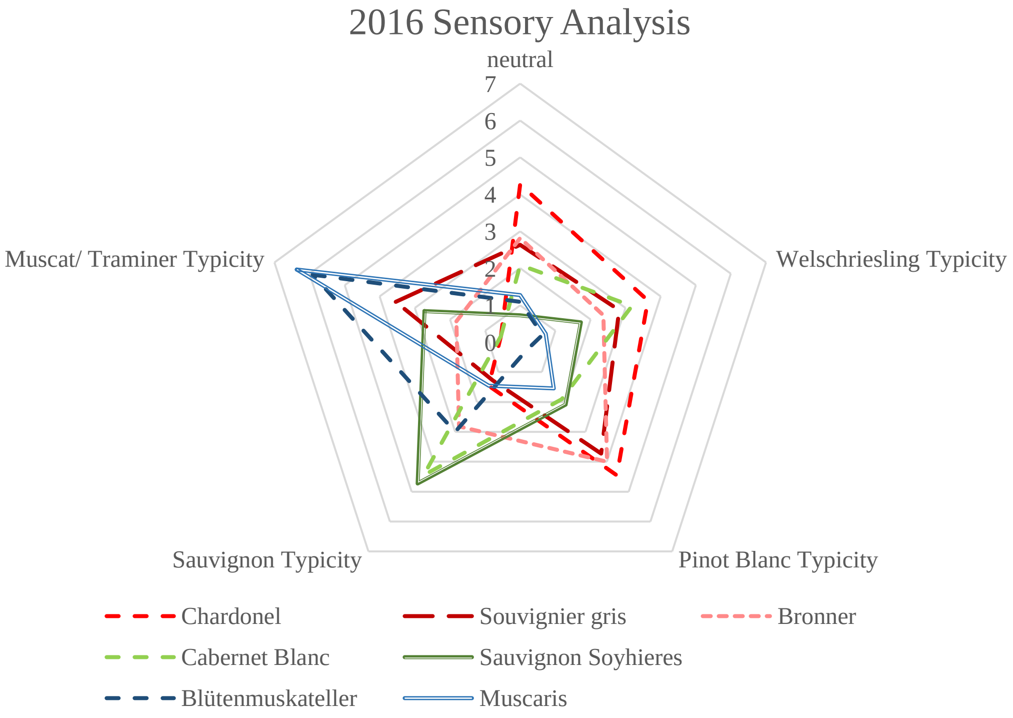


Figure 126: Spider plot of the results of the 2016 sensory analysis, wines of the same group marked by same color scheme

The 2016 sensory analysis included more wines than the 2015 one. To avoid a lack of clarity, wines with similar characteristics are displayed in matching color palates (Figure 126). In addition subplots of the groups based on the results of the sensory analysis are depicted in Figure 127. As in 2015, Muscaris and Blütenmuskateller were rated as Muscat/ Traminer type wines. Cabernet Blanc and Sauvignon Soyhieres were rated to exhibit Sauvignon type wines. Three wines, Bronner, Souvignier gris and Chardonel, were rated to exhibit Pinot Blanc characteristics.

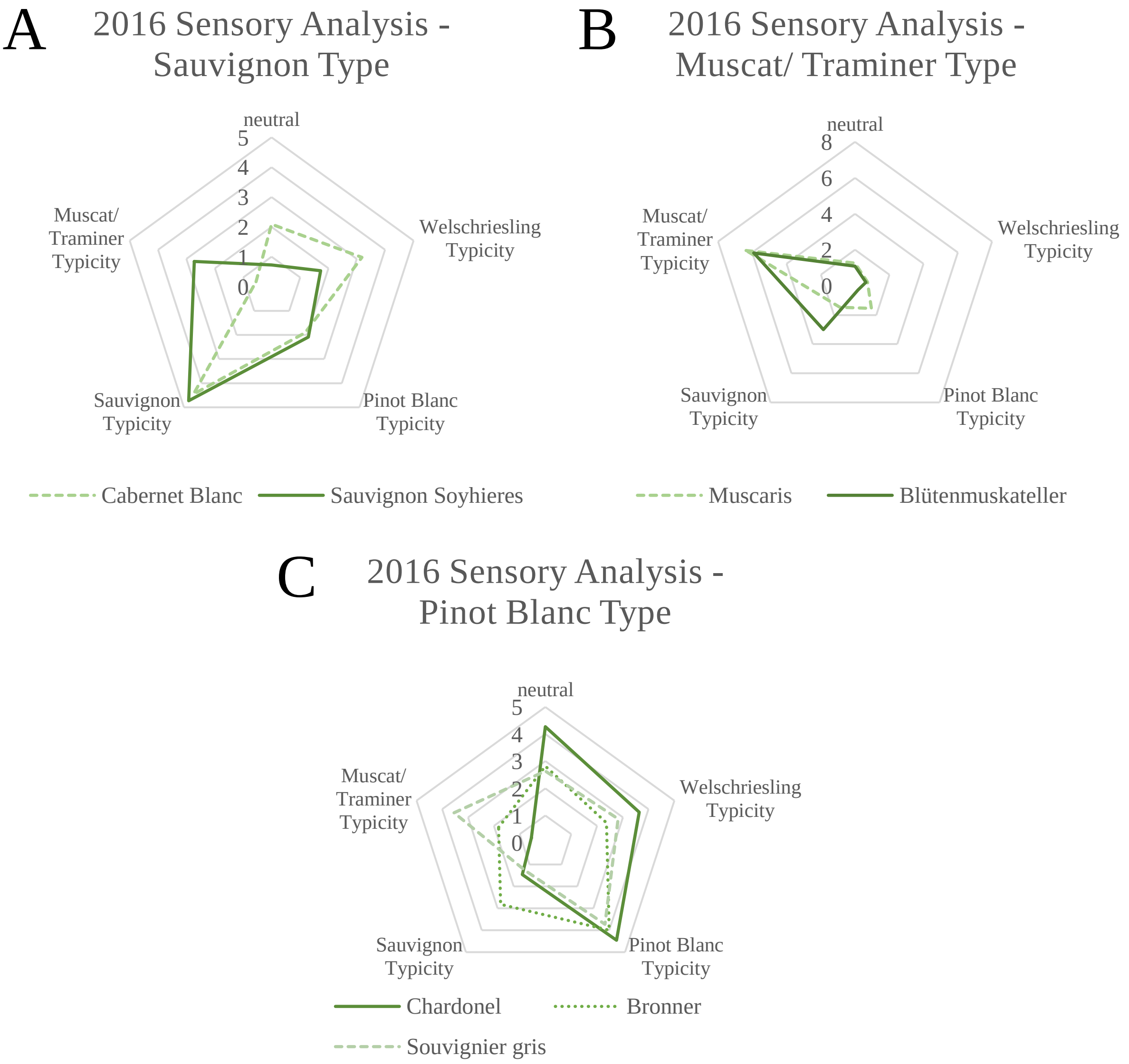


Figure 127: Spider plots of the individual groups (A: Sauvignon Type, B: Muscat/ Traminer Type, C: Pinot Blanc Type) of PIWIs based on the highest scores in the sensory analysis 2016

While the trends are not as clear as in the previous year, they are discernible in the PCA (Figure 128). Wines exhibiting similar characteristics in the sensory evaluations are encircled in the PCA. The Muscat type wines are located in the same quadrant, while the other groups share at least one of the principal components (PC2). Both Sauvignon type wines have IBMP concentrations above the sensory threshold, however, with an OAV of 34 it is more pronounced in Sauvignon Soyhieres than in Cabernet Blanc (OAV 14).

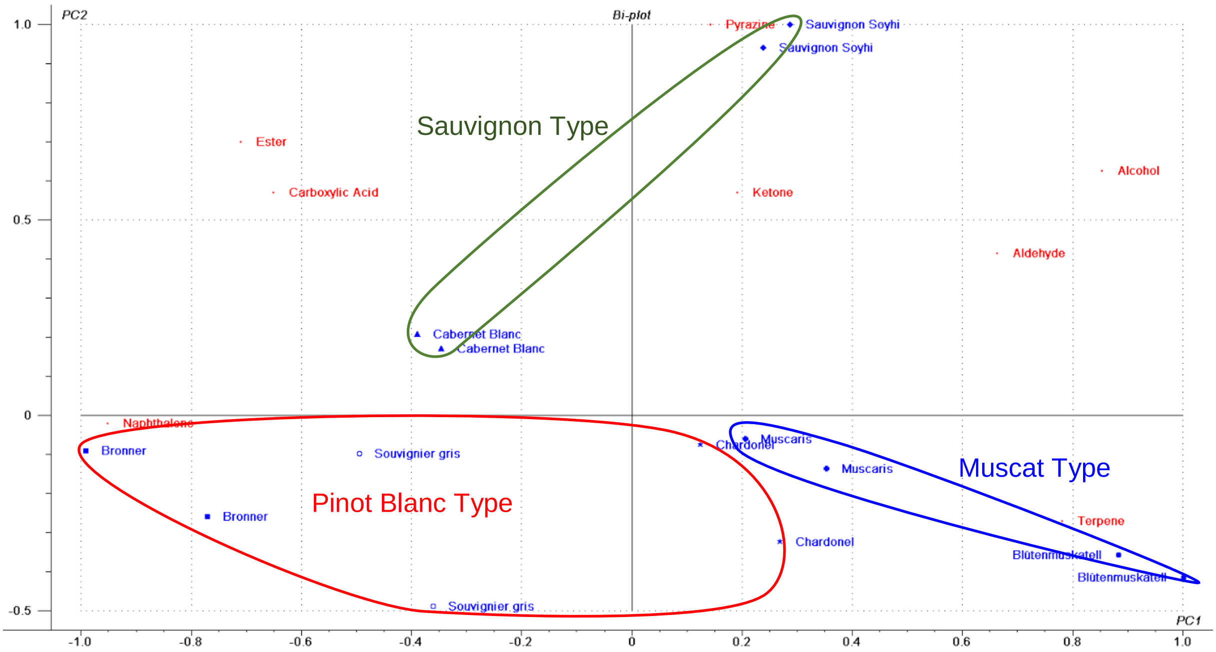


Figure 128: Scores and Loadings PCA plot of the PIWI wines included in the 2016 sensory analysis, groups based on sensory analysis encircled, based on the OAVs of the compound groups (calculated from concentrations based on methyl nonanoate)

In the 2017 sensory analysis wines were additionally assessed based on their Riesling typicity. For more clarity, wines with similar characteristics are again marked with lines in a matching color pattern in the spider plot (Figure 129) and shown in sub-plots (Figure 130). Sauvignac scored the same in Pinot Blanc and Sauvignon typicity, it is therefore depicted in two colors.

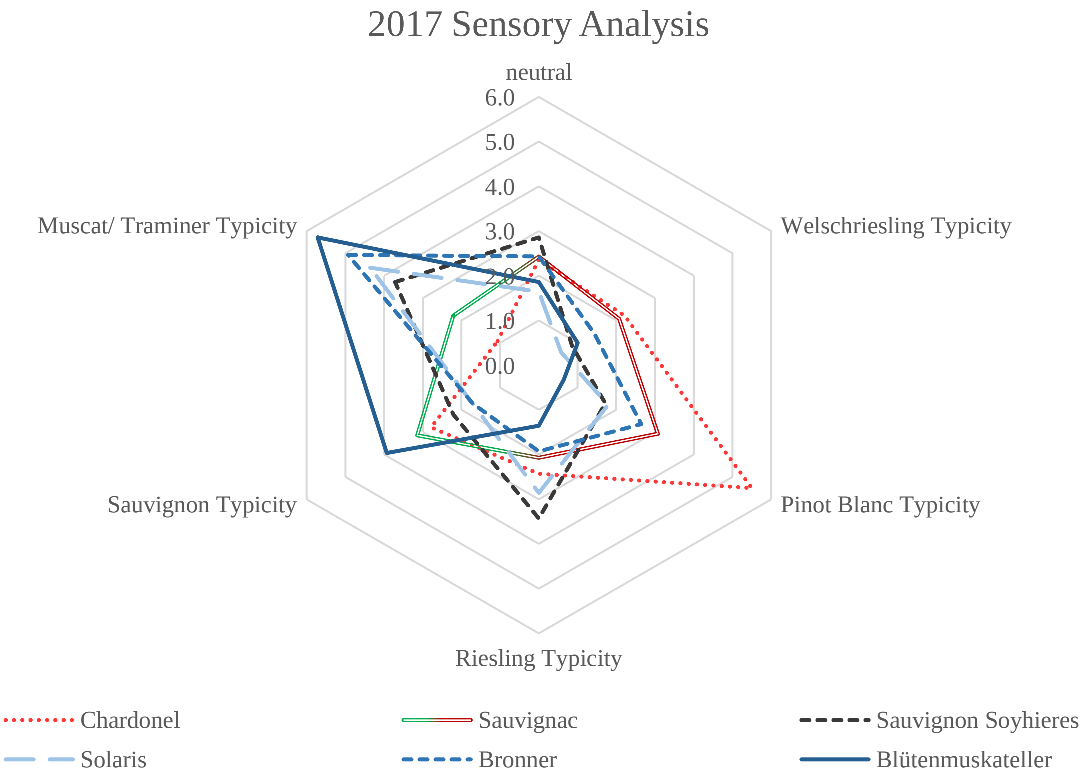


Figure 129: Spider plot of the results of the 2017 sensory analysis, wines of the same group marked by same color scheme

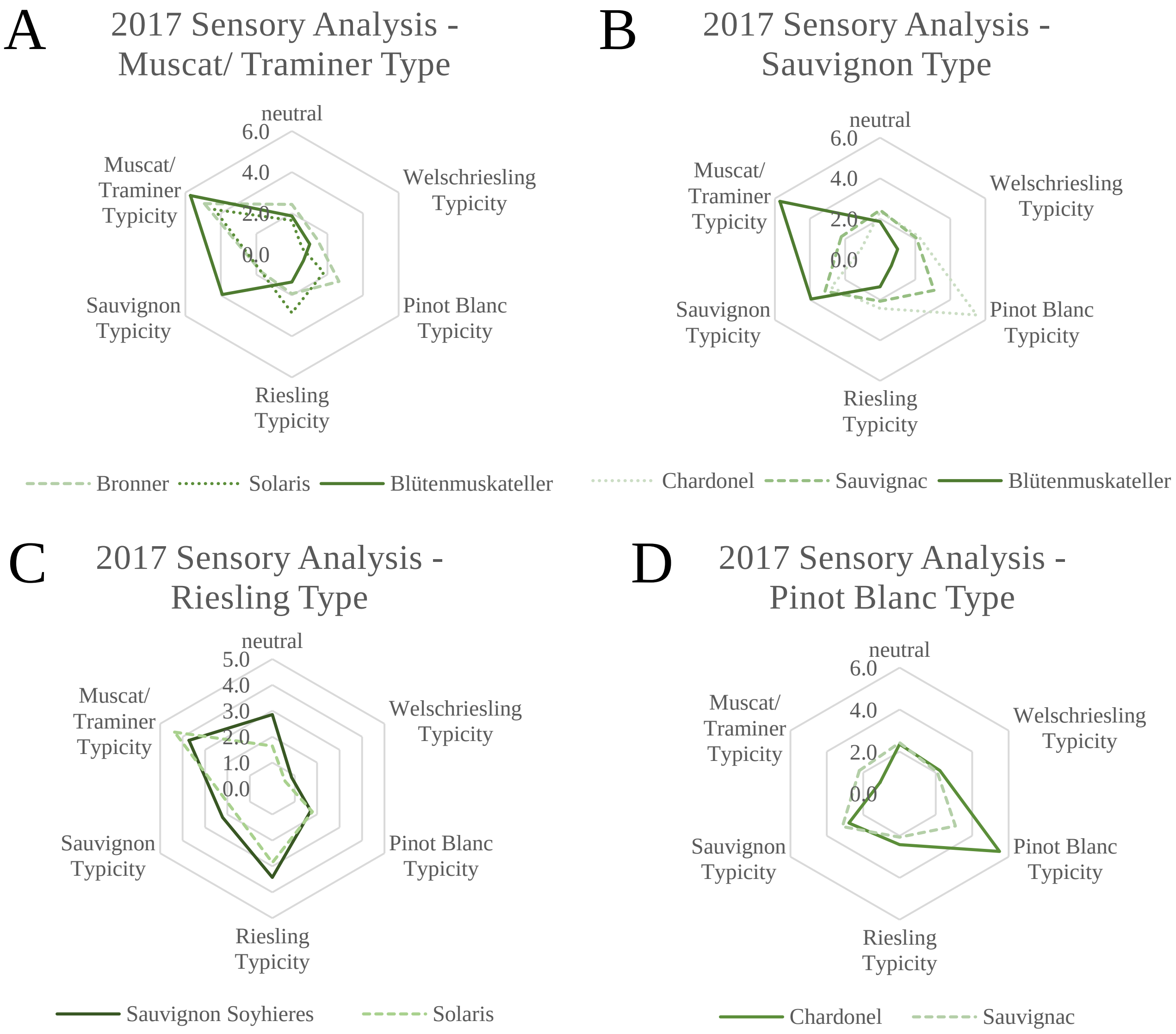


Figure 130: Spider plots of the individual groups (A: Muscat/ Traminer Type, B: Sauvignon Type, C: Riesling Type, D: Pinot Blanc Type) of PIWIs based on the highest scores in the sensory analysis 2017

Figure 131 shows the scores and loadings PCA plot of the wines assessed in the 2017 sensory analysis. Wines rated to exhibit similar characteristics are marked. Pinot Blanc type wines (Sauvignac and Chardonel) were located in proximity to each other. In addition, they, together with Blütenmuskateller, showed the Sauvignon typicity in the sensory analysis. All three align along PC1. Sauvignon Soyhieres and Solaris were the wines with the highest Riesling typicity scores. In the PCA of the OAVs, they are relatively close to each other, compared to their proximity to other samples.

The samples with the highest Muscat/ Traminer scores (Blütenmuskateller, Bronner and Solaris) did not cluster in the PCA.

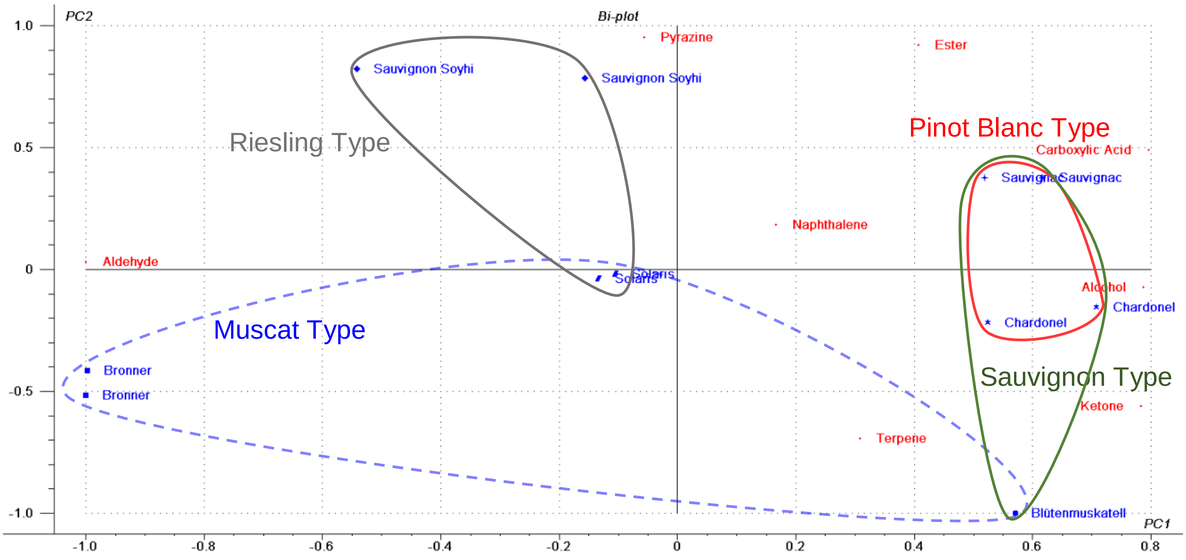


Figure 131: Scores and Loadings PCA plot of the PIWI wines included in the 2017 sensory analysis, groups based on sensory analysis encircled, based on the OAVs of the compound groups (calculated from concentrations based on methyl nonanoate)

The results of the 2018 sensory analysis are visualized in Figure 132. The color scheme is according to the typicity with the highest respective scores. For more clarity, Figure 133 shows the sub-plots of the two samples with the highest scores for each characteristic. Due to the fact that no wine's highest score was for the Muscat/ Traminer typicity, this sub group is not depicted.

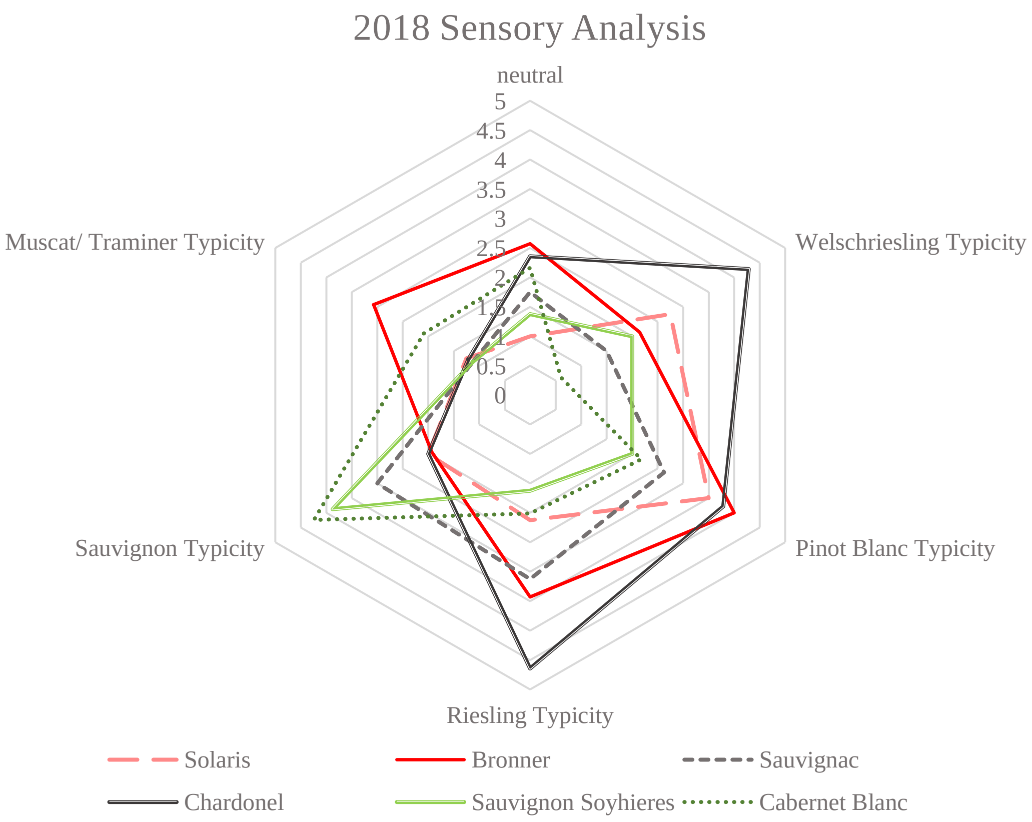


Figure 132: Spider plot of the results of the 2018 sensory analysis, wines of the same group marked by same color scheme

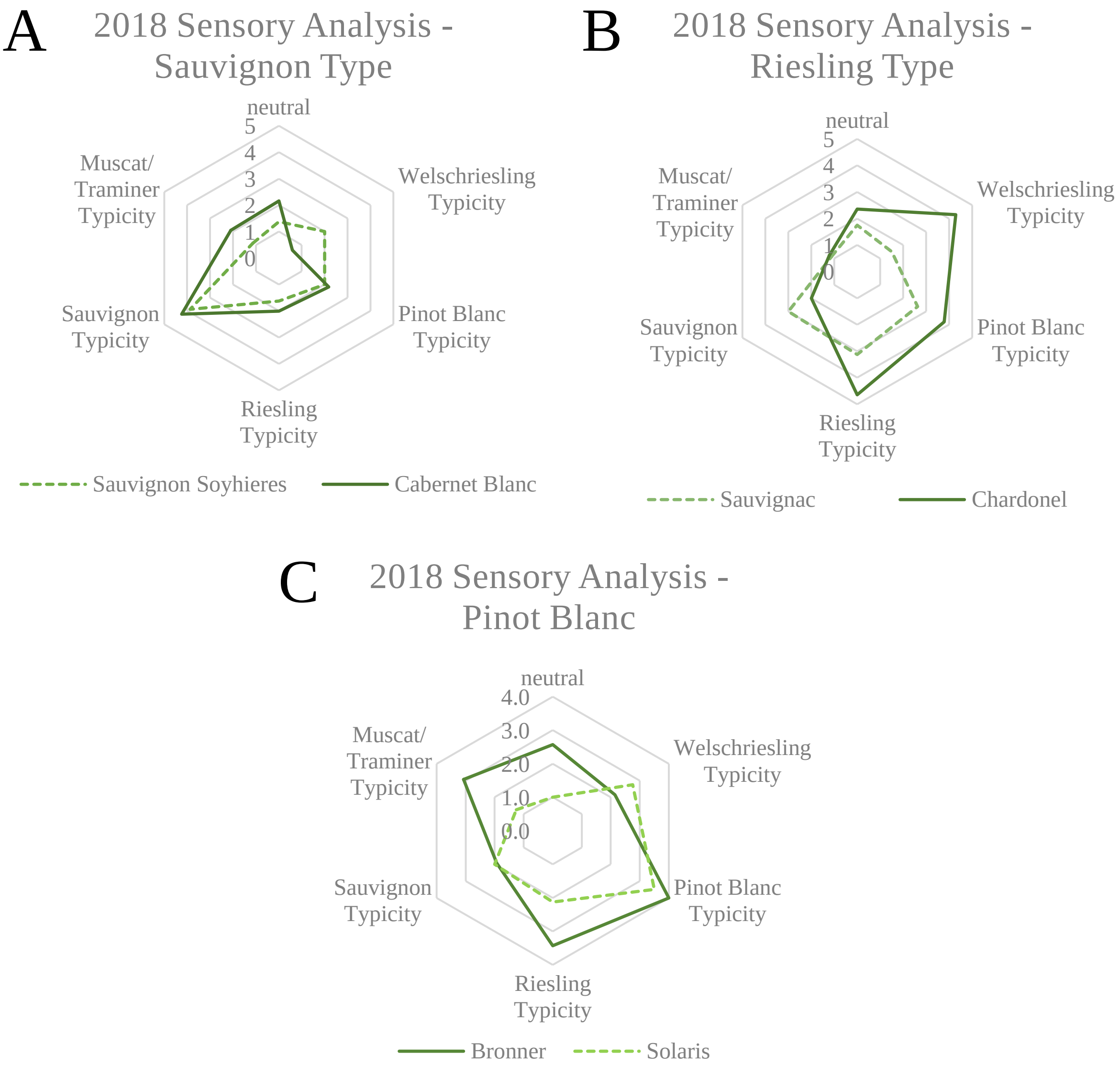


Figure 133: Spider plots of the individual groups (A: Sauvignon Type, B: Riesling Type, C: Pinot Blanc Type) of PIWIs based on the highest scores in the sensory analysis 2018

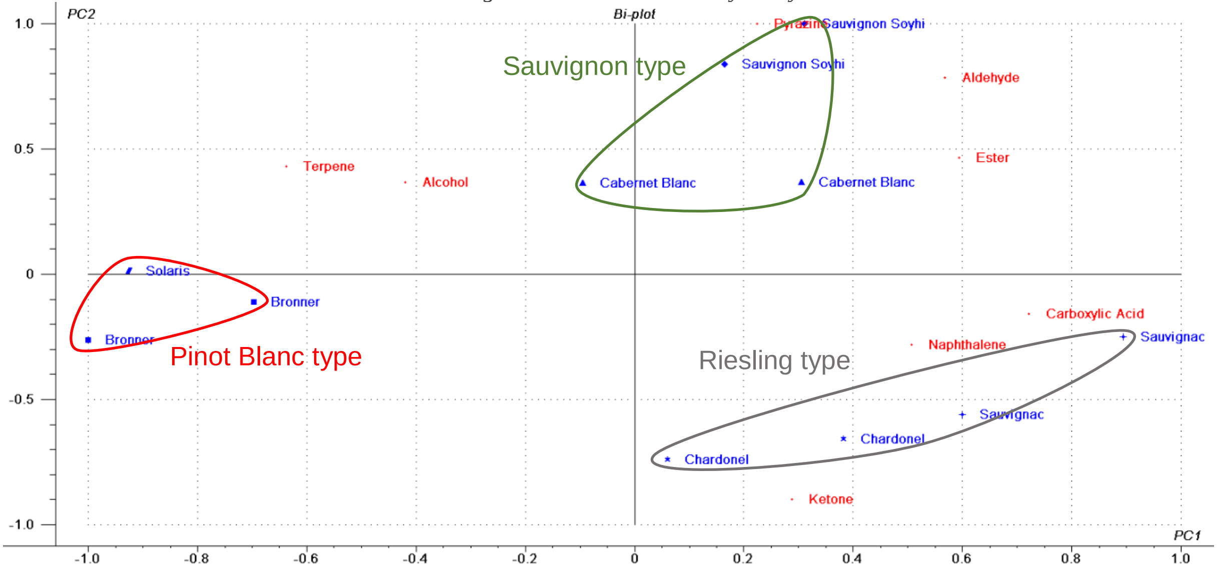


Figure 134: Scores and Loadings PCA plot of the PIWI wines included in the 2018 sensory analysis, groups based on sensory analysis encircled, based on the OAVs of the compound groups (calculated from concentrations based on methyl nonanoate)

Figure 134 show the PCA results of the wines assessed in the 2018 sensory analysis. Samples are grouped based on the sub groups in Figure 133. Cabernet Blanc and Sauvignon Soyhieres are part of the Sauvignon type cluster, Bronner and Solaris form the Pinot Blanc cluster and Sauvignac and Chardonel the Riesling type cluster. No wine highest scoring descriptor was Muscat/ Traminer. One of the reasons for this could be that neither of the Muscat PIWIs were included in the sensory analysis.

Overall, there was a good overlap between the results of the sensory analysis and those of the PCAs. The best results to that effect, were those of the PIWIs with Muscat cultivars in their pedigree (Blütenmuskateller and Muscaris) as well as the PIWI cultivars with higher IBMP concentrations. This was particularly true for Sauvignon Soyhieres and Cabernet Blanc, which scored high on Sauvignon typicity in the sensory evaluations, with the exception of 2017. In this year no wine’s highest score was based on its Sauvignon characteristics. One reason for this could be the fact that the overall IBMP concentrations were the lowest that year (Figure 123 B). In addition, Cabernet Blanc wines from that vintage were not included in the sensory analysis or available for instrumental analysis.

In the end, wine is a natural product and while a standardization of the wine making process can lower the influence of fermentation, this is not the case for the raw material. The composition of volatile compounds, especially primary aroma compounds, depends on many factors including the climatic conditions, which change from year to year. This results in differences between the wines from the same cultivars from different years which can be observed, both in the sensory and the instrumental data.

Conclusion for the Characterization of Styrian PIWIs

In conclusion, the identification and quantification of certain volatile compounds can help to predict the sensory characteristics of a wine. For this it is important to know what the main character impact odorants for specific varieties are in order to adjust the analytical method. For some compounds that are present in higher concentrations and higher odor thresholds, like terpenes or esters, GC-MS is sufficient. This is especially true for wines produced from cultivars with high amounts of varietal compounds. This was demonstrated in the first part of the experiment, where the PIWIs with Muscat heritage could be distinguished from the others based on their terpene compounds (Figure 116, Figure 117 and Figure 120).

For other compounds with lower threshold concentrations, like pyrazines, more sensitive methods are needed to allow a more nuanced insight. The concentration of IBMP, which was determined using GC-MSMS, allowed for the identification of a second group of wines with Sauvignon characteristics. In five out of the nine PIWIs, above threshold concentrations were found. Among these, three groups divided based on the mean IBMP concentration were observed, one with the two Muscat cultivars, one with Cabernet Blanc and Sauvignac and a third group with Sauvignon Soyhieres.

Another factor that has to be taken into account is the influence of the fermentation conditions on the final wine. Looking at the overall distribution of peak areas and OAVs (Table 48) the most influential compound groups are secondary aroma compounds, like esters, carboxylic acids and higher alcohols. This shows the large impact the winemaking process itself has. In this project, a standardized winemaking procedure was used. And while statistical differences in the concentration of four quantified ethyl ester between the cultivars and vintages were found (Figure 121 and Figure 122), those did not follow a clearly discernable pattern, unlike in the case of IBMP. This further illustrates the difference in the impact of primary and secondary compounds have on the PIWI wines in this project.

A comprehensive characterization of the wines can help to raise awareness for these new cultivars among both, winemakers and consumers. For the first group the cultivation of PIWIs can have some advantages, like a reduced need for plant protection agents and the connected reduction of work. However, planting new cultivars also requires a significant investment, of both, time and money. Characterization efforts can help to gain a better understanding of the product and in turn facilitate the winemakers as they get comprehensive information on what to expect from their wines. When it comes to consumers, most people are likely to buy what they know. Being able to describe wines from new cultivars can help them find wines with similar characteristics to those from traditional varieties and lower negative preconceptions.

10. Conclusion and Outlook

The main goal of this thesis was to examine different facets of wine using mostly gas chromatography-based analysis techniques. In addition, the impact of viti- and vinicultural choices on the final product was examined in several case studies. Over the course of the work, several new methods were developed or improved.

Aroma is one of the most important quality indicators of wine. Understanding how it is influenced by choices made in vineyard and cellar is important for winemakers, who want to provide a high-quality product. In addition, the chemical analysis of wine can be used to identify compounds responsible for off-flavors and can help to uncover fraud. Gas chromatographical methods have long been a standard tool for this. The choice of the appropriate method depends on the analytical question. In some cases, for example to gain an overview of the composition of the volatile compounds, less sensitive methods, like GC-MS in full scan mode can be used. However, when it comes to off-flavor causing compounds, which often can be detected by the human nose at very low concentrations, more selective and sensitive methods have to be chosen. In this work, methods with varying properties were used to gain a better understanding of wine as a product and some of the factors responsible for its final aroma.

The influence of nature and nurture on the product was examined using Riesling wines produced by three vintner from three regions, known for high quality wines of this variety. With the exception of one origin, the vinification had the more significant impact in this experiment. Therefore, it is useful to keep fermentation and maturation parameters relatively constant when the impact of viticultural techniques is supposed to be examined.

However, in some cases these wine style-based differences are what distinguishes wines of different origins from each other. Using several different analytical techniques, including elemental and aroma analysis, it was possible to differentiate between sparkling wines from three countries. This possibility to distinguish wines from different origins based on their chemical composition has previously been used to identify wine fraud.

In addition, the sparkling wines were analyzed for the presence of two off-flavor compounds, 2,4,6-Trichloroanisole and 2,4,6-Tribromoanisole. As both compounds can be detected by consumer in very low concentrations of only a few ng/L (ppt) in wine, especially sensitive and selective methods are required. As part of this thesis an easy and reliable method was developed and optimized. This final method can be readily used for the routine analysis of TCA and TBA in wine samples and can also be applied to matrices other than wine.

When examining the impact of viticultural factors on primary (varietal) aroma compounds, another possibility to minimize influences is the used of unfermented grape juice or must. This approach was used to study 2-isobutyl-3-methoxypyrazine. This compound is characteristic for cool climate Sauvignon Blanc and its concentration remains stable over the course of fermentation. The findings from the experiments are in agreement with previous studies, which link the concentration to the overall ripeness of the grapes as it decreases over the ripening process. It was also shown that the concentration depends on the individual growing conditions and can vary from year to year. This yearly variation was further illustrated in the wine from fungus resistant grape varieties.

Using different instrumental analytical methods is necessary to gain a deeper understanding of wine as a product and how its aroma can change, depending on several factors. A combination with statistical tools, like principal component analysis, can help to illustrate the results and simplify them, especially when dealing with large amounts of data. For example, in the analysis of wines from PIWI cultivars, this combination allowed the differentiation of wines with Muscat heritage from the others, based on a higher concentration of terpenes. In combination with additional data, like the IBMP concentration, the results of the instrumental analysis were consistent with those of sensory evaluation of the same wines. A comprehensive instrumental analysis of wines can therefore help to describe the sensory impression of certain wines.

In conclusion, it was shown that instrumental analytical techniques can help to understand the impact of viti- and vinicultural measures on the final product in an objective and reliable fashion. The use of suitable techniques can also be used to detect wine faults and fraud.

Wine is a product that has fascinated humans for millennia. For winemakers it is crucial to understand the potential of their products and communicate it to their customers. Sound analytical techniques in combination with sensory analysis can facilitate this. Since also the wine industry is facing challenges associated with climate change, the use of more resistant varieties will get more important in the near future. To support winemakers to fulfill consumer expectations, especially when it comes to newer, less well-known varieties, methods to comprehensively analyze wines will be just as important in the future as they are today.

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Appendix

Table 52: Results of the ICP OES measurements of the Wurzelwerk Riesling wines, in [mg/L]

	1	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**
Al 167.078	0.20 ± 0.01	0.18 ± 0.00	0.09 ± 0.01	0.23 ± 0.00	1.08 ± 0.01	0.14 ± 0.00	0.11 ± 0.00	0.72 ± 0.01	0.72 ± 0.01
B 249.773	3.08 ± 0.04	2.52 ± 0.03	2.26 ± 0.01	2.55 ± 0.06	2.35 ± 0.02	2.04 ± 0.03	2.55 ± 0.03	2.37 ± 0.04	2.06 ± 0.04
Ca 183.801	83.4 ± 1.61	81.3 ± 2.12	78.1 ± 1.88	78.1 ± 4.76	82.6 ± 1.40	60.9 ± 18.0	75.0 ± 4.12	61.6 ± 1.41	69.8 ± 1.34
Fe 238.204	0.41 ± 0.00	0.56 ± 0.01	0.21 ± 0.00	0.50 ± 0.00	1.12 ± 0.01	0.26 ± 0.00	0.29 ± 0.00	0.77 ± 0.01	0.38 ± 0.00
K 766.491	505 ± 8.95	457 ± 4.41	426 ± 2.83	469 ± 2.29	390 ± 6.21	349 ± 5.26	460 ± 2.48	373 ± 4.22	338 ± 1.94
Mg 285.213	90.0 ± 1.52	85.9 ± 1.31	89.5 ± 0.44	84.9 ± 0.77	84.0 ± 1.36	82.5 ± 0.52	84.8 ± 0.63	88.3 ± 0.77	82.3 ± 0.71
Mn 257.611	1.26 ± 0.01	1.51 ± 0.02	1.59 ± 0.01	0.88 ± 0.00	1.04 ± 0.01	0.64 ± 0.01	0.61 ± 0.01	0.67 ± 0.00	0.44 ± 0.00
Na 589.592	41.3 ± 3.13	39.2 ± 0.63	33.5 ± 0.59	30.3 ± 0.59	38.0 ± 0.63	26.9 ± 0.99	29.0 ± 0.35	38.2 ± 0.35	30.8 ± 0.28
P 177.495	301 ± 6.40	272 ± 3.76	262 ± 1.31	269 ± 3.33	158 ± 2.88	149 ± 1.48	238 ± 2.27	156 ± 2.17	113 ± 1.28
S 180.731	123 ± 2.17	119 ± 0.45	144 ± 1.55	110 ± 2.53	87. ± 1.01	109 ± 1.18	142 ± 1.24	165 ± 4.01	163 ± 3.44
Zn 213.856	0.87 ± 0.01	1.03 ± 0.04	0.87 ± 0.01	0.99 ± 0.01	0.97 ± 0.01	0.93 ± 0.01	0.74 ± 0.03	0.89 ± 0.03	0.52 ± 0.01

Table 53: Results of the ICP MS measurements of the Wurzelwerk Riesling wines, in [µg/L]

	1	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**
^{47}Ti	1007 ± 42.6	998 ± 30.4	1005 ± 19.1	1072 ± 20.3	707 ± 8.45	693 ± 11.3	1041 ± 21.8	765 ± 8.98	595 ± 2.24
^{58}Ni	20.8 ± 0.44	18.1 ± 0.20	16.7 ± 0.24	21.8 ± 1.16	25.5 ± 0.55	14.1 ± 0.66	22.1 ± 0.29	20.7 ± 0.97	14.0 ± 0.53
^{59}Co	1.37 ± 0.05	1.94 ± 0.03	0.86 ± 0.03	1.64 ± 0.07	7.61 ± 0.10	1.50 ± 0.02	1.76 ± 0.07	6.07 ± 0.08	2.67 ± 0.02
^{60}Ni	21.4 ± 0.15	18.3 ± 0.29	18.0 ± 0.15	22.9 ± 0.73	26.8 ± 0.38	14.8 ± 0.78	23.5 ± 0.40	22.1 ± 0.92	15.7 ± 0.38
^{118}Sn	18.1 ± 0.27	12.9 ± 0.24	14.9 ± 0.18	17.7 ± 0.68	12.7 ± 0.30	10.7 ± 0.38	13.8 ± 0.34	13.2 ± 0.28	14.8 ± 0.52
^{206}Pb	41.5 ± 1.18	36.1 ± 0.49	33.6 ± 0.63	35.4 ± 0.82	23.2 ± 0.65	12.0 ± 3.25	16.6 ± 0.92	15.0 ± 3.82	8.96 ± 0.26
^{207}Pb	48.1 ± 1.59	41.9 ± 0.62	39.1 ± 0.52	41.2 ± 0.98	26.3 ± 0.97	13.9 ± 3.85	19.2 ± 0.98	16.9 ± 4.40	10.1 ± 0.26
^{208}Pb	46.0 ± 1.24	40.1 ± 0.71	37.6 ± 0.49	39.8 ± 0.60	25.6 ± 1.01	13.4 ± 3.68	18.4 ± 0.96	16.5 ± 4.26	9.94 ± 0.22

Table 54: Peak areas of volatile organic compounds of the Wurzelwerk Riesling wines 1-6; RSD in %

	1	2	3	4	5	6
Butanoic acid	1099271 ± 9.28	1780760 ± 5.87	1516556 ± 2.24	1576507±4.58	2423345 ± 2.43	1178260 ± 2.22
Hexanoic acid	38728882 ± 16.5	62024155 ± 0.95	55916080 ± 3.67	41933455±1.16	12904008 ± 5.49	41769296 ± 7.01
Octanoic acid	336150745 ± 6.71	227292680 ± 4.23	209718071 ± 4.12	343072241±4.97	258439241 ± 5.27	227632248 ± 2.99
Decanoic acid	163496157 ± 19.1	145986093 ± 9.59	127415087 ± 4.81	186911963±8.66	134601935 ± 7.04	85364750 ± 9.56
Butanoic acid, ethyl ester	19561727 ± 14.2	30241874 ± 3.00	26779078 ± 4.07	27662445±4.72	42136543 ± 4.18	23218890 ± 4.23
Hexanoic acid, ethyl ester	550265347 ± 6.31	781445415 ± 2.58	749700787 ± 4.17	673041592±5.10	817207921 ± 4.17	674374160 ± 4.43
Octanoic acid, ethyl ester	2325878394 ± 2.00	1768157122 ± 2.86	1753733909 ± 4.72	2415261257±5.02	1920950801 ± 5.30	1786962170 ± 5.12
Decanoic acid, ethyl ester	891562662 ± 6.61	661445779 ± 11.3	625922251 ± 13.1	918586683±13.2	646936878 ± 14.0	555182193 ± 15.1
Dodecanoic acid, ethyl ester	53074606 ± 15.9	39030625 ± 6.60	20511334 ± 9.07	53641780±5.91	25865417 ± 4.77	10881434 ± 12.8
Propanoic acid, 2-methyl-, ethyl ester	4015131 ± 16.2	3531131 ± 2.70	3658011 ± 2.45	3281071±6.55	2213145 ± 3.68	5297593 ± 5.71
Butanoic acid, 2-methyl- ethyl ester	6124059 ± 14.3	3310951 ± 3.33	3099221 ± 5.90	4131999±5.44	2123676 ± 4.40	3143484 ± 3.95
Butanoic acid, 3-methyl- ethyl ester	4925260 ± 15.4	4875065 ± 5.87	4568564 ± 4.27	4370769±5.02	5086219 ± 4.53	6126157 ± 4.30
Acetic acid, hexyl ester	6241933 ± 11.5	5839070 ± 4.46	4534801 ± 4.60	8390847±4.77	10623534 ± 4.82	n.d.
Acetic acid, 2-phenylethyl ester	11502900 ± 7.02	2068730 ± 3.95	2001035 ± 4.95	10750214±2.06	3929700 ± 4.77	2851025 ± 5.54
Decanoic acid, methyl ester	4219166 ± 3.18	3954240 ± 7.18	2552171 ± 6.73	4874516±5.24	5113351 ± 6.48	2997134 ± 8.03
Ethyl 9-decenoate	11053390 ± 7.28	2141652 ± 5.24	3139465 ± 7.34	26136289±12.6	2952918 ± 8.01	2704290 ± 3.74
Octanoic acid, 3-methylbutyl ester	5714129 ± 6.60	3019632 ± 7.93	2834432 ± 12.8	6565554±12.5	4231051 ± 13.8	4289288 ± 14.6
1-Butanol, 3-methyl-, acetate	13231289 ± 13.1	14837718 ± 3.85	15863811 ± 4.19	19883642±5.16	34437940 ± 4.97	24928599 ± 6.13
1-Butanol, 2-methyl-, acetate	2234930 ± 15.6	3033183 ± 5.85	3539845 ± 6.07	2686889±4.04	2889061 ± 1.30	4357501 ± 11.1
1-Hexanol	46117240 ± 13.7	60767089 ± 4.09	55656276 ± 3.88	40553464±3.24	43029738 ± 4.50	37860391 ± 10.9
1-Octanol	4223111 ± 8.88	2381256 ± 9.49	2184888 ± 5.49	4914102±6.79	2647674 ± 5.93	6445859 ± 3.81
2-Phenylethanol	562761788 ± 5.33	114819650 ± 6.28	96776113 ± 4.86	478233860±4.89	158045542 ± 3.94	205316954 ± 4.63
α-Terpinene	1950031 ± 8.36	2356816 ± 8.48	1723138 ± 21.0	2085722±14.4	2552787 ± 11.5	1655187 ± 17.0
o-Cymene	3718305 ± 7.13	4945619 ± 2.97	4067479 ± 7.71	3666039±9.16	4209998 ± 6.16	3259313 ± 7.04
α-Terpinolen	3298252 ± 9.63	2526083 ± 10.2	2017144 ± 9.95	4010614±10.8	4764374 ± 8.48	2882694 ± 8.87
Hotrienol	8742839 ± 8.22	8664189 ± 9.37	5657110 ± 11.0	10075166±6.08	11801119 ± 9.51	8287553 ± 15.7
alpha.-Terpineol	5757363 ± 5.84	5999877 ± 10.3	4693346 ± 7.05	9194961±7.01	11928221 ± 8.05	8374346 ± 10.8
Geranyl ethyl ether 1	1173668 ± 7.43	1075017 ± 7.09	555513 ± 3.06	2422551±12.0	4639721 ± 12.0	2148444 ± 12.5
Nerol Oxide	24706821 ± 6.89	28177295 ± 6.31	22287333 ± 6.25	26928646±5.27	29406032 ± 6.98	26706361 ± 9.71
1, 8-Cineol	n.d.	n.d.	n.d.	420255±6.86	421552 ± 7.11	373766 ± 6.53
Rose oxide	346897 ± 5.95	388536 ± 9.49	298595 ± 7.82	393464±9.66	258842 ± 14.8	267642 ± 17.2

Table 54 continued

Vitispirane	97767696 ± 4.03	98969857 ± 4.08	91461925 ± 4.83	98790942±6.41	85561509 ± 5.42	83444688 ± 7.11
1,2-dihydro-1,1,6-trimethyl-Naphthalene	93160735 ± 4.66	99601892 ± 5.73	85720870 ± 9.36	103217206±10.9	93664616 ± 8.72	87511980 ± 9.12
2-Furancarbox-aldehyde	12981685 ± 15.4	12390933 ± 2.99	9042555 ± 12.0	12736483±2.96	11248979 ± 3.03	8899889 ± 1.00
γ-Butyrolactone	2054639 ± 7.51	1908282 ± 6.38	1447943 ± 12.2	1916531±16.9	2260906 ± 4.59	1747395 ± 6.95
Benzenemethanol	1828405 ± 15.7	1659635 ± 6.03	1190418 ± 7.05	1658566±5.41	2543607 ± 4.04	1585772 ± 8.08
2-Nonanone	3693570 ± 13.4	2491820 ± 4.82	54956881 ± 3.97	4089009±4.61	8825441 ± 4.34	40411564 ± 5.52

Table 55: Peak areas of volatile organic compounds of the Wurzelwerk Riesling wines 7-9; RSD in %

	7	**8**	**9**
Butanoic acid	1402671 ± 4.89	1461540 ± 7.84	1073488 ± 4.07
Hexanoic acid	41403654 ± 4.04	45865322 ± 6.30	39760838 ± 4.04
Octanoic acid	330179825 ± 3.15	190366654 ± 3.72	187569899 ± 6.18
Decanoic acid	146521496 ± 9.77	95292428 ± 6.62	89899235 ± 9.47
Butanoic acid, ethyl ester	27478058 ± 4.30	27258912 ± 5.17	22270938 ± 4.73
Hexanoic acid, ethyl ester	624354878 ± 5.02	647342048 ± 5.21	619113568 ± 5.35
Octanoic acid, ethyl ester	2377034461 ± 3.90	1653279496 ± 5.96	1654016998 ± 5.94
Decanoic acid, ethyl ester	964155869 ± 6.00	539921190 ± 16.3	577462594 ± 12.4
Dodecanoic acid, ethyl ester	67601207 ± 2.11	47202332 ± 1.43	20642484 ± 4.72
Propanoic acid, 2-methyl-, ethyl ester	3765331 ± 3.55	5097333 ± 5.76	4560173 ± 3.61
Butanoic acid, 2-methyl- ethyl ester	4417804 ± 5.20	3728320 ± 4.96	2834991 ± 5.93
Butanoic acid, 3-methyl- ethyl ester	4626210 ± 3.88	6471587 ± 5.27	5583034 ± 5.40
Acetic acid, hexyl ester	7372861 ± 3.74	n.d.	n.d.
Acetic acid, 2-phenylethyl ester	14327369 ± 5.37	1793407 ± 7.97	2156238 ± 9.10
Decanoic acid, methyl ester	4718794 ± 6.57	4522663 ± 8.14	2938992 ± 9.38
Ethyl 9-decenoate	11574349 ± 8.81	2183909 ± 6.63	7728191 ± 13.4
Octanoic acid, 3-methylbutyl ester	6895392 ± 4.51	2467651 ± 19.6	2825951 ± 13.2
1-Butanol, 3-methyl-, acetate	21856175 ± 5.24	19783497 ± 7.21	21402092 ± 5.01
1-Butanol, 2-methyl-, acetate	3116608 ± 8.27	4104842 ± 7.77	3329054 ± 5.40
1-Hexanol	33108603 ± 5.43	44769019 ± 13.9	21440217 ± 6.03
1-Octanol	4967441 ± 5.28	4392090 ± 3.95	2190848 ± 9.03
2-Phenylethanol	560070685 ± 3.43	99143768 ± 4.68	106513268 ± 3.84
α-Terpinene	2429954 ± 15.1	2826963 ± 34.9	2099195 ± 14.9
o-Cymene	4767881 ± 6.78	6377639 ± 8.78	4973913 ± 7.11
α-Terpinolen	4790533 ± 7.38	4507871 ± 20.3	3318136 ± 8.00
Hotrienol	9026697 ± 9.17	9932156 ± 13.2	7415928 ± 15.7
alpha.-Terpineol	11866457 ± 9.91	12917339 ± 8.03	9325122 ± 8.53
Geranyl ethyl ether 1	3928811 ± 11.4	3707186 ± 12.1	2488851 ± 13.3
Nerol Oxide	24131778 ± 7.47	30985935 ± 9.61	25432023 ± 9.26
1, 8-Cineol	470292 ± 1.63	668900 ± 11.1	517494 ± 5.22
Rose oxide	392446 ± 7.98	378894 ± 12.6	170288 ± 11.5

Table 55 continued

Vitispirane	100131124 ± 6.65	123055415 ± 6.19	103225936 ± 6.29
1,2-dihydro-1,1,6-trimethyl-Naphthalene	100966124 ± 8.69	126955628 ± 9.80	105821598 ± 10.0
2-Furancarbox-aldehyde	13214243 ± 3.52	12364703 ± 6.62	7768304 ± 5.08
γ-Butyrolactone	1476518 ± 2.77	1661791 ± 7.19	1645723 ± 4.41
Benzenemethanol	2441870 ± 3.16	2867145 ± 5.55	2053117 ± 3.64
2-Nonanone	5316351 ± 3.88	14145052 ± 6.88	44222403 ± 7.11

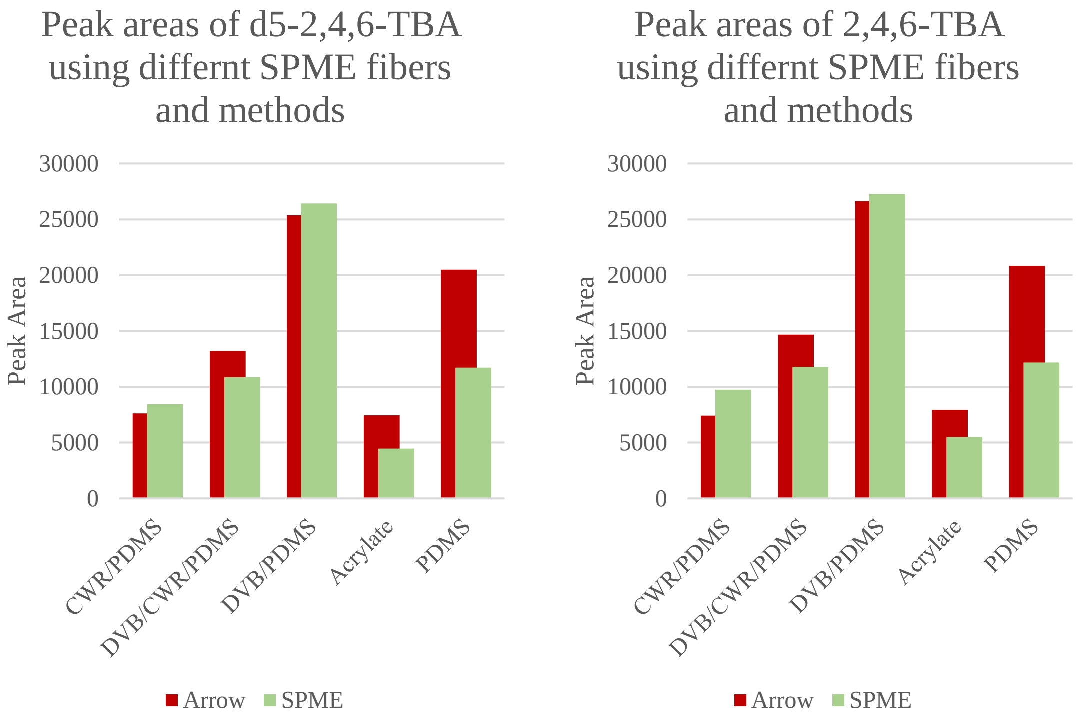

Figure 135: Results of the SPME method and fiber comparison for TBA and d5-TBA; enrichment was done at 80°C for 20 min; data labels are the percentages compared to the largest peak area; values are the mean of two measurements

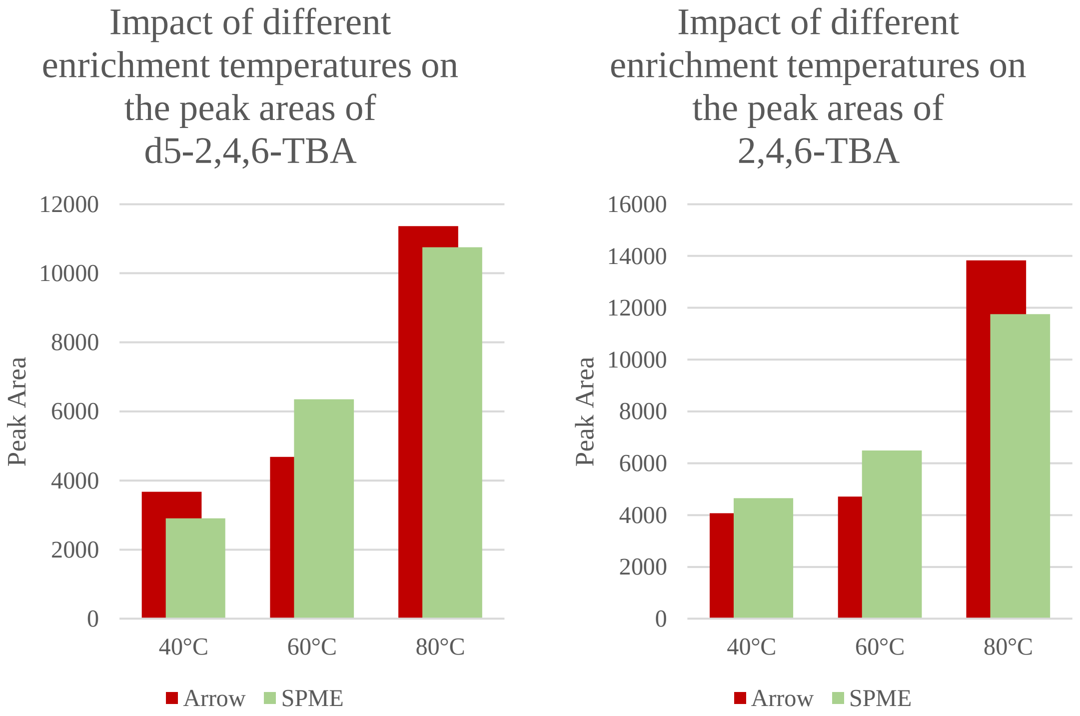

Figure 136: Results of the enrichment temperature comparison for TBA and d5-TBA using the DVB/CWR/PDMS fibers; enrichment time was 20 min; values are the mean of two measurements

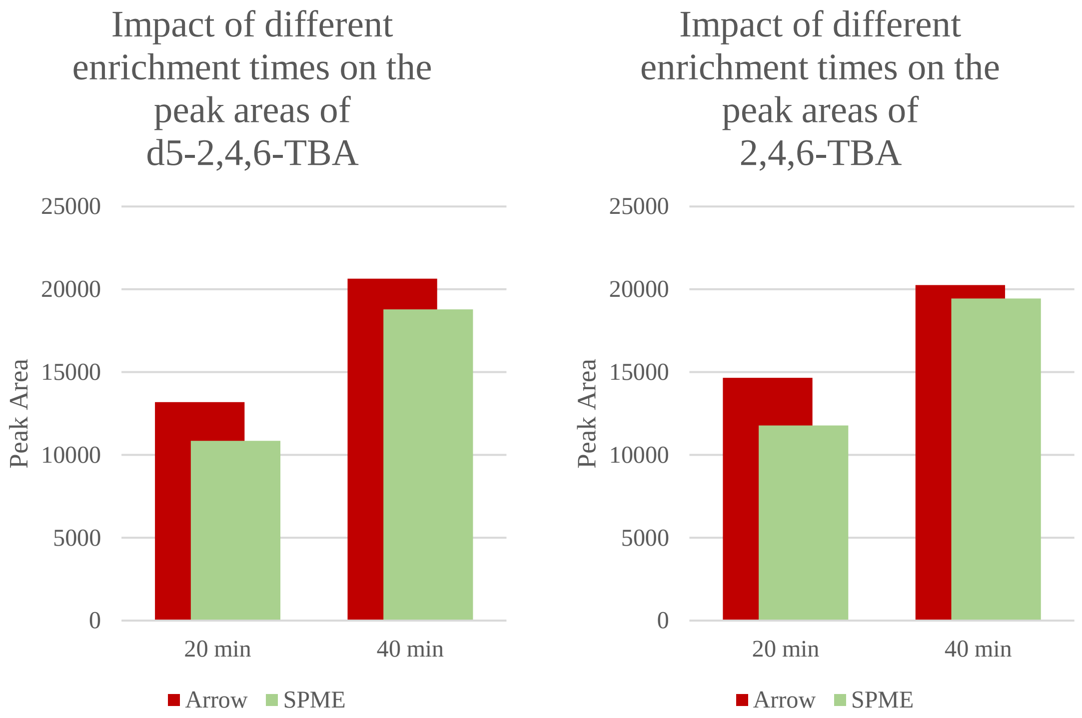

Figure 137: Results of the enrichment time comparison for TBA and d5-TBA using the DVB/CWR/PDMS fibers; enrichment temperature was 80°C; values are the mean of two measurements

Table 56: Quantification results of the ester measurements of the sparkling wines; mean values from two measurements

	Ethylhexanoate [mg/L]	**Ethyloctanoate [mg/L]**	**Ethyldecanoate [mg/L]**	**Sum Esters [mg/L]**
Brut Zéro	1.2	1.0	0.08	2.3
Le Grande Saumur	1.3	1.4	0.18	2.9
Montargull	1.0	0.9	0.06	1.9
Gran Reserva Vintage Cava	0.7	1.1	0.06	1.8
Cava Reserva Brut Natur	1.0	1.0	0.06	2.1
Cava Brut Natur	1.3	1.1	0.11	2.5
Francesco I	1.2	1.1	0.11	2.5
Tenuta Villa Crespina Miolo Brut	0.9	0.8	0.05	1.8
San Christoforo Brut	0.9	1.4	0.07	2.4
Blanc de Blancs	0.9	0.9	0.05	1.8
Monopole Blue Top Brut	1.1	1.5	0.08	2.6
Moet Imperial Brut	1.1	1.3	0.07	2.4
Yellow Lable Brut Champagne	1.4	1.2	0.10	2.7
Reserve Brut	1.2	1.7	0.08	2.9

Table 57: Quantification results of carboxylic acids, higher alcohols and glycerol measurements of the sparkling wines

	Butyric acid [mg/L]	**Hexanoic acid [mg/L]**	**Octanoic acid [mg/L]**	**n-Decanoic acid [mg/L]**	**Methanol [mg/L]**	**n-Propanol [mg/L]**	**Ethyl acetate [mg/L]**	**Isobutanol [mg/L]**	**Isoamyl alcohol [mg/L]**	**Glycerol [g/L]**
Brut Zéro	2.3	27.2	50.2	90.3	40.5	33.8	46.9	19.1	163.5	0.2
Le Grande Saumur	3.1	29.5	51.8	90.8	49.2	31.4	54.4	15.4	120.4	4.7
Montargull	2.6	26.6	50.1	54.3	97.4	52.0	78.5	22.8	231.8	0.3
Gran Reserva Vintage Cava	1.0	22.9	39.3	55.5	50.9	28.0	40.2	20.8	167.7	0.3
Cava Reserva Brut Natur	3.7	33.0	48.4	39.5	95.5	44.6	63.4	19.1	260.1	0.3
Cava Brut Natur	3.2	27.1	44.4	68.0	62.5	21.5	34.2	12.7	131.9	0.1
Francesco I	4.6	26.7	44.4	52.8	31.3	69.3	37.0	15.6	130.4	3.6
Tenuta Villa Crespina Miolo Brut	2.0	18.5	27.9	37.8	49.5	66.2	33.0	17.3	128.3	6.2
San Christoforo Brut	3.1	34.3	40.0	37.5	90.6	118.0	70.7	13.6	246.9	2.1
Blanc de Blancs	2.8	28.7	37.5	26.5	80.7	98.9	81.1	31.1	279.0	0.2
Monopole Blue Top Brut	2.7	27.8	49.1	72.7	35.6	47.5	37.8	23.8	129.1	6.1
Moet Imperial Brut	2.7	27.2	46.7	75.6	31.2	46.0	33.3	23.5	134.3	3.6
Yellow Lable Brut Champagne	3.5	26.7	58.8	89.2	31.1	38.0	47.0	18.3	126.6	4.9
Reserve Brut	3.3	31.2	55.2	67.3	31.8	45.5	33.8	21.4	128.9	4.7

Table 58: Peak areas of selected compounds from the aroma profiles of the sparkling wines; mean values from two measurements

	Furfural	Ethyl-2-Methylbutyrate	Isoamyl acetate	Nonanal	Phenyl ethanol	Ethyl phenylacetate	TDN	Damascenone
Brut Zéro	858294	161045	340973	4030	5430162	231032	200382	4131
Le Grande Saumur	585887	134063	1985520	14883	8982692	205022	112007	11533
Montargull	5622596	586898	368296	20018	7719060	439603	922110	16783
Gran Reserva Vintage Cava	3676522	411417	366867	24069	7560180	546076	889225	14848
Cava Reserva Brut Natur	2445754	476991	225904	15127	10246909	288541	961231	20666
Cava Brut Natur	4516226	195051	385503	24821	9114458	208209	733038	18749
Francesco I	515258	129837	345937	6914	2644277	125980	19922	7299
Tenuta Villa Crespina Miolo Brut	3116850	556824	268989	7560	3900301	200736	67351	8976
San Christoforo Brut	2537883	411593	526997	7551	5206890	182256	69281	9085
Blanc de Blancs	2049507	234158	346857	18381	5465273	1033319	40625	14190
Monopole Blue Top Brut	3287438	302009	325398	7658	5499054	263456	89027	11819
Moet Imperial Brut	1526177	264538	349722	17084	3532719	195902	82120	9942
Yellow Lable Brut Champagne	1411559	183256	155903	19483	4237647	167004	168910	13898
Reserve Brut	1099805	110588	139664	15759	3118935	165792	51331	7842

*Table 59: Compounds identified in PIWI wines, RI data from flavornet (Arn and Acree 1998) and NIST database (https://webbook.nist.gov/), sensory threshold from van Gemert (2011) in artificial wine matrix, unless otherwise indicated: *...water*

Compound name (NIST 11)	Other names	rt (min)	RI (calc)	RI Lit.	Compound class	Aroma descriptor	Aroma group	Threshold µg/L
Isobutyl acetate	isobutyl acetate	10.47	792	776	Ester	fruit, apple, banana	Fruity	1605
2,3-Butanediol, [S-(R*,R*)]-	butanediol	10.85	795	806	Alcohol	fruit, onion	Fruity	668000
Butanoic acid, ethyl ester	ethyl butyrate	11.43	800	804	Ester	apple	Fruity	20
Acetic acid, butyl ester	butyl acetate	11.89	812	816	Ester	pear	Fruity	1830
Propanoic acid, 2-hydroxy-, ethyl ester	ethyl lactate	11.95	814	815	Ester	fruit	Fruity	146000
1-Pentanol, 4-methyl-	Isohexanol	12.70	834	875	Alcohol	nutty	Roasted/ caramelized	3000*
Butanoic acid, 3-methyl-	isovaleric acid	12.60	832	877	Acid	sweat, acid, rancid	Unpleasant	1500
Furfural	furfural	12.68	834	829	Aldehyde	bread, almond, sweet	Roasted/ caramelized	15000
1-Propanol, 3-ethoxy-	ethoxypropanol	12.92	840	833	Alcohol	fruit	Fruity	100
Butanoic acid, 2-methyl-, ethyl ester	ethyl methylbutyrate	13.22	848	846	Ester	apple	Fruity	74
Butanoic acid, 3-methyl-, ethyl ester	ethyl 3-methylbutanoate	13.36	852	854	Ester	fruit	Fruity	3
3-Hexen-1-ol, (E)-	(Z)-3-hexenol	13.47	855	858	Alcohol	grass	Green/ fat	1000
1-Hexanol	hexanol	13.93	868	851	Alcohol	resin, flower, green	Green /fat	8000
1-Butanol, 3-methyl-, acetate	isoamyl acetate	14.24	876	876	Ester	banana	Fruity	30
3-Heptanol[1]		14.93	895		internal std.			
Hexanoic acid, methyl ester	methyl hexanoate	15.93	923	1000	Ester	fruit, fresh, sweet	Fruity	80*
Butyrolactone	γ-butyrolactone	15.59	913	915	Ketone	caramel, sweet	Roasted/ caramelized	20000
Butanoic acid, 3-hydroxy-, ethyl ester	ethyl 3-hydroxybutanoate	16.30	933	935	Ester	marshmallow	Roasted/ caramelized	20000
Benzaldehyde	benzaldehyde	17.47	966	960	Aldehyde	almond, burnt sugar	Roasted/ caramelized	5000
Hexanoic acid	caproic acid	17.90	978	1019	Acid	sweat	Unpleasant	3000
Hexanoic acid, ethyl ester	ethyl hexanoate	18.61	998	1002	Ester	apple peel, fruit	Fruity	5
Acetic acid, hexyl ester	hexyl acetate	19.05	1011	1014	Ester	fruit, herb	Fruity	1000
1,3-Cyclohexadiene, 1-methyl-4-(1-methylethyl)-	α-terpinene	19.50	1025	1012	Terpene	lemon	Fruity	80*

Table 59 continued

p-Cymene	p-cymene	19.74	1032	1027	Terpene	solvent, gasoline, citrus	Chemical	66000
1-Hexanol, 2-ethyl-	2-ethylhexanol	19.59	1027	1032	Alcohol	rose, green	Floral	300*
D-Limonene	(+)-limonene	19.93	1037	1030	Terpene	citrus, mint	Herbal/ spicy	10*
2-Furancarboxylic acid, ethyl ester	ethyl 2-furoate	20.44	1053	1056	Ester	balsamic, fruity, floral, orchid	Roasted/ caramelized	16000
β-Ocimene	(E)-β-ocimene	20.36	1050	1038	Terpene	sweet herb	Herbal/ spicy	34*
ç-Terpinene	γ-terpinene	20.91	1066	1074	Terpene	gasoline, turpentine	Chemical	2500*
1-Octanol	octanol	20.95	1068	1072	Alcohol	chemical, metal, burnt	Unpleasant	10000
Cyclohexene, 1-methyl-4-(1-methylethylidene)-	δ-terpinene	21.94	1097	1090	Terpene	pine, plastic	Chemical	200*
1,5,7-Octatrien-3-ol, 3,7-dimethyl-	hotrienol	22.22	1106	1101	Terpene	hyacinth	Floral	110
Linalool	linalool	22.09	1102	1100	Terpene	flower, lavender	Floral	30
Octanoic acid, methyl ester	methyl octanoate	22.75	1123	1041	Ester	orange	Fruity	200*
Phenylethyl Alcohol	2-phenylethyl alcohol	22.72	1122	1118	Alcohol	honey, spice, rose, lilac	Floral	10000
2H-Pyran, tetrahydro-4-methyl-2-(2-methyl-1-propenyl)-	(-)-cis-rose oxide	23.14	1135	1117	Terpene	sweet, rose	Floral	0.5*
Octanoic acid	octanoic acid	24.43	1176	1279	Acid	sweat, cheese	Unpleasant	10000
Octanoic acid, ethyl ester	ethyl octanoate	25.15	1199	1198	Ester	fruit, fat	Fruity	2
Nonanoic acid, methyl ester[1]		25.86	1223		internal std.			
Isopentyl hexanoate	isoamyl hexanoate	26.62	1249	1244	Ester	fruity, banana, apple, pineapple, green	Fruity	320*
(-)-Carvone	(-)-carvone	26.87	1257	1254	Terpene	mint	Herbal/ spicy	50
Geraniol	geraniol	26.86	1257	1276	Terpene	rose, geranium	Floral	20
Butanedioic acid, hydroxy-, diethyl ester, (ñ)-	diethyl malate	27.16	1267	1270	Ester	brown sugar, sweet	Roasted/ caramelized	10000
Acetic acid, 2-phenylethyl ester	β-phenethyl acetate	27.07	1264	1260	Ester	rose, honey, tobacco	Floral	250
2-Undecanone	undecanone	27.98	1295	1296	Ketone	orange, fresh, green	Fruity	7*

Table 59 continued

Nonanoic acid, ethyl ester	ethyl nonanoate	27.93	1293	1294	Ester	fruity, rose, waxy, rummy, winey, natural, tropical	Green/ fat	377*
Decanoic acid, methyl ester	methyl decanoate	28.77	1323	1324	Ester	oily, winey, fruity, floral	Green/ fat	6*
n-Decanoic acid	decanoic acid	29.93	1365	1373	Acid	rancid, fat	Unpleasant	15000
1, 1, 6-Trimethyl-1, 2-dihydronaphthalene	dehydro-ar-ionene	30.25	1377	1389	Naphthalene	licorice, kerosene	Chemical	2
Decanoic acid, ethyl ester	ethyl decanoate	30.81	1397	1398	Ester	grape	Fruity	200
Octanoic acid, 3-methylbutyl ester	isoamyl octanoate	32.14	1448	1450	Ester	sweet, oily, fruity, green, soapy, pineapple, coconut	Fruity	125
Dodecanoic acid	lauric acid	34.86	1555	1567	Acid	fatty, coconut, bay	Green/ fat	10000
Dodecanoic acid, ethyl ester	ethyl laurate	35.78	1592	1581	Ester	leaf	Green/ fat	500

Table 60: Average concentrations and cumulated sum of four ethyl esters measured in PIWIs

	Year	Ethyl butanoate [mg/L]	Ethyl hexanoate [mg/L]	Ethyl octanoate [mg/L]	Ethyl decanoate [mg/L]	Sum C4-C10 [mg/L]
Bronner	2015	0.30 ± 0.03	1.00 ± 0.06	1.50 ± 0.02	0.51 ± 0.04	3.32 ± 0.07
	2016	0.37 ± 0.04	1.15 ± 0.13	1.60 ± 0.04	0.57 ± 0.02	3.69 ± 0.23
	2017	0.30 ± 0.06	0.87 ± 0.05	1.30 ± 0.05	0.25 ± 0.01	2.72 ± 0.14
	2018	0.37 ± 0.03	0.84 ± 0.03	1.07 ± 0.01	0.17 ± 0.00	2.45 ± 0.07
	2019	0.43 ± 0.03	0.92 ± 0.06	1.46 ± 0.01	0.49 ± 0.08	3.30 ± 0.06
Solaris	2015	0.33 ± 0.05	0.99 ± 0.08	1.54 ± 0.04	0.54 ± 0.07	3.40 ± 0.09
	2016	0.24 ± 0.02	0.52 ± 0.03	0.79 ± 0.03	0.32 ± 0.02	1.88 ± 0.06
	2017	0.47 ± 0.04	1.53 ± 0.08	1.90 ± 0.06	0.60 ± 0.06	4.49 ± 0.17
	2018	0.42 ± 0.02	1.16 ± 0.02	1.89 ± 0.03	0.64 ± 0.04	4.11 ± 0.08
	2019	0.52 ± 0.05	1.36 ± 0.08	2.06 ± 0.04	0.83 ± 0.07	4.77 ± 0.10
Blüten-muskateller	2015	0.31 ± 0.04	1.05 ± 0.11	1.78 ± 0.09	0.87 ± 0.07	4.00 ± 0.10
	2016	0.26 ± 0.02	0.81 ± 0.05	1.65 ± 0.01	0.73 ± 0.04	3.45 ± 0.04
	2017	0.34 ± 0.00	1.29 ± 0.01	2.00 ± 0.07	0.70 ± 0.06	4.33 ± 0.13
	2018	0.32 ± 0.00	1.01 ± 0.01	1.86 ± 0.09	0.82 ± 0.09	4.01 ± 0.17
	2019	0.31 ± 0.02	0.84 ± 0.04	1.68 ± 0.08	0.90 ± 0.05	3.73 ± 0.19
Sauvignon Soyhieres	2015	0.33 ± 0.02	0.84 ± 0.10	1.33 ± 0.08	0.55 ± 0.07	3.05 ± 0.14
	2016	0.32 ± 0.04	0.89 ± 0.06	1.56 ± 0.08	0.70 ± 0.02	3.46 ± 0.18
	2017	0.43 ± 0.01	1.42 ± 0.13	1.93 ± 0.12	0.71 ± 0.07	4.50 ± 0.07
	2018	0.43 ± 0.03	1.26 ± 0.06	2.35 ± 0.09	0.92 ± 0.02	4.96 ± 0.19
	2019	0.44 ± 0.03	1.03 ± 0.05	2.05 ± 0.09	1.03 ± 0.12	4.54 ± 0.25
Souvignier gris	2015	0.39 ± 0.04	1.25 ± 0.11	2.08 ± 0.09	0.67 ± 0.09	4.38 ± 0.15
	2016	0.38 ± 0.04	1.29 ± 0.07	2.09 ± 0.04	0.81 ± 0.03	4.57 ± 0.14
	2017	0.35 ± 0.03	0.58 ± 0.08	1.11 ± 0.05	0.23 ± 0.01	2.27 ± 0.12
	2018	0.42 ± 0.04	1.04 ± 0.07	1.82 ± 0.07	0.69 ± 0.06	3.97 ± 0.15
	2019	0.52 ± 0.06	1.41 ± 0.19	2.32 ± 0.11	0.80 ± 0.04	5.05 ± 0.33
Muscaris	2015	0.35 ± 0.03	1.22 ± 0.08	2.08 ± 0.07	0.85 ± 0.02	4.50 ± 0.19
	2016	0.35 ± 0.04	1.17 ± 0.12	1.97 ± 0.14	0.93 ± 0.06	4.43 ± 0.33
	2017	0.34 ± 0.02	1.06 ± 0.07	1.37 ± 0.06	0.36 ± 0.01	3.14 ± 0.16
	2018	0.45 ± 0.05	1.38 ± 0.16	2.14 ± 0.06	0.84 ± 0.09	4.82 ± 0.08
	2019	0.41 ± 0.01	1.15 ± 0.11	2.11 ± 0.11	1.31 ± 0.08	4.98 ± 0.16
Sauvignac	2015	0.45 ± 0.01	1.34 ± 0.00	2.22 ± 0.08	0.95 ± 0.03	4.96 ± 0.08
	2016	0.40 ± 0.04	1.29 ± 0.09	2.25 ± 0.07	1.00 ± 0.08	4.94 ± 0.11
	2017	0.47 ± 0.08	1.53 ± 0.23	2.07 ± 0.04	0.69 ± 0.03	4.76 ± 0.32
	2018	0.39 ± 0.02	1.42 ± 0.04	2.44 ± 0.03	1.02 ± 0.02	5.26 ± 0.02
	2019	0.52 ± 0.02	1.27 ± 0.05	2.28 ± 0.07	1.10 ± 0.09	5.17 ± 0.17
Cabernet Blanc	2015	0.25 ± 0.01	1.20 ± 0.04	2.24 ± 0.08	0.93 ± 0.08	4.63 ± 0.14
	2016	0.29 ± 0.02	1.14 ± 0.08	1.77 ± 0.02	0.71 ± 0.02	3.90 ± 0.11
	2018	0.24 ± 0.01	0.71 ± 0.01	1.46 ± 0.03	1.27 ± 0.07	3.68 ± 0.11
	2019	0.39 ± 0.03	1.03 ± 0.05	1.56 ± 0.02	1.07 ± 0.04	4.04 ± 0.10
Chardonel	2015	0.41 ± 0.02	1.32 ± 0.15	2.11 ± 0.05	1.01 ± 0.09	4.85 ± 0.06
	2016	0.35 ± 0.01	1.20 ± 0.04	1.98 ± 0.04	0.71 ± 0.01	4.24 ± 0.09
	2017	0.37 ± 0.03	1.55 ± 0.01	1.97 ± 0.12	0.63 ± 0.01	4.53 ± 0.16
	2018	0.39 ± 0.03	1.40 ± 0.08	2.36 ± 0.04	0.98 ± 0.04	5.13 ± 0.12
	2019	0.57 ± 0.06	1.46 ± 0.12	2.20 ± 0.09	0.99 ± 0.06	5.23 ± 0.13

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Activities and Publications by Year

2021

Investigating the Winemaking Potential of Enchantment, a New Vitis Hybrid Teinturier Cultivar
Mayfield, S. E., Threlfall, R. T., Leis, D., Howard, L. R., Leitner, E. & Clark, J. R., 1 Apr 2021, In : American Journal of Enology and Viticulture. 72, 2, p. 194-207 14 p.
Research output: Contribution to journal › Article

2020

Wine from fungus resistant grape varieties: Aroma analysis with GC-MS to evaluate typicity
Leis, D., Leitner, E. & Renner, W., Sep 2020, eFOOD-Lab International, 2020, 3, p. 14-18 4 p.
Research output: Contribution to specialist publication › Article

Separation behavior and microstructure of emulsified, two-phasic E. coli bioreaction mixtures
Lauß, B., Rapp, C., Leis, D., Nidetzky, B. & Kratzer, R., Mar 2020, In: Colloid and Interface Science Communications. 35, 100248.
Research output: Contribution to journal › Article

2019

Characterization of Sauvignon Blanc Must from Different Origins
Leis, D., Renner, W., Maslov, L. & Leitner, E., 25 Jun 2019.
Research output: Contribution to conference › Poster

Impact of Foliar Grapevine Application of Inactive Dry Yeast on Aroma Profile of Chambourcin Wine
Leis, D., Mayfield, S., Threlfall, R. & Leitner, E., 25 Jun 2019.
Research output: Contribution to conference › Poster

Einfluss des Erntezeitpunkts auf die Konzentration von Isobutylmethoxypyrazin von steirischen Mosten der Rebsorte Sauvignon
Leis, D., Renner, W. & Leitner, E., 27 May 2019.
Research output: Contribution to conference › Poster

Influence of Harvest Time on the Concentration of Isobuyl-Methoxypyrazine of Styrian Sauvignon Blanc Must
Leis, D., Renner, W. & Leitner, E., Apr 2019.
Research output: Contribution to conference › Poster

Charakterisierung von Weinen aus pilzresistenten Rebsorten
Dorothea Leis (Speaker), Wolfgang Renner (Contributor), Erich Leitner (Contributor)
26 Mar 2019
Activity: Talk or presentation › Talk at conference or symposium › Science to science

Characterization of Wine Aroma Using Different Analytical Methods
Dorothea Leis (Speaker)
8 Jan 2019
Activity: Talk or presentation › Talk at conference or symposium › Science to science

Einfluss des Erntezeitpunkts auf die Konzentration von Isobutylmethoxypyrazin von steirischen Mosten der Rebsorte Sauvignon
Leis, D., Renner, W. & Leitner, E., 2019, *ALVA Tagungsbericht 2019: Weinbau und Klima.* p. 414-416 3 p.
Research output: Chapter in Book/Report/Conference proceeding › Conference contribution

2018

Characterisation of Wines Produced from Fungus Resistant Grape Varieties
Leis, D., Renner, W. & Leitner, E., Aug 2018, *Flavour Science: Proceedings of the XV Weurman Flavour Research Symposium.* p. 511-514 4 p.
Research output: Chapter in Book/Report/Conference proceeding › Conference contribution

Vergleich verschiedener SPME Faser Materialien für die Analyse von flüchtigen Spurenverbindungen
Dorothea Leis (Speaker)
25 Apr 2018 → 27 Apr 2018
Activity: Talk or presentation › Poster presentation › Science to science

2017

Integration of whole-cell reaction and product isolation: Highly hydrophobic solvents promote in situ substrate supply and simplify extractive product isolation
Leis, D., Lauß, B., Macher-Ambrosch, R., Andreas, P., Nidetzky, B. & Kratzer, R., 2017, In: Journal of Biotechnology. 257, p. 110-117
Research output: Contribution to journal › Article

Characterisation of Wines Produced from Fungus Resistant Grape Varieties
Dorothea Leis (Speaker)
18 Sep 2017 → 20 Sep 2017
Activity: Talk or presentation › Poster presentation › Science to science

Influence of origin and vinification on the aroma of certain Riesling wines
Dorothea Leis (Speaker)
28 Aug 2017 → 1 Sep 2017
Activity: Talk or presentation › Poster presentation › Science to science

13. ASAC JunganalytikerInnen Forum
Dorothea Leis (Speaker)
13 May 2017
Activity: Talk or presentation › Talk at conference or symposium › Science to science

Quantifizierung von High Impact Aroma Komponenten in Wein
Dorothea Leis (Speaker)
3 Apr 2017 → 6 Apr 2017
Activity: Talk or presentation › Poster presentation › Science to science