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Table of Contents

1 Introduction	6
2 Chemical Groups of Volatile Compounds	7
2.1 Phenols	7
2.2 Esters	7
2.3 Terpenes	8
2.4 Methoxypyrazines	9
2.5 C ₁₃ - Norisoprenoids	10
2.6 Volatile Sulfur Compounds (VSC)	11
3 The Process of Winemaking	14
3.1 Maceration and Pressing	17
3.1.1 White wine	17
3.1.2 Red wine	18
3.2 Alcoholic Fermentation	19
3.3 Malolactic Fermentation	28
3.4 Fining	33
3.5 Barrel Aging	37
3.5.1 White wine	37
3.5.2 Red wine	42
4 Detailed characterization of some wine examples	52
4.1 White wine	52
4.2 Red wine	56
5 Off flavors in wine	70
6 Prediction of wine sensory properties from compositional data and consumers acceptability, and expert quality judgement of red and white wines	77
7 Abstract	84
8 Zusammenfassung	85
9 References	86
10 Curriculum vitae	90

1 Introduction

Wine consists of numerous components which contribute to its special aroma, mouthfeeling and taste.

Many of these substances have already been discovered, their structure analyzed and their quantity measured. A lot of studies have also focused on the precursors of these components to detect their origin.

Some of them are already present in grapes, some arise during alcoholic or malolactic fermentation, some derive from aging in oak-barrels or form during bottle aging.

Different grape varieties, cultivation techniques, yielding circumstances, pressing conditions and yeast strains effect the wine composition as much as fining treatments, storage conditions, the wood of the barrel, the length of bottle storage or the quantity of oxygen and other compounds in the fermented must.

However, there are lots of factors influencing the composition of the wine volatiles and this review presents the newest research results concerning aroma and flavor components of white and red wine.

But still, there are many odorants undetected and much more studies in different subjects are necessary to complete the research about the volatiles in wine. Specially the interactions between the volatiles, between volatiles and for example polyphenols, yeast lees or cask-wood and their reaction products are hardly understood. But also in terms of linking quality criteria to distinct flavor compounds, more work is necessary. All those results are in the end helpful for winemakers to produce high quality wine and to avoid the production of off-flavors.

This review will focus largely on the different volatile aroma compounds in white and red wine. Different groups of components will be presented, the process of wine-making illustrated and different grape varieties characterized. Furthermore a chapter will be dedicated to off-odors and the prediction of aroma properties from the chemical composition of the grape must or of wine before its storage.

2 Chemical Groups of Volatile Compounds

2.1 Phenols

Just a few volatile phenols are present in grape must. They are more often occurring as non-volatile conjugates which are released during fermentation, through enzymatic or acid hydrolysis. Although contributing to the varietal aroma of a few grape varieties, phenolics appear to be more generally important as a source of potential flavorants in the form of hydroxycinnamic acid esters.^[1] Also coumaric and ferulic acid esters are important. During fermentation, cinnamic acid derivatives are formed, such as vinylphenols (4-vinylguaiacol and 4-vinylphenol) and ethylphenols (4-ethylguaiacol and 4-ethylphenol). They are giving a “spicy”, “smoky”, “pharmaceutical”, “phenolic”, “clove-like” odor to the wine. Quantities over 400 µg/L ethylphenols or 725 µg/L vinylphenols can evoke feelings of off-flavors.^[1] Red wines generally show higher amounts of those substances than white wine. Eugenol brings a “spicy” note to the wine, while guaiacol in higher concentrations may influence the aromatic bouquet in a negative way.

Oak derived phenolics are mainly derivatives of benzaldehyde and cinnamaldehyde. Also vanillin is a common constituent of wines, aged in oak barrels. They are formed through a break-down of lignin from the oak wood. Furfural is deriving from oak toasting, but also from the decomposition of components like fructose in wine. Its odor can be described as “almond-like”.

2.2 Esters

Esters form as condensation products between the carboxyl group of an organic acid and the hydroxyl group of an alcohol or phenol.

Numerous esters are produced by yeasts during fermentation and factors such as low fermentation temperatures (~10°C) can influence the formation of fruity esters, like isoamyl acetate (banana-like), isobutyl acetate (raspberry-pear-like) or hexyl acetate (general fruity aroma) in a positive way. Higher fermentation temperatures (~15-

20°C) evoke the production of higher molecular-weight esters such as ethyl octanoate, ethyl decanoate and phenylethyl acetate. The longer the hydrocarbon chain gets, the more “soap-like” the odor becomes. And esters with C₁₆-C₁₈ fatty acids are already known to have a “lard-like” flavor.

Esters represent the largest group of constituents in wine. Over 160 different esters are already known. But although quantitatively important, the group of esters is often not significantly important to flavor and sensory attributes. Many of them are found in trace amounts and/or have a low volatility or mild odors. The general sensory attribute linked to esters is “fruity” aroma. But there are still other components like β -damascenone that contribute to a “fruity” aroma in wine. For example the addition of low levels of β -damascenone and β -ionone to a model wine enhanced the fruity note, produced by volatile esters, but the addition of high levels of β -damascenone and β -ionone altered the sensory properties of the wine, and changes the aroma note to “raisin” or “plum”.^[2]

2.3 Terpenes

Terpenes generally consist of isoprene units, that form mono-, sesqui-, di- and triterpenes, respectively. Monoterpenes are present in most grape varieties, specially in Muscat and Riesling families. They mainly occur as non-volatile glycosides, or as di- or triols, that can be liberated during fermentation through yeast glycosidase enzymes or acid hydrolysis. Free terpenes hardly occur in grapes, because they are volatile. The liberated monoterpenes, as linalool for example, contribute significantly to the “floral” and “citrus” aroma of the wine. Terpene composition cannot be influenced by geographical modifications, but different yeast strains are capable to convert for example geraniol to nerol and citronellol. Also linalool, which has a “muscaty”, “iris-like” flavor can be transformed to α -terpineol, which is characterized by a “musty”, “pine-like” scent.^[1] Especially for wines like the Muscat-variety this change in terpene-structure is important, because their aroma composition mainly consists of terpenes.

Also Gewürztraminer is a variety, that contains a certain terpene, (-)-cis rose oxide. It is a fermentation product of the reduction of geranyl diol to citronellyl diol, that causes the acid catalyzed cyclization to form cis- and trans-rose oxide.^[3]

Shiraz is known for its “spicy”, “peppercorn” aroma. This derives from a very potent aroma compound, the sesquiterpene rotundone. It is also present in black or white peppercorns and its odor activity value is relatively high. Although approximately 20% of the panellists in the study of Wood et al. ^[4], could not detect this compound even at the highest concentration tested (4000 ng/L). Therefore, the sensory experience of two consumers enjoying the same charge of Shiraz wine may be very different.^[4]

Another study showed that the development of “eucalyptus” aroma, derived from 1,8-cineole, is not dependent on the vineyard being surrounded by *Eucalyptus sp.* trees, but on precursors like α -terpineol or limonene, that can form 1,8-cineole under acidic conditions.^[5]

2.4 Methoxypyrazines

Pyrazines are cyclic nitrogen-containing compounds.

In the late 1960s 3-isobutyl-2-methoxypyrazine (IBMP) was already known to contribute to the aroma of bell pepper. Its olfactory threshold was also detected (2ng/L) and it was one of the most potent odorants at that time. IBMP is a main odorant in the varieties Sauvignon Blanc, Cabernet Sauvignon Merlot and Carmenere. Its flavor can be described as “green bell pepper” at lower concentrations and as “herbaceous” in higher concentrations (> 15 ng/L in white wines, >25 ng/L in red wines). High methoxypyrazine-levels can also mask fruity notes in wine. Another pyrazine, 3-isopropyl-2-methoxypyrazine (IPMP) has also been identified and its flavor is more “earthy” and “asparagus-like”.

The concentration of methoxypyrazines in berries depends on climate, sun exposure and vine vegetative growth and yield.^[6] The levels usually decrease during maturation and attain a basal level before harvest. IBMP is very sensitive to sunlight and temperature, but also to the period of rainfall as the study of Belancic and Agosin, shows.^[6] IBMP levels were lowest with heavier winter rains, the highest number of days below 0°C in winter and the highest summer temperatures. In comparison, IPMP concentrations showed different levels than IBMP. Its concentration seemed to depend on both, climatic factors and location.

IPMP has also been identified as constituent in the effluent of *Harmonia axyridis* (Coleoptera: Coccinellidae) (HA), also known as lady beetle or lady bug. If HA beetles are incorporated in grapes, they are also fermented with the grapes and they therefore contribute to the wine aroma as much as other components and can cause the wine having higher intensity scores for flavors like bell pepper, asparagus, earthy, herbaceous or peanut than wines without lady beetles.^[7] Studies also show that methoxypyrazines are not the only constituents responsible for vegetal aroma. Therefore, even with optimized enological and viticultural practices to reduce the MP-levels, vegetal wines may still result. The fact, that until now no biosynthetic pathway for MP has been identified, although some amino acids like leucine have been suggested to be precursors for MP, leads to the idea that MP are a product of genetic factors. This theory is backed up by Bowers and Meredith^[8], who showed, that Cabernet Sauvignon grapes are derived from a cross between Sauvignon Blanc and Cabernet Franc, indicating that there may be a yet unidentified genetic basis for methoxypyrazine formation in these varieties.^[9]

2.5 C₁₃- Norisoprenoids

C₁₃- Norisoprenoids are a diverse group of potential impact odorants that are derived from grape carotenoids. As the monoterpenes, C₁₃- norisoprenoids are said to be bound glycosidically and describe non-volatile precursors. Through acid hydrolysis or fermentation the free aroma compounds such as β -damascenone or β -ionone are released. The hydrolytic release of the free form of β -damascenone during vinification is dependent upon the concentration of precursors in the wine, but this is not necessarily directly related to the initial concentration in the fruit itself.^[2] It was proved that the release of β -damascenone and β -ionone was not dependent on the level of their precursors in the grapes, but on the release during fermentation and wine aging. For example heat treatment doubled the free β -damascenone-levels in wine. Daniel et al.^[10] worked on a study supporting the theory of β -damascenone being formed by acetylenic alcohol and acetylenic diol that act as precursors at pH 3-3.2. It was proven that higher β -damascenone-levels in a wine have an impact on the sensory properties, but so far there is no evidence that higher levels of β -damascenone have an impact on the quality of a wine. β -damascenone in its free

form is said to have “honey”, “apple” or “plum” note, while β -ionone is more associated with a “violet” scent. As mentioned above, low levels of both, β -damascenone and β -ionone enhance the fruity characters of wine, but high levels of them change the aroma of the wine to “raisin” or “plum”. It may have a direct influence on fruity aromas by lowering the odor thresholds of fruity esters, or indirectly by rising the perception threshold of IBMP, which is able to mask the sensory attributes deriving from fruity esters when it is present in higher concentrations. Altogether, the aroma contribution of β -damascenone is depending on the complexity of the wine-matrix.

Carotenoids are not only transformed into β -damascenone and β -ionone, but also for example to 1,1,6-trimethyl-1,2-dihydronaphthalene (TDN) or (*E*)-1-(2,3,6-trimethylphenyl)buta-1,3-diene (TPB). TDN gives a “kerosene” note to aged Riesling wine and TPB is said to contribute a strong “green” or “cut grass” aroma to white wines, specially Semillon. Thus, they are considered as off-flavors in wine.

The degradation of carotenoids is not well understood so far, therefore it is only possible to suggest, that enzymes like *carotenoid cleavage dioxygenase* enzymes are involved in the transformation of the precursors into the free volatiles.^[9]

2.6 Volatile Sulfur Compounds (VSC)

VSC are sulfur containing organic compounds. They can be classified into five different families according to their structure: thiols, sulfids, polysulfides, thioesters and heterocyclic compounds. However, the more practical and useful classification is according to their volatility: compounds with a boiling point below 90 °C (volatile compounds) or compounds with a boiling point over 90°C (less volatile compounds).^[11] They usually occur in trace amounts in wine, but their perception thresholds are generally very low. Therefore, also very low concentrations of those compounds can give high sensory intensities. Mostly, the aromatic properties of VSC are considered as detrimental to wine quality, because of the offensive flavor characteristics (off-flavors) they provide. Their odor can be generally described with terms like “cabbage”, “garlic”, “onion” or “rubber”. Some of the descriptors can be attributed to specific compounds, such as “rotten egg” from hydrogen sulfide (H₂S), “putrid”, “garlic” or “onion” from methanethiol (MeSH) and “(canned) corn” or

“(cooked) asparagus” from dimethylsulfide (DMS) at higher concentrations. But not all of the sulfur compounds have a negative impact on the wine aroma. For example DMS in lower concentrations can give a pleasant “black currant” aroma and has been shown to enhance fruity notes in the presence of other volatile wine components.^[12] Even hydrogen sulfide or carbon disulfide (CS₂) are not necessarily thought of as negative contributors to wine aroma, but may also contribute to the bouquet of a young wine and add complexity to the wine aroma at levels low enough for not being perceived as a fault. Some others are typical for a certain variety and contribute actively to the particular aroma of these wines. Examples therefore are the “strawberry” aroma derived from 4-mercapto-2,5-dimethyl(2H)thiophen-3-one, “boxtree” aroma from 3-mercaptohexylacetate, “cat urine”-like scent from 4-mercapto-4-methylpentan-2-one or “cooked leeks”-aroma from 3-mercapto-3-methylbutan-1-ol^[11]

Sauvignon Blanc for example contains VSC like 4-mercapto-4-methylpentan-2-one, 3-mercaptohexylacetate and 3-mercaptohexan-1-ol. Also in red varieties like Merlot or Cabernet Sauvignon VSC like 3-mercapto-2-methylpropanol, 3-mercaptohexan-1-ol and 3-mercaptohexyl acetate can be found. They may originate from enzymatic processes or non-enzymatic processes. Enzymatic processes can be the degradation of sulfur-containing amino acids, the formation of fermentation products, or the metabolism of some sulfur-containing pesticides. Non-enzymatic processes include photochemical, thermal and other chemical reactions of sulfur compounds during winemaking and storage.^[11]

Microorganisms have two pathways for metabolizing sulfur compounds. There is the sulfate assimilatory reduction pathway, where sulfate is taken up and used for the biosynthesis of for example sulfur-containing amino acids like cysteine or methionine. The other pathway is the sulfate dissimilatory reduction pathway, in which the sulfate is reduced of the respiratory pathway to sulfite or sulphide. The sulfur-containing amino acids cysteine and methionine can also be degraded to sulfides and subsequently other volatile sulfur compounds.^[13] It is known that methionine is metabolized with formation of its fusel alcohol (3-methylthio-1-propanol or methionol), its acetate (3-methylthiopropyl acetate), its ethyl ester (ethyl-(3-methylthio)-propionate) and 3-ethylthio-1-propanol.^[11]

3-mercaptohexanol (3-MH) has been identified as an aroma impact compound in Petite Arvine wine from Switzerland. Its contribution to the wine aroma is described

as “rhubarb” and “grapefruit-like”. It has also been found in yellow passion fruit, grapefruit, guava, tomato leaves and rhubarb. 3-MH showed different aroma characteristics at low concentration (“grapefruit-like”) or at moderate (“passion fruit” and “rhubarb”) or at high concentrations (unpleasant sulfur note).^[14] A low concentration of 3-MH could be detected in almost every white wine, but only in Sauvignon Blanc and Petite Arvine wine it was detected in higher concentrations and it is therefore perceived as a distinct key aroma compound.^[14]

The precursors of the aroma active volatile thiols are S-cysteinyl-conjugates. The cleavage of the precursor to the active aroma compound is usually mediated by the C-S-lyase-activity of *Saccharomyces cerevisiae* yeast during the fermentation process. Similarly, enzymatic β -lyase-activities found in yeast or bacteria may convert these precursors into aroma active compounds. Quantification of such precursors in grapes or musts could allow assessment of the aromatic potential of Sauvignon Blanc or other *V. vinifera* cultivars as quality control.^[15]

3 The Process of Winemaking

Winemaking or vinification is a complex series of processes which formally starts with the harvest of the grapes and ends with the final product, the wine itself. As a result of the numerous steps, necessary to produce a wine, it can be influenced by many factors and circumstances. Therefore, it is very hard to produce the same bottle of wine twice. Every vintage, every vineyard, every grape variety and even every yeast strain or oak barrel contribute in a certain and unique way to the aroma of a wine.

As mentioned above, vinification starts with the harvest of the grapes. The next step, the crushing of the grapes to get its juice is the same for white and red wine, but afterwards, the further way splits up into red and white wine-making.

White wine is then macerated. During this process flavor compounds and other constituents, that contribute to a wines taste, can be extracted. Enzymes that are released from ruptured cells may already hydrolyze macromolecules to smaller forms, making them usable for bacteria and yeast. Maceration for white wine is carried out for maximum a few hours. The free running juice is then united with the juice, released by soft pressing, and mostly these two fractions are fermented together. Further fractions are fermented separately. Fermentation can be implemented by indigenous yeasts, deriving from the grape, or by inoculated yeast strains with known fermentation-characteristics. The maceration of red grape varieties is taking a longer time and it is proceeded simultaneously with the alcoholic fermentation. For red wines the alcohol is necessary to extract the phenolic constituents of the grape. These are tannins from seeds and skins, necessary for astringency, anthocyanins for the colour and flavor compounds for the bouquet and volatile phenolic compounds. After partial or completed fermentation, the free running juice is taken and can be united in portions with the pressing fractions, thus sustaining the special taste and flavor characteristics of the red wine type. Rosé wines are made from red grapes, but the maceration and fermentation time is much shorter than for red wines, because of the lighter colour of Rosé wines.

After this step of alcoholic fermentation, the next one, the malolactic fermentation (MLF) is proceeded, if desired. Many red wines are benefitting from MLF. In cooler regions it is recommended to carry out MLF, because the resulting wines usually

contain a higher acid level than wines from warmer climates. During this step, the tart tasting malic acid is converted into the softer tasting lactic acid. White wines are usually not undergoing MLF, because of their milder aroma. They are more probably developing undesired off-flavors. Also wines that are produced in warmer regions do not undergo MLF. They are not containing very high levels of malic acid, like the wines from cooler regions, so MLF can only change the aroma profile, and this is mostly not desired. There are several techniques to prevent spontaneous malolactic fermentation, such as addition of sulfur dioxide, early clarification or storage under cooler conditions.^[1] Red wines, that are stored in oak barrels during MLF are said to contain more “fruity” and “oak” aroma. Wines can also be stored in oak barrels after the MLF to get a more complex aroma composition. This is famous not only for red, but also for some white wines.

After several weeks or months the wine is racked, which means, that the wine is separated from the solids, that already settled out by spontaneous or natural clarification. The solids mainly consist of yeast and bacterial fragments, grape cells, precipitated tannins or proteins and potassium tartrate crystals. Racking helps to avoid the production of off-flavors, because storage on the lees may lead to continued fermentation or microbiological spoilage. During racking, the wine is getting in contact with oxygen, which is tried to be held out of the whole winemaking-process, because of the oxidation, that may contribute to oxygen-derived off-flavors, but small amounts of oxygen help to oxidize hydrogen sulfide in the wine and they also aid in stabilizing the colour of the wine.

Before the wine is bottled, fining treatments are necessary to remove the rest of the solids, like dissolved proteins and others. Historically, fining agents like egg white, blood or milk have been used, or are still in use, but nowadays more modern alternatives are available, like bentonite, gelatine, casein, carrageenan, kieselsol and others. They are binding and therefore removing the solids by electrostatic, adsorbent, ionic or enzymatic interactions. Tannins and other polyphenols may also be removed, which results in a reduction of astringency, but also in a reduction of healthy, antioxidant flavonoids.

In the last step, the wine is bottled and stored. At bottling, generally a small amount of sulfur dioxide is added to reduce oxidation and microbiological spoilage. The wines can be stored under cool conditions for several months or years. This proceeding helps blended wines to “harmonize”. It is also carried out to reduce the

effects of “bottle sickness”, which describes the “trauma” due to oxygen exposure during bottling. Tannins and anthocyanins continue to form aggregates and precipitate, visible on the bottom of the bottle. The sediments generally indicate the winemaker that the wine is mature. Acids and alcohols combine to form esters, flavor-precursors are hydrolyzed to form active aroma compounds and aldehydes are oxidized. At the “peak” of a wine’s maturity, where the complexity of its composition reaches maximum levels, the wine is ready to consume. Too long aging time can badly influence the composition in terms of developing “hollowness” and “weakness” of the aroma and a stronger acidity.

3.1 Maceration and Pressing

3.1.1 White wine

During maceration the crushed grapes release their juice. Through the breakdown of the grape cells, enzymes are liberated and influence the composition of the juice. For winemakers, the higher quality of free running juice is precious in comparison to the pressed juice. But also the pressed fractions can still be used to produce quality wines. At the same time, the amount of pressure applied and the period of skin contact will affect the extraction of aroma compounds and precursor.^[16]

In general, maceration is carried out to raise the levels of flavorants. Many wine varieties are given several hours of skin contact after crushing and before pressing. Flavorants, such as monoterpenes, are concentrated in and just under the skin. Their concentration in the juice increases with longer skin contact and therefore longer maceration period. Their oxidation may also be influenced by a short maceration time at higher temperatures. Not all of the volatile compounds increase with higher maceration temperatures. The production of esters for example increases at a maceration temperature up to 15°C, but then declines at higher temperatures.^[1] Longer skin contact not only raises the amount of flavorants in the juice, but also the quantity of polyphenols. Therefore, the risk of a long maceration period lies in the different mouthfeeling and taste of the wine. Astringency and pH increase as well as the aroma precursors and flavorants.

Although the maceration process is fully oxidative, winemakers try to reduce oxygen contact with the wine. One possibility is the addition of SO₂, but this procedure may have a negative effect on the MLF and the aging process. Alternatively, the use of low temperatures, inert gases, and antioxidants such as ascorbic acid help to lower oxidation. White grape juices treated this way retain their fresh colour, and more of their primary fruit flavors find their way into the finished wine. The addition of SO₂ disrupts the cell membrane and may inhibit the function of polyphenol oxidases. It also delays the inception of alcoholic fermentation and malolactic fermentation. Whenever those qualities are desired, sulfur dioxide is added. Other antioxidants like ascorbic acid were used for a long time, inhibiting the browning effect, but they also delay the oxidation during bottle aging and should therefore be avoided.

If the maceration is carried out at cool conditions and just for a short time, the resulting wine is characterized as young, fresh and fruity. The longer the maceration takes, the deeper the colour of the wine and the fuller its flavor gets. After pressing the winemaker can initiate the fermentation process.

3.1.2 Red wine

The maceration in red wines is primarily focused on the extraction of colour polyphenols and tannins instead on flavor compounds.

The first step in treating red grapes for winemaking is the crushing. Usually the red grapes are destemmed before fermentation, because of the high tannin-content in the stems. But not only tannins, also the scent of a vegetal aroma is given to the wine with fermenting grapes and stems. But sometimes the winemaker decides to leave the stems in the grapes, if the grape must contains too little tannin. The extent of crushing differs with the grape variety and the winemakers preference. The most common practice is to remove the stems and split the berries. Some varieties as Pinot Noir or Syrah are left in whole berries, and macerated at cooler or cold temperatures, which leads to a longer, slower fermentation and less extraction of bitter substances from the grapes. For high-extract wines as Port wines, grapes are crushed to release as much compounds as possible.

At this point it is possible to add SO₂ to suppress the growth of native yeasts and bacteria, which may lead to a “wild yeast fermentation”, which is hardly desired, because of its unpredictable results.^[17] Most yeasts and bacteria are very sensitive to sulfur dioxide. SO₂ also binds with anthocyanin pigments in freshly crushed grapes, making them more soluble. Winemakers can also choose to make other additions like acid, sugar, or other nutrients such as nitrogen or they add whole grapes to this mixture, which fortifies the total phenolic content and increases astringency in a wine that would otherwise be too soft. Nitrogen supplementation can fortify the yeast metabolism and therefore the volatile compounds production like for example esters.^[18]

After crushing or splitting the grapes, the fermentation can be initiated. In red wine-making the fermentation takes place before pressing. There are numerous methods of fermenting the must and numerous studies have been focusing on this topic.

3.2 Alcoholic Fermentation

Yeast is usually already present on the grapes, often visible as a powdery appearance on the surface of the grapes. The fermentation can be done with this indigenous yeast, but the risk of unpredictable results is rather high. Therefore, cultured yeast is often added to the must.

One of the main problems with the use of wild ferments, apart from the unpredictable aroma composition of the resulting wine, is the failure for the fermentation to remain incompleting. Some of the sugar remains unfermented, which makes the wine taste sweet. This is a problem when a dry wine is desired. Wild yeast strains may also lead to the production of unpleasant and undesired acetic acid production as a by product.

Studies with the aroma production of wild yeasts have been conducted and the volatile products have been analyzed. Verginer et al.^[19] have taken a closer look at the Austrian variety “Blaufraenkisch”, a red cultivar, of the vintage 2006, and its grape-associated microorganisms. But by now no certain “wild yeast fermentation”-character could be defined. The effects on the sensory impressions of the different isolated microorganisms were ranged via principle component analysis (PCA) in a 2D pattern and a clear distinction between the cultures (bacteria, fungi, yeast) was visible. Altogether, 30 pure cultures of grape-associated microorganisms with interesting sensory properties, according to their phylogenetic origin, were identified. They found out, that all of the identified bacteria belong to the genera *Exiguobacterium* and *Paenibacillus*. The fungi comprise *Aureobasidium* and *Cladosporium* genera. For three isolates the composition of volatile organic compounds (VOC) was analyzed in detail. To differentiate between volatiles, derived from the microorganisms and volatiles that were emitted from the culture medium, the results from the inoculated sample were compared with a non-inoculated sample and the remaining substances ranged between 34 and 45 different analytes, depending on the sample. The substances could be identified by a MS-library and their contribution to the aroma of a wine was measured. In the *Paenibacillus*-sample, 43 known substances were produced, 15 of them already had an odor description in the literature. Alcohols as 2-phenylethanol (rose-like), aldehydes as 2-phenylacetaldehyde (whitethorn), ketones as 2-pentanone (sweet, ether, fruity) and also sulfur-containing substances as dimethyl disulfide (onion, cabbage, putrid). Some of the detected substances are described as aroma compounds that generally

occur in red wine. The *Sporobolomyces roseus*-sample showed 34 substances. 13 of them could be identified. In general, there were found more alcohols and esters than in the previous sample, but altogether they were relatively similar. In the last sample, inoculated with *Aureobasidium pullulans* a total amount of 45 substances was detected. Odor descriptions were found for 24 of them. Alcohols and aldehydes together made more than two-thirds of these aroma compounds.^[19]

Varela et al.^[17] were also focusing on “wild” yeasts, found on grapes. They were comparing wines from the same grape and vintage, produced under the same conditions, except for the fermentation. One pair of wines was fermented with the indigenous yeasts and the other pair was inoculated with a starter culture of *Saccharomyces*. All the wines were analyzed for volatile and non-volatile compounds of grape and yeast origin.

Starter culture yeast strains are often used, because they produce a predictable sensory profile of the wine. But some winemakers refrain from using those yeasts because of this predictability. Wild yeast fermentations are often associated with an extended lag phase and fermentation time, higher residual sugars and with unpredictable by-product and off-flavor formation. But these fermentations are also associated with greater wine body, unusual or odd aromas and flavors, creamy texture and greater complexity.^[17] *Saccharomyces*-strains are not totally suppressing the indigenous strains, but limiting their growth to a certain extent. So, they still have an influence on the composition of the wine. Non-*Saccharomyces* species are more present in the beginning of the fermentation. Some strains are sensitive to ethanol and some decline during the rise of *Saccharomyces*. Species as *Candida stellata* or *Candida colliculosa* persist during fermentation and often complete fermentation, even when *Saccharomyces* is present. Other populations have only shown weak fermentation properties and are therefore overpowered by the inoculated yeasts. Varela et al.^[17] compared different wines, but no statistically significant differences were found, only slight trends were observed. H₂S tended to be higher in the uninoculated wines, whereas no trend for dimethyl sulfide or carbon disulfide was detectable. Of the terpenes, only α -terpineol was found to be higher in uninoculated wines, but it was suggested that concentrations of DMS, CS₂, linalool and α -terpineol are more dependent on the grape juice than on the yeast. They could not be defined as “wild yeast fermentation” character. Significant differences were noted in contents of the higher (fusel) alcohols 2-methylpropanol and 2-

methylbutanol, the acids 2-methylpropanoic and 2-methylbutanoic acid and the ethyl esters ethyl 2-methylpropanoate, ethyl decanoate and ethyl dodecanoate. They seem to exist in higher concentrations in “wild yeast” fermentation wines. But even though higher alcohol content in uninoculated fermentations exceeded the content in inoculated fermentations, the levels were still under their threshold value. An interesting fact is that the levels for 2-methylpropanol, 2-methylbutanol as well as the acids 2-methylpropanoic and 2-methylbutanoic acid are significantly higher for wild yeasts. Those compounds arise from the degradation of the amino acids valine and isoleucine (Ehrlich-pathway). The observations suggest that in indigenous yeasts, this Ehrlich-pathway is very active for valin and isoleucine metabolism. Some of the volatile compounds associated with the sensory descriptors in Chardonnay wines, such as the alcohols 2-methylpropanol and 2-methylbutanol, the ethyl esters ethyl 2-methylpropanoate, ethyl hexanoate, ethyl decanoate, the acetate ester 3-methylbutyl acetate and the acids hexanoic and octanoic acids were not only significantly different, but also assisted in distinguish between inoculated and indigenous yeast fermentation wines. In general, the wild yeasts in this study managed to produce 17 compounds in a concentration over their sensory threshold. In comparison, the inoculated yeast produced 20 aroma substances over their sensory thresholds. But only 3 of them were just produced in indigenous yeast fermentation. Further work needs to be done to link 2-methylpropanol, 2-methylbutanoic acid and ethyl dodecanoate to the “wild yeast fermentation” character. Even if these three volatiles in pure form are not considered to be very desirable odors, they might be in combination with other aroma compounds and contribute to the overall aroma of native yeast wines.^[17]

The fermentation with *Saccharomyces*-strains is much more predictable, but nevertheless the resulting aroma profile can be modified by certain factors. Different yeast strains and species, different fermentation techniques or temperatures and changes in the nutrient concentrations can produce different aroma compounds.

In a study of Miller et al.^[18] the production of seven volatile esters was monitored concerning seven different *Saccharomyces*-strains during fermentation of Chardonnay juice. Most of the esters do not derive from the grapes, but are formed by yeasts during fermentation. Two pathways are known for ester production. One pathway is leading over the reaction of Co-A with higher alcohols. The alcohols derive from amino acid decarboxylation and deamination, which form aldehydes,

that are subsequently reduced to higher alcohols. The other pathway is the synthesis of fatty acid esters. They are formed by enzymatic ethanolysis of acyl Co-A, which is produced during synthesis or degradation of fatty acids.^[18] Three of the seven observed yeast strains in the study were known as “low”, “medium” and “high” ester producers. A further study was taken on them to observe the effects of different nitrogen supplementation on the ester production.^[18]

Nitrogen is one of the most important nutrients for fermentation by yeasts. But there is not only one nitrogen-source for yeasts. Both, ammonium ions and α -amino acids can serve as a source of nitrogen and higher levels of them are able to increase the metabolic rate of the fermenting yeast, which results in higher levels of yeast derived esters. Ammonium ions tend to be the preferred nitrogen-source of yeasts. Addition of ammonium ions to a model grape juice increases the utilization of nitrogen by yeasts but may delay and reduce utilization of amino acids.^[18] Amino acids can be directly and indirectly involved in the production of volatile esters. The direct involvement implies that the actual amino acids serve as precursors for different esters. An indirect involvement signifies the support of cellular metabolic activities, which also leads to an increased amount of esters in the wine. Since utilization of ammonium and α -amino nitrogen is interrelated, it follows, that the effect of disturbing the quantitative balance between those two nitrogen-sources may lead to a different ester production profile.

For acetate esters, significant differences were visible due to different yeast strains. Ester production levels were examined during the whole fermentation process, but in all cases, the highest concentration of esters was detected at the midpoint of the fermentation, when suddenly weight loss initiated. Ethyl acetate and isoamyl acetate concentrations remained stable, whereas hexyl acetate clearly decreased. After the midpoint of the fermentation it's formation rate was less than the amount of volatilization and hydrolysis. In the end, the content of hexyl acetate was too low to be above its aroma threshold. Ethyl acetate was as well below it's aroma threshold, but isoamyl acetate was above odor threshold levels in all yeast strains.

Varying the yeast strain also had a clear influence on fatty acid ethyl esters. The rates of accumulation, maximum concentrations and final concentrations of fatty acid ethyl esters were monitored for different yeast strains. Ethyl butyrate generally remained stable after reaching maximum concentrations. Ethyl hexanoate concentrations were quite similar to hexyl acetate levels. After reaching it's

maximum at the midpoint of fermentation, the concentration began to decrease. The theory behind is the same as for hexyl acetate. The formation of ethyl hexanoate after the midpoint of fermentation was lower than the volatilization and hydrolysis, therefore its levels went down. Ethyl octanoate in most strains had just a slight decrease after reaching its top levels. Nearly the same was with ethyl decanoate.

Nitrogen supplementation and the effects of it on the volatile ester production was investigated on three of the seven yeast strains. Diammonium phosphate (DAP) and amino acids (AA) in a level, still within the suggested rate for fermentations were added to the grape must and the fermentation was carried out under typical conditions.

The results of this study ^[18] were more or less predictable. The effects of nitrogen source on the kinetics of ester production were dependent upon the yeast strain utilized in the fermentation. Only one of the three strains was sensitive to nitrogen supplementation, the other two strains were therefore left apart in the further experiments of this study. The dynamics of the ester formation varied of course as well with the kind of supplementation (DAP, amino acids or no supplementation). Concerning ethyl acetate, the highest maximum and final concentrations were found for DAP treatment, the lowest for AA treatment. Isoamyl acetate showed similar results and additionally, DAP treatment yielded in the fastest isoamyl acetate production in comparison to the other proceedings. Hexyl acetate concentrations reached similar levels in the end, although maximum concentrations were higher for DAP addition than AA addition. Ethyl butyrate contents did not differ greatly between the methods, ethyl hexanoate had lower concentrations in AA supplementation than in the control or DAP group. Ethyl octanoate and ethyl decanoate were not influenced positively by nitrogen supplementation. Altogether it was clearly visible that amino acid addition as a nitrogen source for yeasts did not have a great influence on the production of volatile esters, whereas in the groups of DAP addition an increase of utilized ammonium and formation of more esters could be seen.

Amino acids are said to be precursors for esters, in particular acetate esters. But the results in the study of Miller et al.^[18] showed that amino acid supplementation did not result in higher ester concentrations. Therefore, some other biological pathway for the formation of esters has to exist. Biosynthetic processes can also entail the formation of esters and DAP supplementation would increase the activity of those

processes because of providing the necessary amounts of nitrogen for the biosynthetic formation of esters.

A recent study of Hernández-Orte et al.^[20] showed that wines from musts, supplemented with ammonium, are richer in ethyl lactate and 3-hexenol and wines supplemented with amino acids are richer in γ -butyrolactone and isobutanol. From the sensory point of view, must supplementation with ammonium results in a decrease in “sulfury” notes and in an increase in “citric” flavor. The effect of amino acid supplementation on the wine composition depends on the yeast strain. In one case, the composition was similar to the composition of wines, supplemented with ammonium ions; in the others, an increase in “fruity” and “fusel” notes was obtained.^[20]

Alcoholic fermentation by yeasts is not only influencing esters formation, but also for e.g. volatile thiols in Sauvignon Blanc. Dubourdieu et al.^[21] were working on the significance of yeasts in the development of volatile thiols. They have chosen to observe the amounts of four thiols namely as 4-mercapto-4-methylpentan-2-one (4MMP), 4-mercapto-4-methylpentan-2-ol, 3-mercaptohexanol and 3-mercaptohexyl acetate. These thiols are all absent in grapes or musts, but they are present in wines, mainly Sauvignon blanc wines, after running through alcoholic fermentation.^[21] 4MMP was already found to have an odor threshold of 0.8 ppt and it's presence in wine is about 40 ppt. So it definitely has an impact on the flavor of Sauvignon blanc. It's flavor can be described as “boxwood” and “broom-like”, but it was also found to contribute to “guava”-flavor of several wines. 3-mercaptohexyl acetate (3MHA) has an odor threshold of 4 ppt and it's appearance in wine is as much as several hundred ppt. 3MHA has a complex odor, combining “boxwood”, “grapefruit peel” and “passion fruit”. 3-mercaptohexan-1-ol (3MH) also has “grapefruit” and “passion fruit” notes like 3MHA and it is present in every Sauvignon blanc wine, always above its perception threshold. 4-mercapto-4-methylpentan-2-ol (4MMPOH) has a more limited role in the aroma of Sauvignon blanc, its concentration is mostly below its sensory threshold. 3-mercapto-3-methylbutan-1-ol (3MMB) was also mentioned, but like 4MMPOH its sensory properties are defined by a very high odor threshold. Undeniable, 4MMP and 3MH have a big influence on the aroma of Sauvignon blanc, whereas the other volatile thiols mentioned above are more adding a complexity to the aroma than a certain sensory attribute.

Interestingly, tasting grapes or must of Sauvignon blanc, leads in a certain extent to the perception of the typical aroma of the wine. Enzymes of the oral flora can transform the precursors into aroma substances, if the must is kept long enough in the mouth. 4MMP, 4MMPOH and 3MH can be generated out of non-volatile Sauvignon blanc must with the cysteine conjugate β -lyase-activity of gastrointestinal bacteria. This strongly indicates that there is a precursor for these volatile thiols, having a structure of S-cysteine conjugate.^[21] Those cysteine conjugates can form volatile thiols by cleaving the C-S linkage to cysteine. This is simply done by a β -lyase, or during fermentation by *Saccharomyces cerevisiae* strains. There are differences in yeasts strains, concerning the release of volatile thiols, depending not only on the amount of sulfur-containing precursors, but also on the yeast itself. Some *Saccharomyces bayanus* var. *uvareum* strains have been reported to have a high capability to release volatile thiols from their cysteine-conjugates. But they are also capable to produce higher levels of β -phenylethyl alcohol and its acetate, which are masking the typical aroma of Sauvignon blanc. Therefore, interspecific hybrids between *S. cerevisiae* and *S. bayanus* var. *uvareum* have been bred and tested. Seven out of nine hybrids showed a great capacity in cleaving the cysteine molecule from the thiol-precursor without exceeding in β -phenylethyl alcohol or acetate production.^[21]

A group of researchers in Australia have already published an article about *S. bayanus* strains in alcoholic fermentation of Chardonnay must and their contribution to the chemical and flavor composition of the wine.^[22] They compared the chemical and sensory analysis of a Chardonnay wine, made with *Saccharomyces bayanus* var. *uvareum* with the same wine, fermented with *Saccharomyces cerevisiae*. In general, fermentation with *S. bayanus* resulted in a greater content of higher alcohols (for example 2-phenyl-ethanol), acetate esters (isoamyl acetate, 2-phenyl acetate) and ethyl lactate. In particular, the sensory descriptive analysis of the *S. bayanus* fermented wines showed that eight of the eleven attributes, chosen to score the wines, were strongly significant for the aroma composition. Those are: “estery”, “pineapple”, “peach”, “citrus”, “orange peel”, “yeasty”, “nutty”, “aldehyde”. The wine made with a *S. cerevisiae* strain had higher scores for “estery”, “pineapple”, “peach” and “citrus”, whereas the other characteristics as “aldehyde”, “nutty”, “yeasty” and “cooked orange peel” were higher for different *S. bayanus* strain fermented wines.^[22]

Surprisingly, the content of malic acid in wines using *S. bayanus* was lower than in wines using *S. cerevisiae* or in the original, unfermented grape must. *S. bayanus* was actually known for the production of malic acid, but this study showed, that prior results were based on the choice of the strains, not on the *S. bayanus* population in general.

In sensory analysis it was noted positively, that *S. bayanus* enhances in particular the attribute “cooked orange peel”, which was also identified as “cooked apricot”.

It’s importance in winemaking is still discussed, but probably its chance lies in the combination with conventional *S. cerevisiae* strains, as already has been tried with *Candida stellata*, or in blending wines made from *S. cerevisiae* on the one hand and *S. bayanus* on the other hand.^[22]

Not only alternative yeast strains, such as *S. bayanus*, but also the “commercial” yeast strains of *Saccharomyces cerevisiae* and their effects on the odorless precursor fractions in grape musts have been studied so far. A study of Loscos et al.^[23] has focused the interest on the determination which aroma molecules are released from odorless precursor fractions during fermentation by the action of yeasts and the assessment of the potential sensory role, played by those molecules in wine aroma.

The effect of the yeast strain on the composition was significant not only for compounds of known fermentative origin, like acetate esters or fatty acid ethyl esters, but also for other compounds with varietal origin, such as (*E*)-2-hexenol, (*E*)-whiskylactone, δ -octalactone, γ -nonalactone, o- and m-cresols, 4-ethyl-2-methoxyphenol, 4-allyl-2-methoxyphenol, 4-vinylphenol, zingerone, actinidiols, 3,7-dimethyl-1,5-octadien-3,7-diol, and farnesol.^[23] The concentrations of those compounds can vary a lot, depending on the yeast strain used.

An addition of a precursor fraction resulted in an increase of compounds of nearly all biochemical origins. However, levels of some compounds also decreased in comparison to the non-supplemented control-sample. The increments of the aroma compounds in supplemented wines and non-supplemented wines were measured and listed and three different patterns were obtained. In the first pattern, increments, caused by supplementation, of nearly the same amount for the three yeast strains were combined. Examples therefore are volatile phenols, vanillins and some norisoprenoids. In the second pattern, increments, that were all positive for the three yeast strains, were combined, as γ -lactones, terpenes and cinnamates, and in the third pattern, increments that were all negative, were combined, which means that the

precursor-supplementation lead to a decrease of aroma substance production. Examples of this are β -ionone, linalool, δ -decalactone or 4-vinyl-2-methoxyphenol. Interestingly, the yeast strains that failed to produce the substances in the supplemented fraction, were the most effective producers of those substances in the non-supplemented fractions.

In terms of sensory effects, only four compounds were found to be present over their sensory thresholds. β -damascenone, β -ionone, 4-vinylphenol and 4-vinyl-2-methoxyphenol. In the case of ethyl cinnamate and 2-methoxyphenol, the increments were close to the corresponding sensory thresholds.

Nevertheless, there is a large number of constituents, that increased with an addition of a flavor precursor fraction and the sum of them may influence the aroma properties of flavor precursor fortified wines. Loscos et. al.^[23] grouped the aroma constituents and characterized the effects, which were very important for the aroma sensory profile of the wines. The groups were norisoprenoids with a “fruity”, “blackberry” aroma, two fractions of volatile phenols, which had a “phenolic” or “dirty”, “unpleasant”, and “medicinal” odor, cinnamates, vanillins, terpenes and lactones. Only the first three groups (norisoprenoids and two phenolic groups) had a significant sensory impact on the wine aroma. Groups were added alone or together with other groups to the neutral wine to obtain results concerning different aroma profiles. Adding the phenolic fractions lead in both cases to an unpleasant smell, particularly in the case of vinylphenols. Only when adding terpenes and cinnamates contemporaneously, a synergic or additive effect was noticed between both groups of odorants. When three or more groups were added together, the smell of the mixture could be significantly recognized. Most of the odorants grouped for this study had a “flowery” and “sweet”, sometimes “fruity” note. Just in case of the phenolic compounds the smell was unpleasant. When all of the groups were added to the neutral white wine, the resulting aroma could be described as mainly “floral”, “sweet”, “fruity” and a little bit “citric”, but very close to the mixture of cinnamates, vanillins, terpenes and lactones in a neutral white wine.

Altogether there have been identified about 20 different compounds contributing to the “floral” aroma of a wine and it has also been found, that most of the volatiles are not derived from a “simple” hydrolytic process, but from yeast metabolism, which has a complex and not yet understood regulation.^[23] These processes still need to be further investigated.

3.3 Malolactic Fermentation

Once the alcoholic fermentation is completed, white wine is in most cases ready to be stored in oak barrels, left in contact with lees or not. Red wine still has to undergo the pressing, which is, apart from few exceptions, carried out after the alcoholic fermentation. Another important step for most of the red, but barely for the white wines, is the malolactic fermentation (MLF). This second fermentation step is still contended and it is the winemakers decision whether the wine is taking this step or not. The main purpose of the malolactic fermentation is to reduce the acidity, because of a transformation of (*L*)-malic acid into (*L*)-lactic acid with the release of carbon dioxide. MLF is carried out by lactic acid bacteria. Numerous different genera such as *Oenococcus*, *Pediococcus* and *Lactobacillus* are capable to conduct MLF in wine. One of the most frequently used species is *Oenococcus oeni*.

Malic acid is characterized of giving a tart-tasting, acid note to the wine. Wines made from grapes of cooler regions often have high levels of malic acid. A certain acidity is necessary to sustain the microbiological stability of a wine. But also the taste of the wine is dependent upon a certain level of acidity. The acid adds a sharpness to the flavors and is detected immediately by a prickling sensation on the sides of the tongue and a mouth watering after taste. A very important factor of a well tasting wine is not only the total amount of acids in the wine, but also the balance between the acidity and sweetness (residual sugar content) and bitterness respectively (tannins and other phenolics). Reducing the malic acid with its strong, tart-like taste and transforming it into lactic acid makes it possible to produce wines of greater palate softness and roundness. If the level of malic acid is too low, the pH level rises and microbiological contamination may occur. Also the taste of the resulting wine can change from a soft and round mouthfeeling to a flat tasting wine with a note of “buttery” aroma, derived from diacetyl (2,3-butanedione), which is released from the lactic acid bacteria (LAB) during fermentation.

However, flavor modifications, evoked by MLF, are far more complex than just an increase of diacetyl. MLF is said to change “fruity”, “flowery” and “nutty” notes and to reduce “herbaceous” and “vegetative” notes.^[24] These modifications are unlikely the result of an increased diacetyl concentration. But the actual mechanisms responsible for the change of the aroma profile are not yet understood. Different authors get varying results concerning the composition of wines, that went through

MLF. Some report an increase of esters and some report a decrease of the same substances. It is likely, that the concentrations of the components vary with the bacteria strain used for the MLF, like the components vary in wines, that went through yeast derived alcoholic fermentation with different yeast strains.

LAB are persistent in wine until their growth medium reaches a pH around 3.0 to 2.9. Thus, the bacteria starts to reduce acidity through transformation of the malic acid (a dicarboxyl acid) to lactic acid (a monocarboxyl acid) by the malolactic enzyme, which is unique and only known in LAB. The metabolism of the bacteria appears to form ATP out of ADP, which may be the source of energy for the LAB. However, the initial energy source is still unknown. LAB not only converts malic acid into lactic acid, but also takes part in the citric acid metabolism and therefore LAB may also influence the acetoin and diacetyl concentration in wine. Amino acids, such as arginine, may serve as well energy source and provide the essential growth factors, necessary for the bacteria. During MLF the LAB gather acetaldehyde and other carbonyl compounds. Their metabolism may slow down the MLF.

Osborne et al.^[25] suggested, that the degradation of acetaldehyde and other aldehydes by LAB in wine may contribute to the decrease of herbaceous aroma.

Ugliano et al.^[24] investigated the general effect of different *Oenococcus* starter cultures on the volatile composition of wine. Altogether, they measured 40 volatile compounds, including esters, alcohols, acids, lactones and sulfur and nitrogen compounds. Significant changes in the composition were observed in the fraction of the volatile esters, as a result of MLF. In particular, the levels of diethyl succinate and ethyl lactate showed the largest increase. Ethyl lactate in wine is present as a mixture of enantiomers. In particular the (*S*)-enantiomer is present in relatively high concentrations. But still, the total concentration of ethyl lactate is somehow under the odor threshold of (*S*)-ethyl lactate. The levels of ethyl fatty acid esters (4-10 carbons) were also relevantly influenced by MLF. Increases between 2-28% for C₄, 22-34% for C₆, 85-89% for C₈ and 20-31% for C₁₀ were noted. Acetate esters, such as the powerful odorant isoamyl acetate, which provides banana notes, rose, as expected, during MLF as well as 2-phenylethyl acetate, although in the end it did not reach its odor threshold. Ethyl 3-hydroxybutanoate levels augmented for all the four *Oenococcus* strains.

As a whole, for all the bacterial strains tested, MLF resulted in a significant change of the overall ester profile of wine, with ethyl fatty acid esters becoming quantitatively the most representative class of esters after MLF.^[24]

There is a great inconsistency of experimental results whether ester production increases or decreases during MLF. However, Ugliano et al.^[24] found an increase for esters in the tested wines. MLF did not have major influences on the concentration of higher alcohols, but important wine odorants such as isoamyl alcohol and 2-phenylethanol, which can be characterized by “herbaceous” and “floral/rose” notes, increased significantly for one of the four *Oenococcus* cultures. No statistically important increases were found for volatile short-chain fatty acids during MLF, which are linked to off-flavors and “cheesy” aromas. But levels of methionol (3-(methylthio)-1-propanol), which provides an odor of “cooked cabbage”, tended to rise through MLF, but were still far below under the odor threshold. Nevertheless, in white wines, methionol can be oxidized to methional, an important contributor to the typical flavor of oxidation-spoiled wines. In the study of Ugliano et al. however, the tested red wine did not suffer from methional caused off-odors.

Summing up the effects of *Oenococcus* cultures on red wine in this study, the concentrations of several esters rose and MLF is getting more and more interest concerning the modification of the aroma profile of wines, considering the positive contribution of the increased components on the total aroma of a wine.

MLF can also appear unmeant by indigenous bacteria strains, that are present in the grape must. Pozo-Bayón et al.^[26] investigated the effects of indigenous lactic acid bacteria on a red model wine, comparing two indigenous LAB strains, *Oenococcus oeni* and *Lactobacillus plantarum*. One of the first differences noticed between the two strains was the metabolic activity of how effective the MLF was carried out. The *L. plantarum* culture needed a higher amount of malic acid to start the MLF. Apart from the metabolic activity, there are significant differences in the volatile composition of the wine fermented with *O. oeni* and *L. plantarum*. In all cases, the levels of ethyl lactate increased up to 4 times in comparison to the wine before MLF, reaching slightly different concentrations, depending on the LAB strain used. The concentration of ethyl decanoate declined. No significant differences in the concentrations of “fruity” esters hexyl acetate and 2-phenylethyl acetate were observed. Fatty acids did not show significant increases either. In this study, seven of the observed 25 volatiles were found to differ, dependend on the LAB strain used for

MLF. These are butyl acetate, ethyl acetate, ethyl decanoate, octanoic acid, decanoic acid, acetaldehyde, and γ -butyrolactone.

So in both, the studies from Ugliano et al.^[24] and Pozo-Bayón et al.^[26], the volatile composition of a wine that went through MLF is dependent on the lactic acid bacteria strain used. In general, the effect of MLF is considered as positive for the wine.

Boido et al.^[27] investigated the effect of different bacterial strains on the composition of Tannat red wine, the most important red wine of Uruguayan viticulture with aromas in moderate intensities of raspberry, plum, quince and small-berry-like notes. The aroma profile was studied during MLF and its development was monitored during bottle aging.

Thirty-seven volatiles, including alcohols, esters, carbonyl compounds and acids were identified and measured before and after malolactic fermentation. Significant differences in the concentrations were noted for 17 of those compounds and the changes in the concentrations of 8 more substances were considered as minor significant. Predictably, the level of ethyl lactate rose for both *Oenococcus oeni* strains. The production of other esters, like isoamyl, isobutyl, hexyl and 2-phenylethyl acetate, which contribute to a wines fruity character, showed very strain dependent differences. Ethyl hexanoate decreased, while with ethyl octanoate and decanoate no significant differences were observed. The levels of diethyl succinate and γ -butyrolactone increased only for one of the two used strains. Several alcohols presented a slight increase, but the sum of alcohols did not show any significant variations in comparison to the control wine. Only 1-hexanol differed denotatively. Of the acids, only butanoic and isobutyric acid had higher levels in the malolactic fermented wines.

Furthermore, the study followed the aging process of the wine in the bottle. Ethyl lactate even more increased during bottle aging, but just in the wines that went through MLF. Ethyl lactate also increased in the control wine, but after one year of aging there was a remarkable difference in the ethyl lactate concentration between the fermented and the non-fermented wine. On the other hand, a major increase of diethyl malate was visible after one year of bottle aging in the non-fermented control wine. This is not surprising at all, considering the fact, that in the non-fermented wine a much higher concentration of malic acid was noticed than in the wine that went through malolactic fermentation. The changes in the acetate levels were higher

for the control wine than for the malolactic fermented wine, probably as a consequence of the higher acidity in control wines, due to the higher content of malic acid. Aging of samples of both, fermented and control wine increased the concentrations of γ -butyrolactone and diethyl succinate, but higher levels were attained in the samples of the malolactic fermented wines.

Altogether, the sensory attributes of the wines were analyzed and decreases of “raspberry”, “black currant”, “apricot”, “green bell pepper” and “cut grass” and increases of “quince”, “butter”, “coffee” and “musk” were noted in the wine, inoculated with one of the two *Oenococcus* strains, while in the wine, inoculated with the other *Oenococcus* strain was observed a decrease of “cherry”, “dried fig” and “plum” and an increase of “apricot”, “cut grass”, “butter” and “yeasty” descriptors. ^[27] The differences between the control and the fermented samples could be correlated with the decrease in the odor activity values of some esters. This is the case of the decrease of the “raspberry” descriptor, which can be correlated with the decrease of isoamyl acetate, ethyl hexanoate and ethyl ester sum. The decrease of the “cherry” and “dried fig” descriptors can be correlated with ethyl ester sum, and the “apricot” descriptor can be matched with isoamyl and hexyl acetate, ethyl octanoate and acetates sum. The increase of the “herbaceous” notes or the “cut grass” descriptor can be explained by an increase of pantolactone acids (butanoic and 2-methylpropanoic acids), methionol and γ -butyrolactone. And the increase in the “butter” descriptor is linked to the diacetyl concentration. Higher “musk” notes can be the consequence of higher vinylphenolic compounds. For the “yeasty” descriptor no possible match was found in the substances dealt with in this study. The study of Boido et al. ^[27] indicated, that an aging period, after vinification, contributed to the change in differences between wines with and without MLF and furthermore between strains.

Malolactic fermentation can be carried out in different vessels, which may contribute to the development of certain aroma compounds. If the MLF is done in an oak barrel, it is very likely, that the resulting wine is containing certain oak-notes, additional to the aroma substances derived from MLF.

3.4 Fining

Fining processes can be carried out at many points in the whole winemaking process. Some winemakers prefer fining their wine before alcoholic fermentation to improve the sensory characteristics of the wine, some fine their wines after fermentation and before storing the wines in oak barrels and others want to fine the wines after barrel and before bottle aging. It is clear, that fining has an influence on the composition of the wines, since a lot of substances are taken out of the wine during fining. Many studies tried to investigate the differences between fined and non-fined wines, and the differences between the certain fining and clarification treatments and their effects on the wines.

The most widely spread clarification treatment, before alcoholic fermentation, is the spontaneous settling at low temperatures.^[28] An addition of pectic enzymes may help in this process to settle the bigger solids faster and to reduce the fraction of pectic substances. Other fining agents, such as bentonite, casein, silica gel, activated charged charcoal and gelatine may be used as well. Their charge is opposite to the charge of the solids in the must, allowing them to precipitate together. During these precipitation processes also other substances, like aroma compounds may precipitate or may be adsorbed onto the precipitate, as well as the content of nitrogen, necessary for yeast growth and fermentation activity, is reduced drastically by the fining agents through adsorption.

Moio et al.^[28] investigated the effects of five different fining processes on the aroma composition of the wine. Spontaneous settling (SS), spontaneous settling with addition of pectic enzyme (E), spontaneous settling with pectic enzyme, followed by filtration (EF), clarification with fining agents after spontaneous settling with addition of pectic enzyme (EC) and filtration of juice, previously treated with fining agents after spontaneous settling with addition of pectic enzyme (ECF). In this study they found a greater loss of glycosides when more grape solids were removed. EC and ECF treatments reported the biggest losses in aroma compounds, although filtration did not seem to have a big influence. A loss of glycolysated aroma precursors in wine results in fewer free volatile compounds, such as linalool or benzyl alcohol, must clarification may also signify a reduction of grape varietal character. A large decrease of glycosylated precursors of geraniol, benzyl alcohol and 2-phenylethanol and a minor decrease of glycosides of linalool and eugenol were

measured. Even if more studies in this topic are necessary, it is obvious, that the chemical composition changed due to the clarification treatment. A bigger loss of volatiles was detected when fining agents such as bentonite, potassium caseinate, charcoal and gelatine were used.

Fining treatments carried out after fermentation and before barrel maturation yield different results than fining treatments before alcoholic fermentation. Jiménez Moreno et al.^[29] studied the influence of a wine turbidity, by analyzing fined and non-fined wines before and after barrel aging. Furanic aldehydes were present in higher concentrations in filtered wines than in unfiltered wines, whilst its reduction products, the furanic alcohols were found in much lower concentrations. Probably, the microbial biomass, which transfers furfural to furfuryl alcohol was removed during the filtration process. The furfural release from the woods was much faster in filtered, limpid wines than in unfiltered, turbid wines. Phenolic aldehydes like vanillin had higher levels in unfiltered wines, suggesting that the filtered wines presented a smaller microbiological activity and therefore the reduction of vanillin to vanillyl alcohol and other non-aromatic compounds took much more time. It is also possible that vanillin, after extraction from the wood, was bound to fermentative lees, which were present in a higher concentration in unfiltered wines. Some lees also showed a big affinity for 4-ethylguaiacol and 4-ethylphenol and retained them from the wine, which resulted in the reduction of off-flavors in the sensory profile of the wines. The filtration treatments had no influence on the oak lactones. These compounds did not show any affinity for the yeast lees during the maturation in the barrels. γ -Nonalactone as well did not show any changes through the filtration process. The only lactone which was effected by filtration was γ -butyrolactone. Its concentration was higher in the unfiltered wine. For the phenolic compounds, 4-methylguaiacol, phenol, *p*- and *m*-cresol presented higher concentrations in the filtered wines, whereas the concentrations of 4-propylguaiacol and eugenol were higher in the unfiltered wines. This indicates, that 4-methylguaiacol, phenol, *p*- and *m*-cresol may be retained by yeasts and other macromolecules, present in higher amounts in unfiltered wines than in the filtered samples. However, 4-propylguaiacol and eugenol did not bind to these molecules during barrel aging.^[29]

Aging wines on the lees is more often carried out for white wines. It refers to the French “sur lies”, which means that the wine is not racked/filtered after fermentation, but altogether put into the bottle. It is said to add a “toasty”, “nutty”, and “hazelnut”

quality and additional depth and complexity especially on the finish. But also a decrease of some oak volatiles, which can be adsorbed on yeast particles, is possible. Loscos et al.^[30] showed, that storage on yeast lees had a clearly visible influence on the chemical composition of the wines in comparison to simple acid hydrolysis, which was carried out in the control samples. Many components are fairly unstable in the samples with lees, at least the half times of many substances are smaller than in samples without lees. Especially in the cases of linalool, β -citronellol, β -damascenone, β -ionone, guaiacol, vanillin, benzaldehyde, ethyl cinnamate, 2-phenoxyethanol and methylbutyric acids the reduction during storage on lees is remarkable. In the cases of α -terpineol, syringaldehyde, homovanillic acid, phenylacetaldehyde and γ -nonalactone the reduction was more moderate. Finally, it is necessary to point out, that lees from different yeast strains yield different patterns and result in different aroma profiles.^[30]

Lambri et al.^[31] examined the effects of bentonite fining on a wines composition and described the interactions of bentonite and aroma-active compounds in their study.

The adsorption properties of bentonite are mainly due to cation exchange action. Bentonite is mostly used to reduce the protein content in wines, which are a potential source of haze. But it is not specific for proteins, bentonite also removes other substances, like flavor components and therefore also the sensory properties of a wine. The most significant reductions were observed for the substances ethyl butyrate, ethyl hexanoate, ethyl octanoate, isoamyl acetate, phenylethyl acetate, β -phenylethanol, 1-hexanol, hexanoic acid and octanoic acid. Ethyl butyrate depleted in all the samples, but its residual concentration was still over its sensory threshold. Ethyl hexanoate removal was significantly affected by bentonite sample and wine style, ethyl octanoate removal was affected by bentonite dose, but not from bentonite type or wine style. Ethyl butyrate and ethyl hexanoate are probably removed by the same mechanism. Interactions between these substances and the proteic hydrophilic colloid are suggested by Lambri et al.^[31], as well as interactions with yeast proteins. Isoamyl acetate levels were reduced as well, but the reduction was not related either to dose or to type of bentonite and did not differ a lot between the wine styles. This leads to the assumption that isoamyl acetate is linked to macromolecules less susceptible to removal by bentonite. Phenylethyl acetate levels varied significantly with bentonite dose and wine. It is possible, that an interaction between the aromatic ring of the molecule and hydrophobic protein sides is taking place. The other option

is that phenylethyl acetate is directly adsorbed onto bentonite and therefore removed from the wine. A similar behaviour was found for β -phenylethanol. Its reduction was affected by bentonite dose, but not by bentonite type or by the wine. It is probably interacting with proteins through the same mechanism. The 1-hexanol concentration was dependent upon both, bentonite dose and type, which indicates a direct interaction with bentonite.

Among the fatty acids, hexanoic acid was affected a lot by bentonite dose and wine style, but the strong octanoic acid removal did not vary a lot with dose and type of bentonite, or with wine style. Octanoic acid is probably linked to proteins, which are removed by bentonite, because of its strong hydrophobicity.

The effect of bentonite fining on the aroma substances in wine is mainly depending on the chemical nature and initial concentration of the volatiles, but there is a proved interaction and modification in a wines composition when it is fined with bentonite.

Once the wine is fined, it can be stored and aged in barrels, or, if this step is not desired, directly bottled.

3.5 Barrel Aging

Oak maturation is an important step in the making process of many dry red and fully-bodied white wines. It increases the complexity of a wine's aroma profile by extraction of additional aroma compounds from the oak wood, the subsequent transformation of these by wine microorganisms and the generation of additional volatile wine components by such microorganisms.^[32]

3.5.1 White wine

Spillman et al.^[32] compared the sensory properties of Chardonnay and Cabernet Sauvignon wines and explored the possible contributions of compounds, associated with barrel maturation to the aroma profile of the wines. They took control samples and stored them in stainless steel vessels for the same period like the wine samples in the oak barrels. Substantial barrel to barrel variations in the composition of the wines resulted from different oak sources, different toasting intensities of the oak barrels or different microbiological activity during the aging process.

For the Chardonnay wine, the most intense aroma in comparison to the stainless steel-stored sample was the “green apple” aroma, which could be correlated to guaiacol, maltol, furfural and furfuryl alcohol and 4-ethylguaiacol. Most of the aroma compounds (guaiacol, 4-methylguaiacol and 4-ethylguaiacol) were strongly associated with a “smoky” character and also had a strong, but negative correlation with the “green apple” descriptor. Vanillin and furfural are also known to derive from the heat, that is necessary for toasting in the process of cooperage, and are therefore incidentally correlated with the “smoky” aroma. Perhaps, these compounds are responsible for the suppression of the “green apple” aroma. Mutual suppression of different aroma compounds is a well known phenomenon, but its occurrence and extent of suppression of fruity characters depends on the degree of barrel toasting. The naturally occurring oak components *cis*- and *trans*-oak lactone and eugenol had no suppressing effect on the “green apple” aroma.

Some of the Chardonnay samples went through malolactic fermentation. A strong correlation between “butter” aroma and the malate consumption was found. This could be associated with the “buttery/caramel” smelling component diacetyl, which

is commonly produced during MLF. “Butter” was also positively correlated to furfuryl alcohol, which increases in wines that went through MLF, and negatively correlated to furfural, which is reduced in this process. This system maybe also works backwards, which means that cooperating products like trans-lactone or others, present in higher concentrations, may inhibit MLF in wine.

The aroma “caramel” was also strongly associated with malate consumption and furfuryl alcohol. Diacetyl may have an impact on this character as well, but there was no obvious correlation found between the “caramel” and “butter” aroma. Higher levels of maltol were also associated with “caramel” aroma, but its concentration in the wine samples were far below its odor threshold. The “caramel” aroma was found to correlate negatively with the natural oak components *cis*-and *trans*-lactone, eugenol, 4-vinylphenol and 4-vinylguaiacol. Reasons of this are unknown, but may be simple masking effects. The “vanilla” descriptor was correlated closest to vanillin, although this association was not statistically significant. There are different opinions about the role of “vanilla” aroma in the bouquet of oak-barrel aged wines. Vanillin plays a significant role in the flavor of barrel-aged wines, although this role is much diminished when wines are fermented in barrel and stored on yeast lees.^[33] On the other hand it has been often concluded that vanillin plays no role in the flavor of barrel-aged wines, because of its low concentration in wine and its high sensory threshold. For the descriptor “allspice” positive correlations with furfural and 5-methylfurfural and negative associations with the MLF were noted. Compounds with known spicy notes like eugenol and 4-vinylguaiacol could not be significantly correlated with the “allspice” descriptor. Coopering heat produced aroma substances like methylguaiacol, furfural and 5-methylfurfural, which were positively associated with a “pencil shavings” aroma and negatively correlated with the microbial degradation products 5-methylfurfuryl ethyl ether and furfuryl alcohol.

The “cinnamon” aroma was correlated with the vanillin, furfural and furfuryl alcohol concentration. The “cashew” descriptor could not be correlated to any of the measured compounds. In this study from Spillman et al.^[32], many compounds could be related to certain aroma descriptors, even though some of the descriptor remained uncorrelated.

Oak wood contains a lot of different substances that find their way into the wine during storage in the oak barrels. Those substances can be derived from oak wood

treatments such as seasoning and toasting during cooperage or from the oak species itself, from geographic location and single tree variations.

Natural products like oak wood have a very complex aroma composition and therefore it is very hard to distinguish the various odorants and match them with a certain aroma descriptor. To investigate the single compounds, gas chromatography-olfactometry (GC-O) was found to help much. Díaz-Maroto et al.^[34] studied the aroma composition of several Chardonnay wines that were aged with oak chips from different regions, such as America, France, Hungary and Russia, and different seasoning (non-toasted and toasted) and they tried to investigate the sensory importance of oak wood volatile compounds, others than oak lactones, and their contribution to the overall aroma of aged wines. The oak woods were extracted and the extracts measured and quantified. For the non-toasted oaks, the oak lactones were highest in the usually used American and French oak woods. Hungarian and Russian oak presented much lower oak-lactone-levels and wine aged with them may get only mild or moderate woody notes. Furfural levels increased during drying and seasoning, mainly when high temperatures were used. Other volatiles, like *trans*-2-nonenal and decanal, were identified in non-toasted oak and they were found to add an unpleasant aroma to the wine, such as “sawdust”. Their concentrations were higher in Hungarian and Russian woods, although toasting may solve the problem.

Toasting the oak woods was followed by significant changes in the chemical composition. The levels of furfural, guaiacol, 4-methylguaiacol and vanillin increased, whilst the levels for oak-lactones drastically decreased during toasting. But *cis*-oak-lactone still remained high for American and French oak. Usually, toasting increases the concentrations of oak-lactones, but if the heat during the process is too high, the oak-lactones will be thermally decomposed. In all the toasted oak samples higher concentrations of *trans*-isoeugenol were measured, and also its isomer *cis*-isoeugenol was found, which hardly exists in non-toasted oak wood, since the compound is formed through thermal degradation of polyphenolic compounds. Levels for eugenol, another polyphenol compound, were expected to rise, but they rose just in cases of Hungarian and Russian oak wood, probably because of over-toasting of the samples of American and French oak woods. Substances that only had significant levels in non-toasted woods were short- and medium-chain alkyl aldehydes, alcohols and acids like hexanol, heptanal, hexanoic acid, 2-octenal, octanoic acid, decanal and nonanoic acid with “green”, “rancid” and “sweaty” odors,

3-octen-1-one with a “mushroom-like” scent and 2,4-nonadienal, with a characteristic “fatty” and “cucumber” odor. In the non-toasted oak woods, compounds as furfural, 5-methylfurfural, 4-methylguaiacol and *cis*-isoeugenol produced very low odor intensities, while *trans*-oak lactone with its “woody/oak”, “vanilla” and “clove” to “coconut” notes presented major odor intensities in American and French oak wood, but poor odor intensities in Hungarian and Russian oak wood. *Cis*-oak lactone produced very high to maximum odor intensities in all the non-toasted samples. It is sensorially more potent than its *trans*-isomer and therefore more important for the characteristic aroma of oak wood. Eugenol, vanillin and *trans*-isoeugenol also produced high to maximum odor intensities.

It is suggested, that *cis* and *trans*-isoeugenol, together with the oak-lactones, are responsible for the highly desired “woody/oak” aroma of wines, especially of those treated wines with wood, poor in oak lactones.^[34]

Toasting the woods was followed by significant changes in the chemical composition, due to the heating treatment during coopering. It was observed that some odorants were only detected in non-toasted oak woods and others were only present in toasted oak woods. The elimination or at least a major reduction of the “fruity”, “fresh” and “floral” odors, as well as some undesirable ones, such as “sawdust”, “mushroom” or “tar” was perceivable after toasting the oak wood. Substances with higher concentrations after toasting were furfural and 5-methylfurfural, since they were formed of the degradation of complex wood carbohydrates, and guaiacol, 4-methylguaiacol, eugenol, *cis*- and *trans*- isoeugenol, formed by thermo-degradation of complex polyphenol compounds. *Cis*- and *trans*-oak lactones presented very high odor intensities in toasted and non-toasted oak woods of America and France, whilst they were hard to detect in Hungarian and Russian samples. For aldehydes, concentrations of hexanal, nonanal and 2,6-nonadienal declined and those of heptanal, decanal, 2-octenal, 2,4-nonadienal and *trans*-cinnamaldehyde in fact went down to a minimum. Only one odor was still remarkable high. *Trans*-2-nonenal concentrations remained high in Hungarian and Russian oak wood samples and it could be correlated with an unpleasant aroma of “sawdust”. Some volatiles, not reported in any previous paper, were found in stronger intensities in Hungarian and Russian wood, like 1-(2-furanyl)-ethanone with a “toasty” note, cyclotene with a “sweet”, “toasty” and “caramel” note and phenyl acetaldehyde as well with a “toasty” scent.

Since the fermentation or storage in oak barrels is difficult to handle and to carry out, new ideas of getting the “woody/oak”-scent into the wine were coming up. For example, the use of oak chips allows the fermentation to be conducted in stainless-steel tanks under the required temperature, and the amount of substances transferred from the wood into the wine can be controlled a lot better. Pérez-Coello et al.^[35] investigated the effect of oak chips on the fermentation of white wines, to determine the quantity of oak chips necessary to produce an acceptable chemical and sensory profile of the white wines.

The wines were fermented with chips from French oak from Allier, Vosges and central France and from American oak. The control control samples were fermented under the same conditions, except for the oak chips that were left apart. The volatile composition presented differences in the concentrations of several alcohols (isobutanol, 2-methyl-1-butanol, 3-methyl-1-butanol and 2-phenylethanol), acetates (isoamyl acetate and 2-phenylethyl acetate) and some esters (ethyl caprylate, ethyl caproate and ethyl lactate). All of those substances had higher levels in the wines fermented with oak chips. This effect can be due to the action of the oak chips, which describe a carrier for yeast cells, similar to the action of immobilized cells. Therefore, increased levels of compounds, such as propanol, isoamyl alcohol, 2-phenylethanol, several esters and acetates, were found. Concentrations of guaiacol were similar for all the tested woods, whereas eugenol levels were higher in the wines fermented with oak chips from central France. *Cis*-oak lactone was present in a much higher concentration than its *trans*-isomer and additionally, the *cis*-oak lactone has the lower perception threshold. Furfural and 5-hydroxyfurfural contents were higher in American oak, but furfural is not a desired component in white wines. Since furfural is reduced to furfuryl alcohol by yeasts, its levels did not rise as much as expected. The content of vanillin was higher in wines, macerated with oak chips, than in wines fermented with oak chips, probably because of the fact that vanillin can be degraded by yeasts to vanillyl alcohol. Characteristics of young, white wines, such as a “fresh” aroma or “unripe fruit” aroma were missing in the fermented wines, maybe because of their degradation during the fermentation process, or because of masking effects from oak derived compounds. Still, small amounts of woody components do not completely mask the attributes of a young wine.

3.5.2 Red wine

Oak barrel maturation is a very important step in winemaking for red wines. It adds many aroma substances to the wine, which contribute to the “woody” notes of a wine.

There are many different aroma substances, but the most important ones are *cis*- and *trans*-oak lactones, *cis*- and *trans*-isoeugenol, eugenol, guaiacol and its derivatives, furfural and its derivatives and vanillin, even if the importance of vanillin is still discussed.

Jarauta et al.^[36] tried to describe the changes in the aroma profile of a wine, aged in oak barrels during this time. Furthermore, they attempted to find certain patterns that they could link to the different chemical, physical and microbiological processes, occurring in the period of wine aging. 70 aroma compounds were monitored during the 2 years of maturation, but only 30 of them showed significant changes. The substances were classified and divided into ten different groups.

Group (1): Genuine wood-extractable compounds. In this group well known wood components like the oak lactones, furfural, 5-methylfurfural, eugenol, guaiacol, and various other volatile phenols were found. The extraction of these compounds follows trends similar to ones, reported in literature, although there are some notable differences. The concentrations of *cis*-oak lactone decreased drastically in the second year of fermentation in the barrels, which indicates, that these molecules are consequently degraded.

Group (2): Compounds likely extracted from the surface of the woods. They are easily extracted into the wine, which means that they lie in the very surface of the wood. They are mostly belonging to the group of fatty acid metabolism by products, such as butyric, hexanoic and octanoic acids, and to the amino acid metabolism by products, such as isovaleric acid, methionol, β -phenylethanol and γ -butyrolactone.

Group (3): Compounds extracted from the wood, but also released or formed from precursors in the wine. Acetovanillone, methylvanillate, ethylvanillate, furaneol, β -damascenone, β -ionone and α -ionone are present in this class. Their levels are not only increasing in the wines, stored in oak barrels, but also in the wines, stored in stainless steel vessels, which indicates, that they are also microbially released of precursors, present in grape must and wine. In case of the α - and β -ionone and β -damascenone, the wood may be able to release small amounts of these compounds,

which would confirm, that carotenoids from wood are also an active source of odorants for wine.^[36]

Group (4): Compounds formed or released by precursors in wine. Linalool and homofuraneol belong to this group, but their levels did not show any relevant differences between the wines from the stainless steel vessels, to the wines from the oak barrels. Linalool is formed from glycosidic precursors or may also be formed from other monoterpenes.

4-Ethylphenol and 4-ethylguaiacol belong to group (5), the compounds formed by microbiological action on wine precursors. The precursors of 4-ethylphenol and 4-ethylguaiacol are the *p*-coumaric and ferulic acids. They are transformed by *Brettanomyces/Dekkera* yeasts in its aromatic successors with “smoky” and “band-aid” aroma descriptors. Their levels tend to decrease between the 6th and 12th month of storage, following a similar behaviour to that of vanillin.

Group (6): Compounds formed by the oxidation taking place exclusively in the oak cask. Examples therefore are sotolon and phenylacetaldehyde. Sotolon is a well known off-flavor in wines, because of its oxidation-spoiled character, as well as phenylacetaldehyde. Both of them are products of the degradation of amino acids. Sotolon is derived from threonine and phenylacetaldehyde comes from phenylalanine, but also from β -phenylethanol.

Therefore, β -phenylethanol is belonging to group (7), compounds that disappear during oxidation, taking place exclusively in oak casks, as well as hexanol and methionol. All three of them are oxidized to their corresponding aldehydes and since aldehydes have smaller odor thresholds than alcohols, this development may have a deep effect on the sensory profile of a wine.

In group (8) there is only one compound, acetaldehyde. It disappears due to condensation reactions with compounds, extracted from the wood, like polyphenols. This is a very important issue for the stabilization of wine colour and in the fining of wine flavor.

Group (9): Compounds disappearing in the wines stored in oak barrels as a likely consequence of sorption processes. The easiest allocation in this class is octanoic acid, but hexanoic, butyric and isovaleric acid behave quite similar. The levels of these components decline significantly in oak barrels, but not in stainless steel vessels.

Group (10): Compounds whose concentration change due to acid and alcohol/ester equilibria. These processes are concomitant with any kind of aging and were therefore not further discussed in the study of Jarauta et al.^[36]

This study demonstrated all the possible interactions of wine and oak barrels and it also showed that the aging process provides significant changes in the concentrations of many substances in wine.

As reported at the white wines in chapter 3.5.1, Spillman et al.^[32] investigated the contribution of compounds, associated with oak barrel maturation, to the aroma profile of wines. They have chosen Chardonnay and Cabernet Sauvignon for their experiment.

One of the major characteristic of Cabernet Sauvignon was “vanilla”. It has just a small correlation with vanillin, higher correlations with guaiacol, furfural, 5-methylfurfural and furfuryl alcohol and the highest correlation was found to be with *cis*-oak lactone. Together with eugenol it is probably responsible for the intensity of the “vanilla” and “oak” character. Another interesting relationship between the oak-lactones and eugenol was found in the correlation with the “berry” aroma descriptor. Since lactones, similar to the oak lactones, are aroma-active in many fruits, the association between *cis*-oak lactone and “berry” notes should not be surprising.^[32] The “earthy” and “band-aid” notes were judged as most intense in the stainless steel control samples, indicating that they are developing either fruit-based, or they are produced by microorganisms that developed in the wine no matter how it was stored. An “earthy” aroma was negatively correlated to the concentrations of *cis*-oak lactone and eugenol, and therefore also with the “coconut” aroma of Cabernet Sauvignon. This may indicate a masking effect. The “band-aid” aroma may derive from 4-ethylphenol, which can be produced by *Brettanomyces* yeasts, an indigenous yeasts, present in wine. Furfuryl alcohol, the natural reduction product of furfural, was strongly correlated to the “coffee” aroma of red wine. This could be explained by the fact that the closely related furfurylthiol is one of the major aroma compounds in coffee and its formation also depends on furfural as basic product. Blanchard et al. investigated the role of furfurylthiol (FFT) in wine.^[37] They found a close relationship between furfural releases from the oak barrel to the medium and FFT levels. This implements that furfural, as a precursor, was converted to FFT during alcoholic fermentation. And the concentration of FFT rose with an addition of cysteine to the medium. This can be explained by the reaction that took place. The

conversion of furfural to FFT requires the replacement of a carbonyl group with a thiol group, which is present in cysteine. However, adding nitrogen in form of ammonium sulfate had little impact on the production of FFT, but the more favourable conditions for high FFT levels are low nitrogen- and high sulfate contents. A strong correlation was also seen between the “coffee” and “vanilla” aroma and the “dark chocolate” aroma. It is possible that cooeping and heat products are responsible for all three of them and that there may be different ways of describing the same group of aroma substances.

Since the oak barrel has an important influence on the composition of the wine, differences in the wood can cause differences in the resulting wine. Usually French and American oak is used to produce the barrels, but lately also Spanish, Russian and Hungarian oak wood made their way into the winemaking process.

Fernández de Simón et al.^[38] compared the chemical and sensory profiles of wines from four different locations, aged in French (*Quercus robur* and *Quercus petraea*), American (*Quercus alba*) and Spanish (*Quercus pyrenaica*) oak barrels. Knowing the composition of the different oak species, in particular the composition of the toasted layer, which is in closest contact with the wine, helps to choose the right barrel for each wine.

They analyzed the 40 most representative compounds in all the wines and found distinct variations in the different wines and different oak barrels. For the furanic derivatives (furfural, 5-methylfurfural and 5-hydroxymethylfurfural) more or less the same levels were reached after 6 months of aging in all the samples. After 12 months certain differences were visible, although in some cases the differences were not statistically significant. It was not possible to find a uniform behaviour for one wood type. Furfuryl alcohol was neither detected in high amounts in non-toasted, nor in toasted wood, since it is a reduction product of furfural. Its levels increased during maturation and had its maximum after 12 months aging, when the study was finished. Furfural levels decreased steadily, but in the end, different amounts for different wines were obtained. The kind of wine with its particular content of sulfur dioxide may be responsible for the differences, since microbial activity causes the transformation of furfural into furfuryl alcohol and sulfur dioxide inhibits any microbial activity. Furthermore, the kind of wood is responsible for a different amount of released furfural content. Since the oak lactones are released from the oak wood, variations regarding their levels in wine were found in the different oak wood

samples. American oak had the highest levels of *cis*-oak lactone, whilst the wines, aged in *Q. petraea*-barrels had the highest concentrations of *trans*-oak lactone. For the Spanish *Q. pyrenaica*, the concentrations of the oak lactones were similar to those from wines aged in American oak. The ratio between the *cis*- and *trans*-isomers is an important marker for many authors and winemakers. American and French oak are easily distinguished by knowing these parameters, since the ratio is usually around 2 for French oak and higher than 5 for American oak. The Spanish oak of *Q. pyrenaica* was located in-between, at a *cis/trans*-ratio of around 4. Phenolic aldehydes, such as vanillin and syringaldehyde were not in every sample above its odor threshold level. Maltol, the origin of “caramel” and also “toasty” notes was highest in American oak wood and in *Q. pyrenaica* wood. Guaiacol and eugenol had the highest concentrations in *Q. pyrenaica*, although the differences observed for guaiacol were not always statistically significant. The rest of the volatile phenols were or extracted from the woods in small concentrations, or formed by microbiological transformation of hydroxycinnamic acids. However, differences can not only be explained by varieties in the wine or oak barrels, but also in the microbiological activity that transforms many of the components into their odor active counterpart. An increase in microbial activity is possibly related to a decrease in sulfur dioxide levels, and low sulfur dioxide levels can promote the transformation of 4-ethyl derivatives to 4-vinyl-derivates, which is the case of 4-ethylphenol and 4-ethylguaiacol. Elevated concentrations of 4-ethylphenol in red wine are often associated with unpleasant aromas such as “phenolic”, “animal”, or “stable”. 4-Ethylguaiacol has less effect on the wine aroma, but still, high levels of it in red wine are linked to “phenolic”, “bacon” and “smoky” aromas. The transformation of these two compounds into their 4-vinyl-derivates is favourable, since the aroma of 4-vinylphenol or 4-vinylguaiacol is described as “pink”, “pepper”, “clove” or “dature” (thorn apple), which suits a red wine much more. For vanillin and syringaldehyde, wines aged in American or French oak showed the highest concentrations. Vanillin is controversially related to “vanilla”, “coffee”, “dark chocolate” and “smoky” aromas, although it seldom reaches its odor threshold levels. Syringaldehyde has even higher threshold levels, which means, that its sensory properties are doubtful.

Altogether, wines aged in *Q. pyrenaica* presented higher “woody” notes and higher wood-wine interactions, as well as “toasty”, “roasty” and “milky coffee” notes. Two of the wines had especially high “spicy” notes. Wines aged in *Q. pyrenaica* were

highly appreciated and got a higher global valuation of the judges, than wines aged in French and American oak wood.

To enlarge their study about American, French or Spanish oak wood types, a part of the group of the latter study presented here, investigated the differences regarding different origins of oak chips. The studied aroma compounds were the same, just the treatment differed from that with the oak barrels.^[39]

The treatment with the oak chips was carried out in stainless steel tanks and the time was set onto 60 days altogether. After 2 weeks, the first samples were taken and important differences regarding furanic compounds were observed in the wines, macerated with the Spanish chips. Since they are formed through thermodegradation from cellulose and hemicellulose during the toasting process, their levels increased with the toasting temperature. All the chips were treated the same way, so the differences in the release of furanic compounds into the wine may be due to differences in the cellulose and hemicellulose content in the tested woods, which are dependent upon geographical origin and botanical species. However, the levels of furfural found in the wines, macerated with the Spanish *Q. pyrenaica* chips after 2 month of treatment were similar to the levels found in wines aged in Spanish *Q. pyrenaica* barrels after one year. In a similar study of Fernández de Simon et al.^[40] a furfural concentration above its sensory threshold was only found in wines, aged in Spanish oak barrels, but not in wines, aged with Spanish oak chips. The latter decrease of furfural and the rise of furfuryl alcohol brought highest concentrations of furfuryl alcohol in *Q. pyrenaica*- staves and in *Q. petraea*- chips. 5-methylfurfural levels were in both studies close to the levels obtained in *Q. pyrenaica* barrels after 1 year. Regarding to the two isomers of oak-lactones (in literature often also named whiskey-lactones), the highest concentrations for the *cis*-isomer in the study^[39] were found in wines macerated with American chips and in the study^[40] for wines macerated with Spanish oak chips. They both agree on French oak chips having remarkably low contents of *cis*-oak lactones. For the *trans*-oak lactone the highest concentrations were observed in wines made with Spanish *Q. petraea* oak chips in the study^[39] and for French *Q. petraea* in the study^[40], but still, this isomer is less aromatic than the *cis*-oak lactone. According to the *cis/trans* ratio, the American chips showed, as expected, a ratio around 7, the French chips were settled around 1.51 and 2.22 and the Spanish chips were expected to be around 4, according to former studies,^[38] but the ratio was surprisingly around 0.75 to 2.35. In general, the

levels of the two isomers obtained were lower than the levels observed for wines aged in barrels for one year.^[38]

Fernández et al.^[41] and Spillman et al.^[42] compared the concentration of *cis*- and *trans*-oak lactones in the wines to concentrations of *cis*- and *trans*-oak lactone in the woods before toasting. Similar concentrations were found. The oak lactones may migrate from the headpieces of the barrel, which have not been toasted, or from the inner layers of the wood, into the wine, or they could even be generated from the chemical precursors that are also extracted from wood.^[43] γ -Butyrolactone levels were found to be more or less the same for all the different chips studied by Rodríguez-Bencomo et al.^[39] and they remained stable after rising in the first 2 months, which indicates, that they are derived from a more external layer of the wood. The concentrations of maltol and cyclotene were higher in the wines stored in staves than in wines treated with oak chips, and both of the aroma substances did not succeed their odor threshold. But nevertheless, they may exercise an additive effect on the overall aromatic profile. For the volatile phenols, ethylphenols and vinylphenols, which can be extracted from the wood, but are more often formed through microbiological processes from hydroxycinnamic acids by *Brettanomyces/Dekkera* yeasts, obtained values much lower in the wines of the study of Rodríguez-Bencomo et al.^[39], than the values obtained from wines aged in American, French or Spanish oak barrels. The levels of 4-ethylguaiaicol were lower in the wines macerated with the Spanish oak chips than in the wines macerated with American or French oak chips whilst no significant differences were found among the wines matured with the different oak chips. The levels of other oak-related volatile phenols, such as guaiacol and eugenol, *cis*- and *trans*-isoeugenol and 2,6-dimethoxyeugenol, were much lower in the Spanish *Q. pyrenaica* chips aged wines than in the American and French chips wines. These results do not coincide with the results obtained from wines aged in oak barrels.^[38] But at least, the values of the wines from the oak chips are proportional to them of the oak barrels, in which *Q. pyrenaica* had a very high aromatic potential from eugenol and guaiacol.^[40] The extraction of the phenolic aldehydes and their derivatives, methyl and ethyl vanillate, acetovanillone, vanillin and syringaldehyde, was clearly related to the geographic origin of the chips. At the end of the maceration, the wines macerated with the Spanish oak chips showed the lowest contents of the phenolic aldehydes, with an exception of ethyl vanillate, for which no statistically differences were found. For

vanillin and methyl vanillate, wines produced with American chips had much higher levels than wines macerated with French oak chips. Even though vanillin reached levels above its sensory threshold in all the tested wines, its levels were below the levels of wines aged in oak barrels. Syringaldehyde did not exceed its threshold, since it is very high in both, the wines macerated with oak chips and in the wines stored in oak barrels.

Altogether, the results indicate, that the geographic origin of the oak wood has an influence on the composition of the wine macerated with these chips and that Spanish oak chips are in their chemical composition more similar to French chips than to American chips.

Not only the type of wood, but also the time of maceration and the size of the oak chips have a big impact on the wine. The statistical differences between the wines aged with chips or in oak barrels become more distinctive, the longer the maceration time takes, and the more wood-wine-interactions occur. Wines aged with American chips are most separated from wines, aged with French and Spanish oak chips in this parameter. The volatile composition of wines aged with chips becomes stable after 70 days of contact, with the greatest evolution between 30 and 70 days. And here, wines macerated with French oak chips are most separated from the other two.^[40]

Bautista et al.^[44] studied the effect of oak powder, oak shavings and oak cubes on wines stored in stainless steel tanks, used barrels, or new barrels. The length of the contact time and the size of the wood chips were changed to verify the right parameters for winemaking. After 3 month, the highest concentrations of furfural were found in the wines aged in new barrels and in wines aged in used barrels with oak cubes. A decrease in its levels from the third to the sixth month was observed, while the concentration remained stable from 6 to 9 months. 5-Methylfurfural had the highest levels in wines aged in new barrels. Besides that, the concentration was higher in used barrels with added chips than in stainless steel tanks with added chips. After bottle storage of 6 months, all the wines showed a decrease in furfural levels, except for the wines aged for 9 months in wood, which presented a slight increase in furfural concentration. Guaiacol levels in wines aged in tanks were very low and after 6 months bottling, guaiacol was not detectable anymore. Similarly, the levels of guaiacol and 4-methylguaiacol in wines aged in oak barrels were very low as well. It has been discovered, that guaiacol and its derivatives are only formed if the toasting temperature is below 230°C. Their concentrations are quite always beneath its odor

threshold levels. According to the *cis*- and *trans*-oak lactones, the levels were of course highest in the new barrels, since the lactones are derived from the oak wood. But apart from that, used barrels and tanks with oak chips reached surprisingly good levels in comparison to the new barrels. After 3 months, the concentrations of *cis*-oak lactone almost reached their maximum in the wines aged with chips or cubes. No statistically significant changes in the concentration were found after 3 and 6 months of bottle aging, although a small increase of *trans*-oak lactone was detected. The control wine in the used barrels without supplementation of oak chips, shavings or powder had the smallest concentrations of these lactones. For the wines aged with cubes or shavings, after 3 month in the barrel, the levels of *cis*-lactone still increased, whilst its levels in the wines aged with chips already remained stable. 4-Ethylguaiaicol and 4-ethylphenol had the maximum concentrations in used oak barrels, since they may harbour yeasts, such as *Brettanomyces/Dekkera* yeasts, which conduct the transformation of the hydroxycinnamic acids, *p*-coumaric and ferulic acid, to the aroma substances 4-ethylphenol and 4-ethylguaiaicol. Old barrels are often containing remains of yeasts and other bacteria, because it is very difficult to clean the barrels through and through. 4-Ethylphenol levels were also high in wines aged in tanks, and even higher in wines aged in tanks plus additional oak cubes. It is possible, that wines, if they are containing low sulfur dioxide levels, are not having enough protection, since sulfur dioxide is inhibiting bacterial and yeast growth. After alcoholic fermentation almost no free sulfur dioxide is present, and if the malolactic fermentation has a late start, high contents of yeast populations may be found and therefore also high contents of 4-ethylphenol. Its maximum levels were attained after 9 months of aging, indicating that their concentrations steadily rise with time. Vanillin is formed by the thermal degradation of lignin in wood, or by microbiological processes, because it was present in tanks without any contact to wood and had higher levels in wines aged in tanks containing oak pieces or in oak barrels. Finer pieces are more combustible and more vanillin is formed, but if the pieces are getting smaller than 5 mm a loss of vanillin, due to evaporation, has been detected. ^[44] The highest quantities of vanillin were detected after 3 months, afterwards its levels decreased, and interestingly it decreased more in wines aged with wood than in wines aged in tanks. The aldehyde vanillin is degraded to its alcohol vanillyl alcohol during fermentation and other non-aromatic compounds. After bottling, small decreases in the vanillin concentration were observed, but just

in the wines aged for 3 and 6 months. The wines aged for 9 months in barrels showed small increases in vanillin quantity after bottling.

De Rosso et al.^[45] focused on an interesting item in their study and analyzed not only red wines, aged in different types of oak wood, but in different types of wood in general. They studied the influence of different wood types on red wine, namely acacia, cherry, chestnut and mulberry and compared the results to the same red wine, aged in oak wood. These woods show very different chemical properties. Acacia is characterized by significant contents of benzene aldehydes, chestnut by richness in polyphenols and relevant release of eugenol and vanillin, cherry by cession of methoxyphenols, mulberry by the lowest volatiles and oak by *cis*- and *trans* oak lactones and polyphenols. Since the barrels of acacia, cherry, chestnut and mulberry woods were untoasted, furfural and 5-methylfurfural were only detected in oak barrels. Guaiacol was not found in a detectable level in any of the samples. Wines aged in acacia showed a constant increase in ethylguaiacol, which has a “spicy” note, and in the end this compound was highest in this sample. Mulberry-aged wine was characterized by a significant decrease in the “fruity” note of ethyl esters and ethylguaiacol, whilst ethylphenol with its “horsy” odor was quantified in very high amounts. Wines aged in cherry were found to contain high ethylguaiacol levels already after 3 months of aging and isoamyl acetate had a significant increase after 6 months with a concomitant increase of acetic acid. In general, it can be said, that the resulting wines of this experiment were not containing favourable amounts of pleasant volatiles.

4 Detailed characterization of some wine examples

4.1 White wine

Lorrain et al.^[46] analyzed Chardonnay wines in order to select odorants thought to have a positive effect on typical Chardonnay wine. The continuative experiment was dedicated to the sensory evaluation of aroma models obtained by supplementation in wines.

The odor activity values (OAV) of the isolated substances were measured, calculating the measured concentration and the odor threshold in the wine matrix. If a substance in a mixture processes a high OAV, it is said to have a close relationship to the actual odor. In order to minimize the complexity of a mixture, it is possible to take the substances with a high OAV to copy a certain mixture. But a very excessive simplification may affect the aroma profile in a bad way. The mixture is becoming more similar to the real composition of a wine, if it contains more substances with a smaller OAV than just a few compounds with a high OAV. The study of Lorrain et al.^[46] is based on results of a further study of Ballester et al. of 2005^[47], where typical aroma compounds of Chardonnay wine were already validated. As a whole, 71 target compounds were measured and categorized in order to produce a good model mixture of Chardonnay wine. The first model contained 6 aroma components, which were selected from the categories primarily prepared. Ethyl butanoate (fruity), octanoic acid (microbiological), phenylacetaldehyde (floral), 4-vinylphenol (spicy), 2-methyltetrahydrothiophen-3-one (chemical), and δ -decalactone (nutty). The second model contained 10 aroma components to reinforce the weight of four aroma odor classes. 3-Methylbutyl acetate (fruity), decanoic acid (microbiological), linalool (floral) and 4-vinyl-2-methoxyphenol (spicy) were added. For the sensory analysis, 24 samples were assessed, 6 of them were the aroma supplemented Chardonnay wines as aroma models and the remaining 15 were additional wines from Chardonnay and other varieties like Sauvignon Blanc, Sylvaner, Marsanne, Pinot Blanc, Chenin, Aligoté and Melon de Bourgogne. The judging panel considered six of the nine Chardonnay wines to be representative of the concept, including two of the three supplemented wines. The third wine was considered as intermediate. As expected, five of the nine other, non-Chardonnay wines, in particular the Sauvignon

Blanc, Sylvaner and Marsanne wine were judged to be bad examples of Chardonnay. It was obvious that the 10-component supplemented wines scored much higher in typicality than the 6-component supplemented Chardonnay wines. A multiple-comparison procedure showed the same result. The 10-compound combinations were clearly better than the 6-compound combinations. However, actually both, the 6-compound and the 10-compound mixtures can be considered as simplified models, but the greater the mixture complexity gets, the more the olfactory sensation was judged as representative of the sensory concept.^[46] So the supplementation of only four well-chosen additional compounds was enough to influence the aromatic balance and to ensure the expected olfactory perception. The results of the study confirmed that the typicality of Chardonnay wine seemed to derive from an association of the 10 aroma compounds ethyl butanoate (fruity), octanoic acid (microbiological), phenylacetaldehyde (floral), 4-vinylphenol (spicy), 2-methyltetrahydrothiophen-3-one (chemical), δ -decalactone (nutty), 3-methylbutyl acetate (fruity), decanoic acid (microbiological), linalool (floral) and 4-vinyl-2-methoxyphenol (spicy), but however, these results should be considered as preliminary findings.^[46]

The influence of geographic origin on aroma descriptors and the aroma composition itself of Spanish Albariño wines have been studied by Vilanova et al.^[48] Albariño is a grape variety of *Vitis vinifera* L., a typical variety of Galicia, in the northwest of Spain. Usually, winters there are humid and summers are dry. The Denomination of Origin Rías Baixas comprises five regions where the Albariño-variety is cultivated, four of them produce higher amounts of this wine. In general, Albariño wines are characterized by “fruity” and “floral” odors. The study of Vilanova et al. tried to establish aroma descriptors that identify the aroma of Albariño wines from four different regions in the Denomination of Origin Rías Baixas.

Twenty-five wines were quantified by judges to obtain the aromatic descriptors of the wines. The number of descriptors resulting from the preliminary analysis was too great, therefore it was reduced to 17. Twelve of the Albariño wines were containing these descriptors. The wines from the different regions were linked to 3-7 descriptors, but the only descriptor that all the wines had in common was the “apple” character. The “lactic” and “toasting” aroma was only detected in the wine that went through malolactic fermentation. “Peach” and “pineapple” were also present in only one of the samples. It was shown that the judges rated wines differently for the most

of the sensory attributes in this study. Six sensory attributes were significantly different between the wines, such as “ripe fruit”, “pineapple”, “banana”, “pear”, “citrus fruit” and “floral”. The study showed clearly, that Albariño wines, obtained from different regions in the Denomination of Origin Rías Baixas, have a distinct aroma composition and that it is very hard to identify a certain Albariño wine flavor for all of the wines.

The study of Skinkis et al.^[49] focused on the comparison of monoterpene constituents in the white grape varieties Traminette, Gewürztraminer and Riesling. So far, Traminette is not well known, therefore this study was dedicated to investigate the sensory properties of this variety and to characterize its aroma composition. Gewürztraminer is known for containing linalool and geraniol as major monoterpene constituents, Riesling varieties produce fruity and floral wines, containing just small amounts of terpenes such as linalool, but high amounts of norisoprenoids, and the interspecific hybrid Traminette is characterized of very “fruity”, “floral” and “spicy” notes. Gewürztraminer and Riesling had 43% and 58.8% lower concentrations of total monoterpenes compared with Traminette, respectively, although the gas chromatograms of Traminette and Gewürztraminer were nearly identical in monoterpene constituents and the relative ratio of many of these compounds was similar. In all three varieties, a total of 15 monoterpenes was found to be in common and 17 were found to coincide in Traminette and Gewürztraminer. One of the most important monoterpene and odorant in Traminette was *cis*-rose oxide. It was found in a nearly three times greater concentration in Traminette than in the other two wines. Its odor can be described as “rose-like”. *Cis*-rose oxide has already been identified as an important aroma compound for Gewürztraminer. But also “spicy” and “floral” notes are characteristic for Gewürztraminer. Sabinene hydrate is responsible for the “spicy” aroma and holds ~20% of the total monoterpenes in Traminette and ~10% in Riesling, which is rather considered as a “fruity” than a “spicy” wine. Nerol and geraniol provide a “floral” and “rose-like” aroma and could be identified in all three varieties. Low levels of these compounds may be explained by a transformation to other aroma compounds, amongst them *cis*-rose oxide. Citrus aromas are mainly derived from monoterpenes like 2- and 3-carene, which represent the major part in Gewürztraminer and Traminette, but only 2-carene was found in Riesling. Altogether, Riesling is mostly composed of linalool, lavandulol and limonene oxide, but also ocimene, 2-carene and β -terpineol. The aroma of Gewürztraminer is strongly

correlated with linalool, geraniol and nerol and the variety Traminette is mostly linked to *cis*-rose oxide, but it is also well correlated with monoterpenes that provide “floral”, “fruity” and “spicy” aroma properties. In addition to monoterpenes, three norisoprenoids were identified, including vitispirane, 1,1,6-trimethyl-1,2-dihydronaphthalene (TDN), and β -damascenone. These three substances were mainly found in Riesling and it is known that they play an important role in the development of the characteristic Riesling aroma. In particular, high concentrations of β -damascenone were found in Riesling wine. It is probably responsible for the “fruity” odor these wines. The results of this study ^[49] indicate that Traminette has a significant aromatic potential, considering the fact that it had the highest concentration of monoterpenes. Further investigation regarding to this variety are necessary to concretize the winemaking techniques, used to modify this reservoir of aroma compounds to yield different wine styles.

Blackman et al.^[50] concentrated on the analysis of Semillon wine of the Hunter Valley region, Australia, to investigate the range of profiles, or styles, typically found for Hunter Valley Semillon (HVS) wines, since no detailed information was available so far. It was validated, whether bottle aging is important for the flavor development of this wine and whether two different styles of HVS exist. It was also verified if young HVS is always “austere” and if certain aroma profiles exist to guarantee additional styles.

HVS is said to be “austere”, “dull”, with “grassy/herbaceous” note and an acid backbone, before developing to a wonderfully rich and succulent wine with a more substantial palate and rich “honeyed”, “toasty” and “nutty” characters. ^[50] 16 wines of different vintages were compared, the youngest wines were 1-2 years and the oldest wines were 10 and 11 years old. All the wines were characterized by panellists and two factors of variance were measured. The first factor was “bottle aging” and the second one was “acid-sweet dimension”. Changes in the descriptors concerning the first factor involved a reduction of “floral”, “confectionary”, “lemon/lime”, “grassy” and “pineapple” notes and an increase of the “developed” characteristics of bottle aged wines, such as “toasty”, “honey” and “orange marmalade”. Those wines with “developed” characteristics were wines aged for 5-11 years, whilst the wines driven by “floral”, “confectionary”, “grapefruit” and “grassy” characters were 1-2 years old at the time of analysis. The second factor of variance, the “acid-sweet dimension” is mainly affected by acidity, with opposite descriptors of “floral”,

“confectionary”, “pineapple”, “honey” and “orange marmalade”, whilst the sugar-associated components are characters like “honey” and “floral”.

HVS wines were then classified into four styles. The group of style 1 is containing just one of the sixteen wines, and it is characterized by high “confectionary”, “floral”, “lemon/lime” and “pineapple” aromas, which are, like mentioned above, characteristic for young, not aged wines. It also had the lowest intensity ratings for “toasty”, “honey” and “orange marmalade”.

Style 2 had a lower perceived sweetness and higher perceived acidity, the “lemon/lime”, “floral”, “lychee”, “pineapple” and “confectionary” were also lower than in style 1, but higher than in style 3 and 4. At the time of analysis, the characters “honey”, “toasty”, and “orange marmalade” were still very low. These wines have a great potential for further bottle aging, because of their acid-sugar-balance, but they also show a high early drinking potential. Style 3 is characterized by the highest perceived acidity of all styles and the characters “pineapple” and “lychee” were lowest of all wine styles. “Floral”, “grassy”, “confectionary”, “grapefruit”, “asparagus” and “lemon/lime” were also lower than in style 1 and 2. Since acid levels are important for aging, these wines seemed to be intended for bottle aging. The category of style 4 only contained wines with a minimum age of 3 years. As expected, the descriptors “honey”, “toasty” and “orange marmalade” were highest for these wines, fruity characters were much lower, although the “lemon/lime” aroma was still present. So as a whole, the presence of different styles of HVS was confirmed in this study and the aging potential is dependent upon the wine style. Just one style was identified to possess both, an early drinking appeal and an aging potential.

4.2 Red wine

Research concerning the characterization of white wines has been elucidated in many analyses before the first studies concerning the characterization of red wines started. The researchers mainly focused on certain compounds in red wine, like 2-methoxy-3-isobutylpyrazine in Cabernet Sauvignon and some “fruit-like” thiols in Cabernet Sauvignon and Merlot, but Kotseridis et al. ^[51] were the first to put the focus on the

general spectrum of aroma substances of Cabernet Sauvignon and Merlot from the Bordeaux region, on their grape juice, the yeast extracts and the wines itself.

Impact odorants of red wines of the Bordeaux region, Cabernet Sauvignon and Merlot, have been identified by GC-O and flavor dilution factors (FD) have been measured. The FD is the ratio of the concentration of the odorant in the initial extract to its concentration in the most dilute extract in which the odor is still detectable by GC-O. Cabernet sauvignon and Merlot wines were compared and their aroma profiles were quite similar. Indeed, the productions of these two cultivars are about the same. Kotseridis et al.^[51] quantified 50 aroma substances in the grape juices, musts and wines. In the grape juice of Cabernet Sauvignon 18 different odorants have been quantified and the highest FD values were obtained by 3-(methylsulfanyl)propanal, (*E,Z*)-2,6-nonadienal and decanal. These compounds are responsible for “vegetal” notes characterized by a “nutty”, “stale” and “baked potato” note for 3-(methylsulfanyl)propanal, a “cucumber” scent for (*E,Z*)-2,6-nonadienal and a “vegetal” note of “green woods” for decanal. In the extract of the yeast fraction, mainly four odorants could be identified. 2-Methyl-3-sulfanylfuran, 3-(methylsulfanyl)propanal, phenylethanal and 2-/3-methylbutanoic acids dominated the aroma profile, providing a “meaty/cheesy” aroma of the dry yeasts. 2-Methyl-3-sulfanylfuran itself can be described with “meaty”, “milky”, “sunflower seeds” and “roasted nuts” characters, 3-(methylsulfanyl)propanal exhibited “meaty”, “vegetal” and “baked potato” aromas, phenylacetal could be linked to the “honey” descriptor and the 2-/3-methylbutanoic acids were reminding of Parmesan cheese.

The 48 aroma compounds, identified in the wines itself, were found to be similar in Cabernet Sauvignon and Merlot and many of the previously reported aroma components of the grape juice were found again in the fermented musts and the yeast extract. In particular, the thiol fraction was important for these two wines, since the thiols add a “fruity” note to the wines aroma. 4-sulfanyl-4-methylpentan-2-one, 3-sulfanylhexyl acetate and 3-sulfanylhexas-1-ol were characterized by “passionfruit”, “grapefruit” and “asparagus” descriptors. From the aldehydes, 3-(methylsulfanyl)propanal, decanal, (*E,Z*)-2,6-nonadienal and phenylethanal. Phenylethanal was already reported as impact odorant in Muscat wines, even if its odor threshold was slightly higher than the threshold of , 3-(methylsulfanyl)propanal, decanal and (*E,Z*)-2,6-nonadienal. The 2- and 3-methylbutanoic acids and acetic acids showed the highest FD of the fatty acids, but the contribution of the

methylbutanoic acids was weak, because of their high odor threshold value. Among the ethyl esters of fatty acids, ethyl-2-methylpropanoate, ethyl butanoate, ethyl-2- and ethyl-3-methylbutanoate and 3-methylbutyl acetate showed the highest FD values and were characterized as fruity aroma descriptors like “banana”, “apple”, “strawberry” and “pineapple”. Furthermore, the four fusel alcohols, 2- and 3-methylbutanol, 3-(methylsulfanyl)propanol and 2-phenylethanol are well known aroma contributors in wine. Furaneol, homofuraneol and sotolon were also detected in the wines. Sotolon was considered to be a specific compound of wines prepared by flor yeast aerobic fermentation, but it was also identified in sweet fortified wines, produced by oxidative aging, and also in young white wines, produced under classical vinification conditions.^[51] The odor of sotolon was described as “curry-like”. β -Damascenone greatly contributed to the Cabernet and Merlot wine aroma by enhancing their fruity notes. For the phenols, 2-methoxyphenol and 2-methoxy-4-vinylphenol were presenting the highest FD values. FD values used for the quantification of the amounts of aroma substances and their influence on the whole aroma of a wine should not be considered as a method to find impact odorants in a mixture, but it should be seen as previous step, to find possible impact odorants in a complex mixture of odorants. Therefore, the study of Kotseridis et al.^[51] does not present actual impact odorants, but shows, which aroma substances do have an impact on the whole aroma composition.

The typical wine in Uruguay, the *Vitis vinifera* Cv.Tannat variety, was studied by Boido et al.^[52] The study presents the composition of this wines, focussing in particular on compounds, peculiar for this variety, like isoprenoids, monoterpenols and the bound forms, with the aim to improve the characterization and classification of young Tannat red wines.

51 volatile compounds, including alcohols, esters, carbonyl compounds, acids, terpenes and norisoprenoids were identified and quantitatively determined. Tannat wines contained remarkable amounts of 2-phenylethanol and low contents of higher alcohol acetates and fatty acid ethyl esters, but it was suggested, that these results come from the yeast strain used in the vinification process. The levels of monoterpenic compounds were all under their respective sensory threshold, as it is usually found for neutral varieties. Only rather high amounts of limonene and geraniol were found. Regarding free norisoprenoids, just very small amounts were detected, but the levels of the bound compounds in form of glycoconjugates were

rather high and made up ~42% of the total level of the volatiles in the Tannat variety. The other volatiles were C₆-alcohols with ~6% and benzenoid compounds with ~51%. The main monoterpene components were *cis*- and *trans*-8-hydroxylinalool, with higher levels for the *trans*-isomer. Other monoterpenes like α -terpineol, linalool, citronellol, nerol, geraniol and others were found in low concentrations, well below their odor thresholds. The most abundant C₁₃ norisoprenoids were vomifoliol, 3-oxo- α -ionol, 3-hydroxy- β -damascenone and their derivatives, yielding in significant amounts of β -damascenone, the potent “rose” and “berry” like odorant. Multiple precursors of β -damascenone are present in the Tannat red wine, but β -damascenone itself was not detected in the experiment, probably because β -damascenone attains its maximum level in wine after one year of aging. This characterization of the Tannat wine from Uruguay was the first concerning this variety, but probably more work has to be done to understand the impact of the individual compounds for the composition of this wine.

Since many components are already known, but not satisfactorily quantified or fully described, Culleré et al.^[53] tried to evaluate the amount of the potential aroma compounds in red wine, that are still unknown, to establish, which odorants can be found above or close to their odor threshold, to determine which odorants can be responsible for the sensory differences between wines and to evaluate the strengths and drawbacks of gas chromatography-olfactometry (GC-O).^[53] Therefore, Culleré et al. analyzed the aroma profiles of six aged Spanish red wines. Of the 85 detected odorants, only 78 could be identified. Two aroma substances detected in this experiment were reported for the first time: 1-nonen-3-one (just a tentative) and 2-acetylpyrazine, a well known component of products that have undergone thermal processing. Another four odorants, 2-methyl-3-furanthiol, 2-furfurylthiol, 1-octen-3-one and (*E/Z*)-2,6-nonadienal could not be determined, because of their too low concentrations. In the set of wines in this study, about 40 substances could be detected, that were above their threshold levels. It is remarkable, that components, present at concentrations well above their threshold levels, could not be quantified in a previous investigation. Potentially different compounds found in the studied six red wines are ethyl phenols. Apart from 4-ethylphenol and 4-ethyl-2-methoxyphenol, two components derived from the yeast metabolism of *Brettanomyces*, the study reveals the existence of 4-propyl-2-methoxyphenol also to occupy an important role in the differences of various red wines. Other components like furaneol and (*Z*)-3-

hexenol, thiols that derived from cysteinic precursors like 3-mercaptohexyl acetate, 4-methyl-4-mercaptopentan-2-one, and 3-mercaptohexanol, some wood-related compounds like *trans*-isoeugenol, eugenol, vanillin, *cis*-oak lactone and guaiacol, and some compounds related either to the metabolism of the amino acids like 2-phenylethanol, 3-methylbutyl acetate and ethyl esters of isoacids, or related to the oxidative degradation of the amino acids, such as phenylacetaldehyde and to a lesser extent sotolon are present in different concentration in the wines and makes it possible to distinguish between them. The relation between the olfactometric data and the quantitative analysis showed good results, and most of the volatiles were detected by both methods. Only a few substances were or detected in the olfactometry or in the GC-O. But even if the results are relatively satisfying, an improvement in the combination of gas chromatography and olfactometry is desired, since the olfactograms are still too complex with a high number of odorants having little importance and small intensities.^[53]

Pinot Noir is one of the oldest wine cultivars, originated in the Burgundy region in France. Recently it has also become famous in Oregon, United States, Australia and New Zealand. The Pinot Noir variety in France is already well studied in comparison to the Pinot Noir variety in Oregon. Therefore, Fang et al.^[54] focused their work on the aroma and chemical composition of the Pinot Noir cultivar in the United States, using different methods to identify the varying compounds. They characterized Oregon Pinot Noir using aroma extract dilution analysis (AEDA),^[54] and stir bar sorptive extraction (SBSE),^[55] since this technique developed and various wines were already characterized by this method.

The AEDA is resulting in FD, flavor dilution factors, indicating possible impact odorants, whilst the SBSE quantifies a wide range of important substances for the Pinot Noir wine. Based on the AEDA, the potential important acids were 2-methylpropanoic, butanoic, 2-methylbutanoic and 3-methylbutanoic acid, transmitting strong sweaty odors. Propanoic, hexanoic and octanoic acids were also found at high AEDA values. The fusel alcohols, 3-methylbutanol and 2-methylpropanol, that give a nail-polish-like scent to the wine, had extremely high AEDA values. They are derived from the sugar catabolism or from the deamination of amino acids. Alcohols like 3-*cis/trans*-hexenol and 1-hexanol give “green” odors and are also present in grapes and must. 2-Phenylethynol with its “rose-like” and “honey” aroma and benzylalcohol with its “floral” notes are reported as key

compounds in Pinot Noir, because of their low odor thresholds and their high levels found in the wines. Also linalool, geraniol, nerol and citronellol are important odorants in Pinot Noir wines. Their levels in the wines were close or above their threshold levels, which indicates their impact on the aroma bouquet of Pinot Noir. Guaiacol, α -terpineol, 4-ethylguaiacol, *p*-cresol and eugenol, which exhibit “spicy”, “smoky” and “medicine-like” aromas also showed potential importance, whilst *m*-cresol, isoeugenol and 4-vinylphenol were present in lower concentrations. Most of these substances are produced by degradation of free odorless polyols or hydrolysis of glycosidically conjugated forms during wine aging. Sulphur containing aroma compounds like 3-methylthio-1-propanol and 3-ethylthio-1-propanol were found to have high AEDA values and a “cooked-potato” aroma, which is actually an unpleasant and undesired flavor. 3-Methylthio-1-propanol can be formed by photodegradation of methionine, 3-ethylthio-1-propanol was already reported to be present in Muscat and Port wines. 3-Mercaptohexanol, a very potent aroma compound of Grenache Rose wines, was also found to have a high AEDA value in the studied Pinot Noir wines. Amongst the esters, ethyl esters are very present in Pinot Noir, transmitting their fruity aroma to the wine. Ethyl butanoate, hexanoate, octanoate and decanoate were found in high concentrations. They can be described as “tropical fruit” aromas. Several aromatic esters were present as well, describing aromas such as “floral”, “cherry”, “stone-fruit” and “dry-plum”. 2-Phenylethyl acetate, ethyl phenylacetate and ethyl-3-phenylpropanoate have been identified as important contributors to the wine. Late stage wines had usually higher levels of oak lactones and γ -nonalactone, with “woody” and “coconut” aromas. The C₁₃-norisoprenoids β -damascenone and β -ionone, which mainly derive from degradation of carotenoids were also found in higher concentrations in the late stage wines than in the early stage Pinot Noir wines. This explains the higher “berry” notes in the late maturity wines. Fang et al.^[54] characterized Pinot Noir wines from Oregon by two methods and both presented similar results concerning the aroma substances in the wines. Therefore, both of them can be used to describe a wines aroma composition. Another useful and novel technique to obtain relatively simple and clean olfactograms is the dynamic headspace sampling technique and further quantitative GC-O. This method has been successfully applied to characterize the aroma profile of young white wines from different varieties. Escuerdo et al.^[56] used this technique to characterize five premium red wines, three Spanish and two Uruguayan wines and

compared them with neutral and dearomatized wines. The authors tried to combine the advantages of the new GC-O strategy with comprehensive aroma chemical analysis and with sensory tests specifically designed to improve our understanding of the role of groups of odorants in the odor nuances of these red wines.^[56] The aroma of the studied wines can be described as mainly “woody”, “sweet-caramel” “raisin-dried fruit” “toasted”, “berry fruit”, “veggie”, and “phenolic”. All of the wines seem to have high scores for the “raisin” note, whilst on the contrary, “sweet-caramel”, “woody”, “toasted”, “berry fruit”, and “veggie” obtained very different results in the different wines. The three Spanish wines were richest in “berry fruit” notes. The “woody” descriptors vary with the time the wine spent in the barrels, except for one wine.

The quantitative composition showed 37 compounds at concentrations higher than their sensory threshold and another 20 at concentrations at least higher than 0.1 times the threshold. For the Spanish wines, the presence and potential importance of the ethyl esters of C₆ and C₇ branched and cyclic acids and the presence of small amounts of isobutyl-2-methoxypyrazine was characteristic. The Uruguayan Tannat red wines were containing higher levels of γ -decalactone and β -damascenone, but had poor concentrations of linalool, ethyl-2-methylpropanoate and ethyl hexanoate. The aroma of these wines is hardly correlated with single compounds. Only in the case of guaiacol and *cis*-oak lactone a clear correlation is visible. The former is related to “toasty” notes and the latter with the “woody” character in wine. For the rest, the brain recognizes only general aromas, such as “fruity” or “sweet”. Therefore Escuerdo et al.^[56] grouped the compounds into four classes, F, for compounds with “fruity” character, V for compounds with “vegetal” notes, S for compounds with “sweet” and “flowery” descriptors and P for compounds with phenolic character. The summation of all the scores of all odorants for “fruity” descriptors correlated with the intensity of the “berry fruit” notes of the wines. Similarly, the summation of the scores of all the compounds with “sweet-floral” aroma can be correlated with the “sweet-caramel” character of the wines. The summation of the odorants with phenolic character was related to the “phenolic” character in the wines, and also the “vegetal” character can be correlated to the “veggie” character of the wines, although the significant level is not reached. The “vegetal” character is mainly attributed to the concentration of isobutyl-2-methoxy-pyrazine (IBMP), but when the two alcohols *cis*-3-hexenol and 1-hexanol were added, the sensory effect was much easier

perceived and a peppery nuance was recognized. These findings indicate, that the three substances may act synergistically. The addition of five compounds with “floral” and “sweet” notes to a neutral wine results in heavy “sweet-caramel” notes, which confirms the importance of these compounds in wine. Guaiacol is responsible for the “toasty” notes, but only the addition to a de-aromatized wine yielded in “toasty” and “smoky” notes. The addition of guaiacol to a neutral wine was not resulting in the same sensory perception, which suggests that the “toasty” note is not only derived from guaiacol, but from a mixture of different volatiles that all contribute to a “toasty” and “smoky” aroma. “Woody” notes are coming from the *cis*-oak lactone, but this was already proved in many studies. The “berry fruit” character is very complicated to identify, but it is the most interesting descriptor from the chemical point of view. Compounds like esters are said to be responsible for the “berry-fruit” notes, but an addition of several esters to a de-aromatized or neutral wine did not result in clear “berry-fruit” notes. Even the mixture of different chemicals and the variation of the ethanol content yielded in unsatisfying results. A composition of nine fruity compounds led to a perception of a strong “apple” aroma. When there is no ethanol in the mixture, the smell can be described as fully “apple-like”, but the intensity of the “apple” aroma decreases when the amount of ethanol increases, and at a level of 14.5% ethanol in the mixture, the “apple” aroma is hardly perceived and the aroma can be described as “sweet” and “fruity” in general.^[56] These results are in disagreement with some previous studies that found a lower level of fruitiness in de-alcoholized wines than in normal wines. In any case, this set of results indicate that the “fruity” characters can be related to more compounds than only to fruity esters. Different substances were checked to be aroma enhancers, amongst them the norisoprenoids β -damascenone and β -ionone, to a minor extend also vanillin-related compounds, such as methyl vanillate, vanillin, ethyl vanillate and acetovanillone and dimethyl sulphide (DMS). Vanillin-related compounds did not have a big influence on the perception of fruitiness in wines, at least the influence was not statistically significant. The role of norisoprenoids was different. Their ability to enhance the fruitiness of esters in wine is remarkable. The addition of low levels of β -damascenone and β -ionone to the mixture of esters brought a clear increase of the “fruity” character, whilst the addition of high levels of β -damascenone and β -ionone resulted in “raisin-dry plum” notes. Surprisingly, this enhancing role was not observed with de-aromatized wines.^[56] Small amounts of

DMS, that cannot be perceived in de-aromatized wines, change the situation, making it possible to perceive complex “sweet-fruity” or “green olive” notes. The presence of higher amounts of DMS renders the mixture more intense, although the “sulfury” notes are more intense as well. The simultaneous addition of DMS, fruity esters and norisoprenoids leads to an increase of the “berry-fruit” notes.^[56] These results are conform with the results presented by Segurel et al. and show once more the complexity of the “berry-fruit” character in wine.^[57]

Since GC-O became a widely used technique for identification and quantification of odorants in wine, many different varieties have been studied and analyzed with GC-O. Botelho et al.^[58] used this analytical technique to identify and quantify the odorants and their intensities in Trincadeira wine clones from Portugal. Forty-one peaks were yielded by the method for the ten clonal wine extracts, only thirty-one were perceived by GC-MS.

According to GC-O analysis, 3-methylbutanoic acid, 2-phenylethynol, 2,5-dimethyl-4-hydroxy-3(2H)-furanone, and 4-vinylguaiacol were the odorants with the highest average intensity scores in all the clones and in all the vintages. Seven esters, well-known compounds responsible for the “fruity” notes in the wines, like ethyl isobutyrate, ethyl butanoate, ethyl 2-methylbutanoate, isoamyl acetate, ethyl hexanoate and ethyl octanoate, were detected by GC-O. Amongst these esters, isoamyl acetate plays an important role in the differentiation among clonal wines. 2-Phenylethanol attained a high average intensity score for all the different clones and vintages. It is mostly produced by yeasts during alcoholic fermentation. Among C₁₃-norisoprenoids, only β -damascenone was detected in both vintages, and it is considered as an important odorant in musts and wines. Monoterpenic compounds were not analyzed in this study, which indicates that the Trincadeira wines are not influenced by monoterpenic aroma compounds. For the lactones, only γ -butyrolactone was detected in GC-O analysis, but in a very low concentration and a low odor intensity. Homofuraneol and 2,5-dimethyl-4-hydroxy-3(2H)-furanone were as well detected by GC-O and described with “burnt sugar” and “candy cotton” notes. Three volatile acids, butanoic acid, 3-methylbutanoic acid and hexanoic acid were also determined by GC-O, but they are basically not important for the aroma wine. The volatile phenols, guaiacol, 4-ethylguaiacol, eugenol, 4-ethylphenol, 4-vinylguaiacol and syringol have been determined and all of them were found in the odorant fraction of Trincadeira wines, except 4-ethylguaiacol, which was not

detected in the 2001 vintage, but in the 2003 vintage, revealing the high statistical effect of the vintage. ^[58] Vanillin, ethyl vanillate and acetovanillone were also detected by GC-O, although vanillin was not found in the 2001 vintage. In general, clonal wines from 2001 were influenced by the same group of odorant components. In particular, these are ethyl isobutyrate, diacetyl, ethyl 2-methylbutanoate, benzaldehyde, 3-methylbutanoic acid and 2-phenylethanol. The wines of the 2003 vintage were not clearly influenced by just one group of components, which classifies the 2003 vintage into two groups. But altogether, a separation between the two vintages was visible, probably due to their different climatic conditions. So it was shown that the vintage is an important factor that influences the aromatic composition of a wine.

Studies involving both, Cabernet Sauvignon and Merlot, already presented similar results for the wines. ^[51] However, only three of the 10-11 most important aroma substances in those wine varieties were found to be in common. β -Damascenone, 2-phenylethanol and 3-methylbutanol. It was uncertain if the lack of agreement derived from the different winemaking conditions or from different grape origins. Therefore Gürbüz et al. ^[59] studied the aroma compositions of the two varieties Merlot and Cabernet Sauvignon, and compared them and see if these wine types have aroma characteristics that can be observed from year to year and from different growing regions. The MS analysis yielded in over 100 peaks, but only 66 were detected, since the smaller peaks did not produce a clean MS spectrum, even with background correction. All the wines were of similar composition, the major differences were quantitative rather than qualitative. Merlot showed approximately twice as much total MS peak areas than the Cabernet wines. To compare the volatiles, the peaks were normalized, in comparison to the largest peak found in the chromatogram, which was the peak from ethyl octanoate in one of the Merlot samples. The other Merlot samples also contained very high ethyl octanoate levels, compared to the Cabernet wines with rather low values for ethyl octanoate. Cabernet wines contained higher levels of ethyl acetate and 3-methyl-1-butanol. The eight largest peaks produced between 81 and 85% of the total volatile non-ethanol peak area. Those were ethyl octanoate, ethyl decanoate, ethyl acetate, 3-methyl-1-butanol, isopentyl hexanoate, diethyl succinate and 2-phenylethanol. Five of the eight substances were esters. In general, esters made a large percentage of the total volatile composition of the wines, ranging from 60-63%, with one exception. The percentage of esters in one

of the merlot samples was at 83%. Of the minor alcohols, 17 were identified with 3-methyl-1-butanol having the largest peak. In total, the peak area of esters was 2-7 times greater than the peak area of the minor alcohols. Aroma compounds, such as phenols, deriving from the wood or from precursors of the grapes, only held a small percentage of the volatiles in the studied wines. Cabernet Sauvignon exhibited the most complex aroma composition, compared to the other samples, but ironically, it had the smallest total MS peak area, suggesting that this wine contained several potent aroma compounds at very low concentrations. The most intense aroma compounds in Merlot and Cabernet Sauvignon were 3-methyl-1-butanol, 3-hydroxy-2-butanone, octanal, ethyl hexanoate, ethyl 2-methylbutanoate, β -damascenone, guaiacol, 4-ethenyl-2-methoxy-phenol, ethyl 3-methylbutanoate, acetic acid, and 2-phenylethanol. Ferreira et al. ^[60] reported that ethyl octanoate, β -damascenone, ethyl hexanoate, 3-methyl-butanoic acid and isoamyl acetate were the most important odors in four young red wines, including Merlot and Cabernet Sauvignon.

To predict the overall aroma of these four wines, it was necessary to classify them.^[59] They were grouped into nine different classes, following the wine aroma wheel. The major category with the most substances was the “fruity” aroma. This was not surprising, considering the great amount of esters in the wines. The categories “green, vegetative and fatty” and “caramel and cooked” were also major aroma descriptors. Most of the aroma compounds could be easily assigned to one group, but several volatiles were difficult to allocate. Some were assigned to two or more groups and some could not be assigned to any of the groups. In this case they were just grouped to the category most similar to the descriptor. For example “rancid” was grouped to the category “green, vegetative and fatty”, as “rancid is often associated with “fatty”.

Comparing the two varieties, Merlot and Cabernet Sauvignon^[59], one of the Cabernet wines was the most distinctive of all the samples. Its relative pattern of the aroma category intensities were about the same to the other wines, but the total odor intensity was about 40-65% higher. Ironically, this wine had the smallest total peak area. It had a much greater number of volatiles than the other wines with remarkably high values for “earthy” and “green and fatty” descriptors, suggesting that the wine had a longer contact time with oak or a greater skin contact time. For Gürbüz et al.^[59] the latter possibility seemed more probable.

One of the most important goals in the study ^[59] was to determine, why there was so little agreement with other analyses. Many of the volatiles were not detected in the current study, but in earlier ones, and many aroma compounds have been detected in this one but in no other study before. Even the most intense odorants vary with different studies. The most likely explanation is, that different extraction procedures, different solvents, extracting times and extraction temperatures influence the chromatograms and that therefore the different results were obtained in different studies. Gürbüz et al. ^[59] showed in their analysis that the geographic origin and the vintage did not have that much influence on the aroma composition of Cabernet Sauvignon and Merlot, but instead, different techniques and procedures modified the composition significantly.

Ferreira et al. ^[61] investigated the composition of premium Spanish red wines and tried to find the relation between the compositional data and quality parameters and if it is possible to link certain substances to the quality of a red wine. Classically, the quality of a red wine is linked to the absence of some defect odors, derived from the grapes, microbial spoilage, cork and other closures, accidental contamination, oxidation, reduction or wood-related problems. Quality should also be dependent upon the presence of positive odors, such as “fruity”, “sweet”, “woody” or “toasty” notes that contribute to a wines overall flavor. Olfactograms of 25 premium Spanish red wines from 11 different denominations and 6 different vintages were drawn and the data were analyzed. After eliminating the noise in the chromatograms, 65 substances remained to be determined. Some of them have been identified in recent or past studies, only two substances were identified for the first time, (Z)-2-nonenal and bis-(2-methyl-3-furyl) disulfide. 11 substances remained unknown. (Z)-2-nonenal was found to contribute to the “green” and “metallic” aroma in wines and it was detected in relatively high amounts in the samples. It has also been reported as a compound in cashew apple, giving a “green”, “metallic”, “mushroom”, and “fatty” odor to the matrix. For bis-(2-methyl-3-furyl)-disulfide it was not possible to obtain a clear mass spectrometric signal from any wine extract, but its odor descriptors were close to those of its monomer 2-methyl-3-furanthiol and it has already been reported in orange juice, high heat skim milk powder and cashew apple. ^[61] It is a very powerful odorant, having a remarkably low sensory threshold. 16 odorants have been found in all the wine samples and only 20 of the 65 volatiles were detectable in 90% of the wines. As expected, the most frequent compounds were consistent of some

well-known fermentation, wood-related or grape-derived compounds, although also lesser known compounds, such as ethyl-4-methylpentanoate, ethyl cyclohexanoate, octanal, 2-acetylpyrazine, 1-octen-3-one, 3-methyl-2,4-nonadione, 2-methyl-3-(methyldithio)furan or (*Z*)-2-nonenal, were present in the samples. Nearly all of these compounds have been reported previously, but there is little or no information available about those substances.

The sensory analysis showed that high quality wines have strong positive odors in comparison with low quality wines, having clear negative odors, such as “dirty”, “reduced”, “oxidized” or “animal”, whereas wines with intermediate scores showed most often a very low aroma intensity and a good taste and aftertaste. A correlation study showed that no single component can be definitely related to quality. Compounds can be classified to build olfactometric vectors with the summations of the GC-O scores of the individual odorants in the category.

A closer look at the differences between high and low quality wines reveals that wines with lower quality often comprise high scores for substances that are usually related with quality problems. One of the wines had high concentrations of the powerful odorant 3,5-dimethyl-2-methoxypyrazine and also high levels of 2,4,6-trichloranisole. Other wines contained high amounts of 4-ethylphenol, 4-ethylguaiacol, 3-ethylphenol or *o*-cresol. The presence of substances like these is responsible for low quality scores of wines. Substances with a description including terms with negative connotations were listed, amongst them two sulfur compounds, methionol and methional, three unsaturated acid derivatives ((*Z*)-2-nonenal, 1-octen-3-one and (*E,E*)-2,4-decadienal, 2-methylbutanal, acetic acid, 3-isopropyl-2-methoxypyrazine and 2-methylisoborneol. For many of them, a negative contribution to a wines aroma has been suggested or reported. In case of 3-isopropyl-2-methoxypyrazine it was demonstrated, that the perception of fruity notes decreases when the concentrations of IPMP is rising.

In general, it was found that the contribution of the “sweet” and “fruity” aroma is an important parameter for the quality of a wine, but it has also been observed, that high and intermediate quality wines show an intermediate level of those descriptors, while lower quality wines show remarkably high scores for the “fruity” and “sweet” descriptors. This explains why a positive correlation between quality and the fruity-sweet aroma was not found.

Altogether it can be said that quality primarily depends on the wine aroma composition and in particular on its content of 3- and 4-ethylphenols, 2,4,6-trichloranisole, o-cresol and 3,5-dimethyl-2-methoxypyrazine, but also on the presence of other compounds with negative aroma descriptors. Secondly, a certain amount of substances with “fruity” and “sweet” descriptors is necessary. This does not mean that aroma substances with “woody”, “toasty” and other descriptors do not have any influence on the quality of a wine. In fact, quality is also related to the amount of residual sugar, polyphenol content or wine alcoholic degree. This model should not be interpreted narrowly. It should be considered as a general outline about the structure of the quality scores of red wine. ^[61]

5 Off flavors in wine

The production of quality wines requires a great attention to possible sources of microbiological, chemical, or oxidation-related spoilage. Among such defects, “earthy” and “musty” odorants are particularly detrimental. Sources for this kind of odors can be contaminated material, such as vats or barrels, closure material like corks, or musty cellar atmospheres. These odors may also derive from fungal or bacterial microflora, leading to spoilage and undesired aroma composition of the wines. “Musty” odors often come from substances like 2,4,6-trichloranisole or 2,3,4,6-tetrachloranisole. Trichloranisole may be derived from spoiled cork closures, whilst tetrachloranisole may derive from the air of cellars where wooden beams have been treated with insecticides containing polychlorophenols.^[62] “Earthy” odorants have been rarely studied in wine, but some compounds are already known to contribute such an odor to the wine, like the natural substances geosmin, 2-methylisoborneol and 2-methoxy-3-isopropylpyrazine, or the synthetic substances 2-ethylfenchol or some tertiary alcohols. In the study of Darriet et al.^[62], geosmin was identified and quantified in red and white wines and also in their musts, indicating that geosmin is an early contaminator in wine. Using GC-O, only geosmin had the same retention times as the odorant zones in the wines. 2-Methylisoborneol, 2,4,6-trichloranisole and 2,3,4,6-tetrachloranisole were injected as well, but did not show the same retention time as the odorant zone. Concentrations of geosmin in the various wine samples ranges from 20 to 300 ng/L, and in most of the samples, the levels of geosmin were above its threshold level. Of the two enantiomers, (-)-geosmin has been found to be the natural form, which possesses an odor threshold in water of 11 times lower than the threshold of (+)-geosmin. Therefore, (-)-geosmin could be the main enantiomer in wines. It could also be detected in wines which ran through alcoholic and melolactic fermentation, as well as in wines aged for several years, indicating that geosmin is a very stable component in wine. The analysis of geosmin in wine showed that it is not derived from cork closures or contaminated barrels as thought, but probably from some microorganisms present on grapes, because it was already present in the grape must before alcoholic fermentation.

Apart from geosmin, many substances contribute to an undesired flavor in wines like those, associated with oxidative degradation of white wines. 1,1,6-Trimethyl-1,2-dihydronaphthalene (TDN) has been reported to contribute to a “kerosene-like” aroma, usually found in old wines from the Riesling variety. Like TDN, Riesling acetal is thought to form in wine by acid-hydrolysis of grape-derived glycoconjugated precursors. A hemiacetal was already isolated and proved to be a precursor for TDN and Riesling acetal. Daniel et al.^[63] investigated the competitive formation of TDN and Riesling acetal under wine storage conditions and proved that Riesling acetal is only an intermediate in the TDN formation, rather than a separate end product, as thought before. They conducted the hydrolysis of hemiacetal and acetal to observe the formation of the two searched compounds. And indeed, at higher storage temperatures, no Riesling acetal was present in the wines, because it was converted into TDN, resulting in higher concentrations of TDN in the hydrolysate. To test the hypothesis, bigger amounts of Riesling acetal were added to the wine samples and the TDN production was observed. The formation of TDN was much higher in the supplemented samples. This data confirmed that Riesling acetal is ultimately converted into TDN during bottle aging at higher temperatures. But also with knowing how TDN is formed in wine, it is unlikely to influence its formation in wine during bottle aging.^[63]

Another compound, known to contribute to an undesired aroma in wines is 2-aminoacetophenone, the “untypical aging off-flavor” (UTA).^[64] It can be formed in the bottle or in the wine cask within a few months after fermentation. UTA is characterized by “furniture polish”, “wet wool”, “mothball”, “fusel alcohol” or “acacia blossom” notes. It was shown, that it cannot be detected in the grape, in the must or in the wine directly after fermentation, but it can be perceived after storage of the sulfurized wines. The biosynthesis of 2-aminoacetophenone is generally linked to the tryptophan metabolism. In particular the tryptophan metabolites kynurenine and indole-3-acetic acid were discussed to be potential precursors for 2-aminoacetophenone in wine. Also 4-(2-aminophenyl)-2,4-dioxobutanoic acid has been discussed as a potential precursor for UTA.

Besides a fermentative formation of 2-aminoacetophenone by *Saccharomyces cerevisiae*, a non-fermentative pathway is possible. It has been suggested that indole-3-acetic acid in a reaction with sulfite results in the formation of 2-aminoacetophenone and the side-products skatole and 2-formamidoacetophenone.

Hoenicke et al.^[64] investigated the possible pathways for the formation of 2-aminoacetophenone in wine, and discussed the possible precursors for it. The first possible pathway was the fermentative formation of 2-aminoacetophenone from kynurenine by *Saccharomyces cerevisiae*. The degradation of kynurenine was observed, but no increase of 2-aminoacetophenone could be detected. Only the formation of kynurenic acid was found. Also the non-fermentative formation of 2-aminoacetophenone from kynurenine by sulfurization was not successful. A model-wine solution was supplemented with kynurenine and the sulfurized, but again, only the formation of kynurenic acid was observed. Taking indol-3-acetic acid as a precursor was leading to a formation of 2-aminoacetophenone, but far below its odor threshold level. Only the non-fermentative pathway from indol-3-acetic acid lead to reasonable amounts of 2-aminoacetophenone. Indole-3-acetic acid was supplemented to a model-wine which was then sulfurized. 2-Aminoacetophenone, 2-oxindole-3-acetic acid and indole-3-aldehyde were formed. The latter two are only oxidation by-products. Traces of 2-formamidoacetophenone and 3-(2-formylaminophenyl)-3-oxopropionic acid were found as well, and they were revealed to be part of the pathway from indole-3-acetic acid to 2-aminoacetophenone. Concluding, the formation of 2-aminoacetophenone by this pathway is most likely after sulfuration, which is indispensable for white wine making. However, 2-aminoacetophenone concentrations vary significantly among the different winegrowing regions and vintages, therefore it may not be the only component contributing to an untypical aging off-flavor.

Odorants, such as 3-(methylthio)-propionaldehyde (methional), eugenol, sotolon and 2,4,5-trimethyl-1,3-dioxolane have also been linked to off-flavor characteristics. Ferreira et al.^[65] employed sniffing analysis to screen odor zones, that can be related with descriptors, previously selected by a trained panel as characteristics of “precocious aging”. In general, oxidative degradation is related with low pH, too high temperatures of storage and high oxygen content. GC-O was performed to identify the aroma compounds, most related to the selected off-flavor descriptors, which were “boiled potato”, “honey-like” and “kerosene”. When comparing a non-oxidation-spoiled wine with an oxidation-spoiled one, differences in the flavor dilution factors (FD) were visible, regarding to the intensities of the three selected odor zones “boiled potato”, “honey-like” and “kerosene”. The FD’s in the spoiled wines were much higher than in the non-spoiled wines. Using GC-MS, it was

possible to link the odor zones to the corresponding molecules, that were methional, phenylacetaldehyde, TDN and sotolon. To promote the aroma degradation of a white wine, an experiment was carried out, putting a white wine sample under forced aging conditions. It was submitted to different oxygen and SO₂ contents, different pH levels and different storage temperatures. The substances were added simultaneously and separately to the wine. After 7 days the methional, sotolon, phenylacetaldehyde and TDN contents were determined. Sotolon was present in concentrations above its threshold only in samples treated with oxygen and stored at 60°C. The highest spoilage of the wines was observed when methional, sotolon and phenylacetaldehyde were added simultaneously to the wine. TDN could not be added to the model wine, because it was only available in unsatisfying purity. Comparing the contribution of the single oxidation-related compounds to the sensory perception, methional was considered to have the most intensive effect on the typical aroma of an oxidation-spoiled white wine.

The role of other aldehydes to the oxidation-spoiled aroma of white wines was investigated by Culleré et al.^[66]. They tried to determine the levels of some aldehydes with potential sensory significance and to evaluate their sensory role. The wine samples were classified into several groups, like young wines or aged red wines and compared to two groups made up by samples of young wines, oxidized in the laboratory, which may represent the accidental oxidation of wine. The data confirmed, as expected, that the levels of most of the aldehydes are linked to the wine-type and therefore to the kind of aging it went through. Only (*E*)-2-heptenal and (*E*)-2-octenal seemed to be an exception, since they had high levels only in the wine samples oxidized in the laboratory. The three branched aliphatic aldehydes analyzed in this study, methylpropanal, 2-methylbutanal, and 3-methylbutanal were found in high concentrations in red wines with a long aging time. Also methional and phenylacetaldehyde were detected in high concentrations in aged red wines. Young wines had in all cases the lowest amounts of these compounds. In the case of (*E*)-2-alkenals, methional and phenylacetaldehyde, the young wines had the lowest levels in comparison with aged red wines and the laboratory-aged wines, although the levels of methional and phenylacetaldehyde were close to the sensory threshold levels in the young wines. The three branched aldehydes were added to a neutral white wine in a similar concentration as it was found in aged red wines, resulting in high values for “sweet”, “orange-like”, and “fusel” notes. Supplementing a neutral

red wine with the same mixture, levels for the “dried fruit”, old wood”, “papery”, “sweet” and “fusel” notes increased significantly. A similar result was obtained when supplementing the neutral wines with (*E*)-2-alkenals. Supplementing a white wine with (*E*)-2-alkenals in a concentration found in slightly oxidized white wines, resulted in increased “papery”, “old wood” and “moldy” notes. Supplementing a young red wine with a mixture of (*E*)-2-alkenals, in particular with high levels of (*E*)-2-octenal, which resembles the most oxidized young wines, resulted in an increase of “dirty”, “dusty”, “closed old rooms” notes, whereas a young red wine, supplemented with especially high levels of (*E*)-2-nonenal resulted in high “dusty” and “rancid” notes. Investigating the two compounds (*E*)-2-octenal and (*E*)-2-nonenal revealed, that surprisingly, even if the added amounts were under their sensory threshold, they were easily perceived in the wines, suggesting a synergistic effect with other wine volatiles, provoking the appearance of “dirty”, “closed room” and “earthy” notes. The addition of (*E*)-2-nonenal brought the appearance of “brandy-like” notes in the wines. However, high amounts of (*E*)-2-alkenals show nasty odor nuances that can be linked to these compounds. Surprisingly, when high amounts of branched aliphatic aldehydes are in the wine, the sensory perception of the nasty odors diminish and the odors of the branched aliphatic aldehydes come in the foreground. These results suggest the importance of the branched aliphatic aldehydes in wines, not only for their contribution of their typical notes to a wines composition, but also because of their help to minimize the sensory effects of (*E*)-2-alkenals. ^[66]

Sotolon (3-hydroxy-4,5-dimethyl-2(5H)-furanone) is considered to contribute in a negative way to the aroma composition of a wine. It brings a “honey” like or “oxidative” note to the wine. It was first discovered in vegetable protein hydrolysates in the late 1960’s with an aroma reminiscent of walnuts. In some studies it was also described to have an intensive “curry” note, and others linked a “dried fig” or “rancio” scent to sotolon.^[67] However, the impact and the biosynthetic pathway of sotolon in wines were unclear. It is known, that sotolon is basically formed under oxidizing conditions, although there are wines with high levels of sotolon, that had not been aged under oxidation conditions. Therefore, its formation in white wines is apparently promoted or triggered by unknown parameters. Lavigne et al.^[68] monitored changes in the sotolon content of a Sauvignon Blanc wine, fermented and aged in new and used oak barrels with or without total lees. The presence of lees in

the wines during 8 months of barrel-aging delayed the formation of sotolon, and therefore the final concentrations of sotolon in these wines were much lower than in the other samples. The capacity of the lees to combine oxygen may explain the effect of preventing the sotolon formation. Sotolon contents were high in bottles with defective corks, indicating that oxygen plays a major role in the formation of sotolon. Forty white wines were sealed with top-grade natural corks and then aged for seven years, and the different levels of dissolved oxygen could be clearly correlated to the levels of sotolon. Also the perception of oxidation-related aromas was dependent upon the presence of sotolon in the wines. This suggests, that the furanone levels in wine are a good marker for the defective aging phenomenon in white wines.^[68]

Pons et al.^[69] first reported about the two enantiomers of sotolon and determined their odor thresholds and the concentrations in the wine. They found out, that the perception threshold of (*S*)-sotolon in wine was 20 times lower than the perception threshold of the (*R*)-sotolon enantiomer, indicating that the (*S*)-enantiomer alone imparts its odor and organoleptic properties to the wine. They also tried to find a pathway, but they could only suggest, that sotolon may be generated as a racemic sotolon from natural enantiomerically pure chiral sources like amino acids, sugars and others. Another possible option is, that sotolon is generated by a stereoselective reaction, resulting in an enantio-enriched form. An exact pathway was not found or proved, but Pons et al. continued the experiments to this topic and proved a biosynthetic pathway for sotolon. Significant decreases of the ascorbic acid levels and increases of sotolon levels indicated an oxidative degradation of ascorbic acid and a formation of sotolon from it, though sotolon is also present in wines untreated with ascorbic acid. One logical hypothesis was, that a by product of the degradation of ascorbic acid acted as an intermediate in the sotolon formation pathway. Because of this approach, the carbon skeleton of sotolon was analyzed and the carbons were identified to derive from ascorbic acid and ethanol. Another pathway was also thought of. Sotolon was assumed to originate from an aldol reaction between compounds with two and four carbon atoms in their skeleton. This approach led to search for 2-ketobutyric acid among the oxidative degradation by products of ascorbic acid. It was also proved that 2-ketobutyric acid derived from ascorbic acid during degradation. 2-Ketobutyric acid was already known to form sotolon with acetaldehyde. In the wine samples both, 2-ketobutyric acid and acetaldehyde, were present. Acetaldehyde can also bind sulfur dioxide, therefore only small amounts of

free acetaldehyde are present in wine. Experiments revealed, that sotolon was formed when acetaldehyde concentrations exceeded 500 µg/L. However, its racemic form indicated, that sotolon may also be formed by the mediation of chiral vectors, like amino acids, in particular proline, which catalyzes aldol reactions in an enantioselective way, but during aging they form racemics.

Acetaldehyde is a known by-product of alcoholic fermentation in wine, but the presence of free acetaldehyde in wine is mainly dependent upon the sulfur dioxide levels in wine, but factors affecting the 2-ketobutyric acid concentrations are more interesting as to the differences in the sotolon content of wines. 2-Ketobutyric acid is an intermediate of the degradation of threonine, forming a 2-keto acid and then *n*-propanol. The choice of the yeast strain has a big influence on the formation of 2-ketobutyric acid at the end of the fermentation process. This may be due to differences in the activity of threonine deaminase. Concerning sulfur dioxide, an addition of it to fermented model solutions, resulted in a 50% decrease in keto acid, irrespective of the yeast strain. The ascorbic acid levels are also strongly correlated with the resulting sotolon concentration. The levels of ascorbic acid in wines, sealed with top grade natural corks, and stored for seven years, ranged from a decrease of 30-100%, depending upon the permeability of the corks.

Altogether, the results of this study showed, that 2-ketobutyric acid via aldol condensation with acetaldehyde, is responsible for the small amounts of sotolon in prematurely aged dry white wines.

6 Prediction of wine sensory properties from compositional data and consumers acceptability, and expert quality judgement of red and white wines

One of the main aims of the wine flavor industry in the last years is to create mathematical models and scales to produce high quality wines. The industry would benefit from the determination of such models and also from robust correlations between sensory and instrumental measurements of aroma compounds. High quality is not only depending on the presence of certain aroma constituents, but also on the absence of off-flavors and of course on the consumers acceptability.

Francis and Newton ^[70] tried to link the compositional data of wine aroma to consumers preferences, although they were aware that the aroma composition of a wine is not dependent upon one or two compounds, but upon a multitude of components. It is proved that much of the consumers interest in wines, especially in white wines, derives from subtle and complex aroma mixtures, and not from typical, strong aroma notes.

Therefore, the ultimate aim for the wine flavor industry would be to quantify a great number of components that indicate whether a wine is highly valued by the target consumer group or not. This has been achieved only for a limited extent of constituents, indicating the complexity of the perception of aroma mixtures. Many volatiles are present in very low concentrations in the wines and therefore they may not have a big influence on the wines aroma. But even if substances are present in concentrations below their thresholds, it is possible that they are perceived, because of synergistic effects with other components. Perception thresholds are mostly an indicator of how much the compounds contribute to the aroma of a wine, but thresholds are different for diverse matrices, including air, water, various model systems and also red and white wine. Odor thresholds are necessary to determine the odor activity value (OAV), a useful parameter to assess the relative importance of individual chemical components present in a sample. If the OAV exceeds 1, the concentration of the component in the matrix is higher than its odor threshold and therefore it can be perceived in a mixture; but it is not said that a component always contributes in the same way and extent to the aroma composition. 4-Mercapto-4-methylpentan-2-one, a potent sulfur component, is often referred to a “boxtree-like”

aroma, but “boxtree” aroma itself consists of either “cat urine” or “passionfruit” or “tropical fruit” aroma. Therefore, at different concentrations of 4-mercapto-4-methylpentan-2-one the aroma perception changes. This values also for dimethyl sulfide, which provides a “cooked/canned asparagus” aroma at lower levels, but at higher concentrations the aroma turns into “blackcurrant” and at even higher concentrations it is perceived as “cooked corn/tomato”.

Substances with an OAV below 1 may also have an impact on the aroma perception of wines. They may interact synergistically or additively. But they may also mask each other, as it is the case of high concentrations of ethyl acetate and other esters. If ethyl acetate is present in high concentrations in a mixture, it is likely that other esters, such as ethyl propanoate and butanoate, propyl, butyl and tert-butyl acetates cannot be perceived, even if they are present in concentrations close to their threshold levels. So the chemical composition not always allows exact predictions of how the sensory perception of a wine will be.

Another study was carried out by Campo et al.^[71] about the prediction of wine sensory properties from compositional data. The composition of six white wines was studied and described in different aroma characters. One term which was found in every sample was the “tree fruit” descriptor. Other terms that also attained high scores, but only in some of the samples, were considered as important descriptors to differentiate the wines, like “tropical fruit” “citric”, “floral”, “muscat” and “sweet”. Campo et al. built models, using these terms, but only seven compounds were introduced in the models and only linalool and 3-mercaptopentyl acetate, previously identified as most discriminant odorants, have high loading weights. Models for “sweet”, “floral”, and “muscat” showed a similar structure, since linalool is the most important positive and 3-mercaptopentyl acetate the most important negative contributor. These three descriptors are somehow opposed to the “tropical fruit” descriptor, which is positively correlated to the 3-mercaptopentyl acetate content and negatively to the level of 3-*sec*-butyl-2-methoxypyrazine. The latter one only had significant effects when the concentration of 3-mercaptopentyl acetate was low. For the “floral” and “sweet” models, linalool and 2-phenylethyl acetate were positively contributing to its aroma, and 3-mercaptopentyl acetate was affecting it in a negative way. The effect of linalool was clear, but the effect of 2-phenylethyl acetate did not furnish any sensory change. Only at low levels of linalool and together with isoamyl acetate, another odorant of the same family which provides a strong “banana” flavor,

a significant change in the wine aroma was perceived. This indicated, that linalool is able to mask the sensory properties of both acetates. The “muscat” model was significantly affected by the addition of acetic acid, but only high amounts of acetic acid could decrease the typical “muscat” note. The “citric” model could not be modelled with the used GC-O data set, demonstrating another limit of the commonly used methods.

Aznar et al.^[72] tried to build up models correlating a matrix of sensory data Y , with one of chemical data X , guaranteeing that the number of cases studied is sufficient to produce robust and reliable models. Thirty-four compounds were measured, most of them reached their sensory threshold, but some of them, like vanillin and several related compounds did not, but were still kept in the study, because there are reasons to think that these compounds can play some role in the aroma composition of wines. Some of these compounds are similar and they are thought to have additive or synergistic effects, such as vanillin, ethyl vanillate, methyl vanillate, and 1-(4-hydroxy-3-methoxyphenyl)ethanone. Others have odor thresholds, reported in literature, that are far below the thresholds measured in this study. Components that share the same biochemical origin and present similar sensory properties as a consequence of their similar chemical structure were combined, in order to reduce the list of substances.

Fifty-seven wines were then analyzed by Aznar et al.^[72] and their sensory performance was characterized with the previously mentioned terms and groups. The citations were counted and model solutions were made with the compounds of the most frequently used terms in the wines. For all the descriptors it can be said, that none of them was univariate. All of them were integrated by at least 4 variables, but most models required between 6 and 10 variables. This result again shows the complexity of the aroma composition of wine.

Confirming this complexity, 27 odorants or groups of odorants intervened in some of the 9 models. The correlations were positive and negative, suggesting that the perception of an aromatic note is influenced not only by the presence of a few components whose aroma form the note, but also by the presence of other odorants that affect negatively in the perception of such aromatic notes.^[72] This is the case of the most important descriptor in the wines tested, the “wood-vanillin-cinnamon” note. It is mainly containing the three most important aromas of wood, *cis*-oak lactone, vanillin and eugenol. Although, its perception is limited by the presence of

4-ethylphenol, 4-ethylguaiacol, acetaldehyde and phenylacetaldehyde. The following descriptor in importance is the “fruity” term, which can be linked to the presence of β -damascenone, but also with the “berry-fruit” notes from 2,3-butanodione, methyl vanillate and β -ionone and the “raisin-flowery” notes from the ethyl esters of the acids 3-methylbutyric, 2-methylbutyric and 2-methylpropanoic acid and β -ionone and 3-hydroxy-2-butanone. The “berry-fruit” descriptors seem to be limited by the same components that are limiting the “fruity” notes, and the “raisin-flowery” components seem to be affected by high levels of 3-(methylthio)-1-propanol and 2-methoxyphenol, which provide a pungent aroma. Modelling the most complex descriptor “toasted-coffee” was difficult and it had the highest percentage of variance. The volatile with the strongest weight was 2-methoxyphenol, but also a lot of other odorants played a role for the “toasted” and “coffee” note, like 2-furfurylthiol, which was already identified as a compound in coffee and is already known to contribute to a “coffee” note in wine aroma, but there was no quantitative data available for this compound. The “sweet-candy-cacao” term was probably dominated by 4-hydroxy-2,5-dimethyl-3(2H)-furanone, but due to the absent data of it, the term could not be fully characterized. Nevertheless, the descriptor was positively related to “sweet” and “fruity” tones, and negatively correlated to the presence of 4-ethylphenol, phenylacetaldehyde and 3-(methylthio)-1-propanol. The characterization of the “animal-leather-phenolic” term was quite easy. It could be clearly related to 4-ethylphenol and 4-ethylguaiacol and its odor was limited by substances like β -damascenone and vanillin. Similarly, the characterization of the “old-wood-reduction” descriptor was quite simple, correlating the single component phenylacetaldehyde to the term. Unfortunately, the term “vegetal-pepper” could not be related to methoxypyrazine, because of a lack of data of these substances. Ethyl esters of the isoacids, fusel alcohols and the isoacids themselves also contribute to a “vegetal-pepper” aroma.

Testing model solutions and linking descriptors to certain compounds are important, but the industry does not know what the consumers preferences are. Therefore recently a strong interest lies in the consumers acceptability of wines to produce wines that are commonly considered as tasty.

Lattey et al ^[73] were the first to determine wine quality from a consumers perspective, including identification of key attributes that push consumers response. Wine producers already made a big effort in the investigations of the sensory

attributes of the wines and how to modify them through the choice of the harvest time, the yeast strain, oak type, or fermentation conditions, but there are only few data available concerning wine sensory attributes and consumer liking scores. It is difficult to use feedback from consumers via the marketplace, which is imprecise and slow and carries the risk of the unreliability of market signals. The aim of this study^[73] was to determine whether Cabernet Sauvignon and Shiraz wines are liked differently, because of a recent decrease in sales of Cabernet Sauvignon and a slight increase in sales of Shiraz wines. the study also shows if the wine types liked by the consumers, are the same that are liked by trained judges.

20 wines were tested, 10 Cabernet Sauvignon and 10 Shiraz wines. Two wines were separated from the rest in the first panel, because of their high notes of “bandaid”, “barnyard”, “metallic”, “leather”, and “reduced”. These wines were found to contain high levels of the *Brettanomyces* yeast products 4-ethylphenol and 4-ethylguaiacol. A separation concerning the attributes “purple” and “colour intensity” and the descriptor “brown” was also visible. Mainly the older wines from the 2002 and 2003 vintage were rated as “brown” wines, which is not surprising, considering the fact that older wines will commonly have a browner, less purple and lighter appearance and a lower intensity of “fruity” notes. Shiraz wines were rated similarly and had high levels of “cooked dark fruits”, “red berries”, “vanilla”, and “persistence”. Two wines were also rated higher in “caramel” and “fruit flavor”, and one wine had a distinct “coconut scent. Other patterns considered the differences in the intensities of “coffee” notes, “pepper” and “spicy” notes or “drying”, “coarseness” and “adhesive” descriptors. 203 consumers tested the 20 wines, and highly significant differences were found among 12 wines. 6 of them were ranged above the mean liking values. In general, one Shiraz and two Cabernet Sauvignon were liked most, whilst two Shiraz and one Cabernet wine were liked least. So, there cannot be defined a clear evidence for a higher liking of Shiraz compared to Cabernet Sauvignon.

The liking scores for the total consumers group were associated with high scores of “confectionary”, “floral”, “red berries” and “fruit flavor”, whereas the wines liked least could be linked to the attributes “smoky”, “bitterness”, “hotness”, “black pepper”, “leather”, “barnyard”, “bandaid”, and “metallic”. But however, there were big differences between the certain clusters. The grouping was based on similar liking patterns. The first group liked wines with high “caramel” notes and low “acidity” and “green flavor” notes, the second one preferred overall Cabernet

Sauvignon wines with higher “green flavor”, “blackcurrant”, “mint” and “acidity” attributes. The third cluster clearly disliked the “pepper” aroma and the largest group of the fourth cluster preferred wines with high “confectionary”, “red berries” and “floral” scents. This cluster also correlated negatively with the “smoky” descriptor. Liking was also linked to the astringent attributes “drying”, “adhesive” and “surface texture”.

The most important negative influences seen on the total consumer sample included “pepper”, “smoky”, “woody”, “hotness”, and “bitter”. Also “earthy”, “bandaid”, “barnyard” and “metallic” attributes, deriving from 4-ethylphenol and 4-ethylguaiacol were generally considered as negative influences on the wine aroma.

Overall, it is clear that consumers reacted differently to the wines, but there are some attributes obviously determined as positive, and others were clearly related to negative features. Interestingly, there was no significant correlation between the liking scores and the price, as well as age, gender and self-reported wine-knowledge. However, the scorings of professional wine judges and winemakers in comparison to ordinary wine consumers differed significantly. The only conformity of the different ratings were due to the *Brettanomyces*-related substances 4-ethylphenol and 4-ethylguaiacol and their sensory attributes, which were in all cases linked to low quality.

Wines that scored highest in the quality test of the winemakers were generally the more expensive ones, with one exception, which was intermediate in price. Wines that were liked from winemakers were darker in “overall colour”, higher in “red berries”, “mint”, “pepper”, “spice”, “woody”, “coconut”, “vanilla”, “fruit flavor”, “surface texture”, “drying”, “adhesive”, “hotness”, and “persistence”. In general, these features derive from more richly flavored wines, with higher fruit, oak and astringency notes.

Wines were scored lower if they had higher “vegetal”, “coffee”, “smoky”, “earthy”, “leather”, “reduced”, “barnyard” and “bandaid” descriptors. For consumers, the “bitterness” feature was significantly negative, but for winemakers it was sometimes even positive, probably because wines with higher astringency can have higher bitterness. Also the “woody” descriptor was considered negative for the consumers, but positive for winemakers.^[73]

This study demonstrated, that quality can be very different for winemakers and professional judges, and for the consumers. A study to investigate further in this

point would be of great value to see where the differences originate and that the wines can be better adjusted on the appropriate group of consumers that the winemaker tries to reach.

7 Abstract

This review was aimed to unite the latest research results concerning wine aromas and therefore mainly articles from 2000-2010 were taken into account.

Whereas the focus of the past centuries lay on the determination of major components in wine, like ethanol, sugar or acid content, studies are now more focusing on wine aroma compounds, in particular on the determination of aroma compounds that are typical for a certain grape variety, like linalool and other terpenols for Muscat-related grapes, methoxypyrazines for Sauvignon varieties, *cis*-rose oxide for Gewürztraminer, 4-methyl-4-mercaptopentanone for Sauvignon blanc or 3-mercaptohexanol for Grenache and Merlot wines. Unfortunately, so far, no method has revealed specific odorants in wines that are typical for only one grape variety. It was only possible to state, that the quantities of certain odorants vary strongly in different grape varieties. Furthermore, a high wine quality is linked to a very complex and subtle wine aroma composition, a bouquet, instead to typical overall flavors of certain wines.

Factors that influence the production of wine aroma compounds, like harvest and pressing conditions, different yeast strains, oak barrels or oak chips and others are discussed in this review, as well as the determination of biosynthetic pathways of aroma compounds, to find out more about their origin and therefore also how to enhance or diminish them. Off-flavors are often mentioned, because greater concentrations of undesired flavor characteristics lead to lower quality wines.

Studies about the quality judgement of wines, consumer acceptability, and also the prediction of wine quality from compositional data came up very recently and especially the wine industry shows a big interest in this topic.

8 Zusammenfassung

Dieser Artikel befasst sich im wesentlichen mit den neuesten Ergebnissen und Publikationen bezüglich Aromen im Wein in den Jahren 2000-2010.

Während in den vergangenen Jahrhunderten die Untersuchung der Grundbausteine von Wein, wie Alkohol-, Zucker- und Säuregehalt im Vordergrund stand, wird heute mehr Aufmerksamkeit auf die Aromakomposition eines Weins gelegt. Besonders die Identifizierung von Hauptinhaltsstoffen, wie Linalool und anderen Terpenen in Muskat-Weinen, Methoxypyrazine in Sauvignon-Arten, cis-Rosenoxid in Gewürztraminer, 4-methyl-4-Mercaptopentanon in Sauvignon blanc oder 3-Mercaptohexanol in Grenache und Merlot Weinen. Jedoch konnten bis heute noch keine eindeutigen Hauptinhaltsstoffe charakterisiert werden, die für alle Weine einer Varietät in annähernd gleicher Konzentration vorhanden sind. Außerdem wird ein Wein meist mehr geschätzt wenn nicht nur ein, oder einige wenige Hauptaromastoffe im Vordergrund stehen, sondern wenn der Wein ein komplexes und feines Aromabukett aufweist.

In diesem Artikel werden weiters die Faktoren, die die Aromakomposition eines Weines beeinflussen können, genauer betrachtet, wie z.B. die Ernte- und Verarbeitungsbedingungen, die Nutzung von unterschiedlichen Hefe-Stämmen, die Zusammensetzung und Zustand der Eichenfässer, bzw. die Größe und Einwirkzeit von Eichenchips, und einige andere. Auch die Ermittlung von biosynthetischen Produktionswegen für diverse Aromastoffe stand im Mittelpunkt vieler Arbeiten. Es ist wichtig, die Ursprünge von verschiedenen Inhaltsstoffen zu kennen, damit man gegebenenfalls ihre Produktion steigern oder abschwächen kann, wie im Falle von Fremdgerüchen. Diese „off-flavors“ können einen Großteil dazu beitragen, dass ein Wein als qualitativ niedrig eingestuft wird.

Zum Thema Qualität wurden erst kürzlich einige Artikel verfasst, die sich genauer mit der Charakterisierung von qualitätsbestimmenden Inhaltsstoffen auseinandergesetzt haben, und auch, ob man von der chemischen Zusammensetzung auf die Qualität eines Weins schließen kann. Besonders die Weinindustrie interessiert sich sehr für dieses Thema, um ihre Weine bestmöglich zu optimieren.

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10 Curriculum vitae

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