Doctoral Thesis

Aroma active sulphur compounds and their precursors in petite arvine wine

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AROMA ACTIVE SULPHUR COMPOUNDS AND THEIR PRECURSORS IN PETITE ARVINE WINE

A dissertation submitted to the
SWISS FEDERAL INSTITUTE OF TECHNOLOGY
ZÜRICH

For the degree of
Doctor of Technical Sciences

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Curriculum Vitae

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A special and cordial thank goes to my family, my parents, my brothers and my partner Martin for supporting and motivating me throughout my studies and my doctoral thesis.
Petite Arvine is an autochthone grape variety used to prepare a very popular wine specialty of the Canton of Valais in Switzerland. The characteristic aroma of Petite Arvine wine is described as fruity and flowery. The aim of this work was to identify the characteristic aroma compounds and to describe their formation during wine making.

By using sensory evaluation, an aroma profile could be established. The typicity of this wine correlated positively with the intensity of the descriptors grapefruit, rhubarb and quince. Using gas-chromatographic methods (GC, GC-O, GC-MS), 3-mercaptohexanol was identified as one of the key aroma compounds, responsible for the grapefruit and rhubarb flavour note of Petite Arvine wine. The concentration of 3-mercaptohexanol exceeded its odour threshold manifold.

The non-volatile precursor of 3-mercaptohexanol has previously been described in literature. The precursor is transformed into free 3-mercaptohexanol by a β-lyase of yeast. In this study, the following aspects of this transformation have been examined:

- Lactic acid bacteria that are responsible for the malolactic fermentation in wine making, have been shown to possess the ability to release 3-mercaptohexanol from its precursor. The impact on the overall aroma of Petite Arvine, however, is minor.

- In order to study the impact of different fermentation parameters on the formation of some flavour compounds, the Youden experimental design was used. This tool has shown to be very effective for this purpose. The fermentation temperature mostly influenced the formation of 3-mercaptohexanol; elevated fermentation temperature being favourable.

- The pathway of the transformation from the precursor to the free thiol was studied by adding synthetic $^{35}$S-3-(hexan-1-ol) L-cysteine to a fermenting grape must. The fermented
must was divided into different water- and organic solvent-soluble fractions. It could be demonstrated, that no free thiol was lost by evaporation during fermentation and that only a small part was adsorbed by the yeast lees. The most important part of the initial radioactivity was found incorporated in water-soluble compounds.

The study of the aroma compounds and their formation revealed that 3-mercaptOHexanol is an important contributor to the characteristic aroma of Petite Arvine wine particularly with respect to the grapefruit and rhubarb notes. The transformation of the non-volatile precursor into the free thiol is very complex and influenced by many factors, but it seems that by choosing the appropriate fermentation temperature and wine making parameters, the release of 3-mercaptohexanol can be influenced positively.
Zusammenfassung


Der Präkursor von 3-Mercaptohexanol wurde in früheren Studien bereits identifiziert. Es handelt sich um ein Cysteinkonjugat, welches durch eine β-Lyase der Hefe während der alkoholischen Gärung 3-Mercaptohexanol freisetzt. In der vorliegenden Studie wurden die folgenden Aspekte dieser Umsetzung untersucht:

- In Modellversuchen konnte gezeigt werden, dass die Milchsäurebakterien, welche für den biologischen Säureabbau in der Weinbereitung eingesetzt werden, ebenfalls eine β-Lyase Aktivität aufweisen. Der Einfluss auf das Aroma des Petite Arvine Weins scheint allerdings gering zu sein.

- Um den Einfluss der Fermentationsparameter auf die Bildung einiger Aromastoffe abzuklären, wurde die Youden Versuchsplanung verwendet. Diese Methode erwies sich als
sehr effizient. Die Fermentationstemperatur hat die Bildung von 3-Mercaptohexanol am meisten beeinflusst, wobei sich erhöhte Temperaturen als vorteilhaft erwiesen.


Chapter 1

INTRODUCTION

Petite Arvine is an autochthone white grape variety, which grows exclusively in the Canton of Valais/Switzerland. Petite Arvine wine has become more and more popular in the last decades; the cultivated area and the production of Petite Arvine wine has considerably increased. The vinification of Petite Arvine wine is delicate and therefore not very systematic; the differences between vintages and producers are remarkable.

Wine makers and enologists describe the characteristic aroma of Petite Arvine wine as fruity (grapefruit, exotic fruit, rhubarb, etc.) and flowery (wisteria, violet). However, the characteristic flavour impact compounds of Petite Arvine and their precursors have not yet been identified. Some preliminary studies carried out at the University of Applied Science in Sion, have led to the assumption that sulphur compounds have a great impact on the characteristic flavour of Petite Arvine wine.

Sulphur containing compounds play a mayor role in food processing. They can be desired as key flavour compounds i.e. in coffee, meat, onion and leek, but also undesired as off-flavours in milk or the sulphurous off-flavour "Böckser" in wines. Sulphurous compounds are usually present only in traces, are volatile and reactive and have in general low odour thresholds which can have an enormous impact on the sensorial properties of a food. The knowledge of the (sulphurous) flavour compounds allows the control of the production of foodstuff by preventing formation of off-flavour and promoting formation of desired flavours.

The aims of this doctoral thesis are:

• to identify the compounds that are responsible for the characteristic flavour of Petite Arvine wine
• to identify the precursors of the flavour impact compounds
• to describe the transformation from the precursors into flavour compounds during wine making

The knowledge of the flavour impact compounds of Petite Arvine wine will help to control the winemaking, to optimise the flavour development and to obtain a more regular wine quality.

The thesis is structured into nine chapters. Chapter 1 deals with the introduction and scope of the thesis. In chapter 2, relevant literature on wine and aroma analyses as well as recent research results particularly related to the presence and analysis of sulphurous flavour compounds are reviewed. Chapter 3 describes the sensorial evaluation of Petite Arvine wines. The subject of chapter 4 is the identification of volatile compounds contributing to the characteristic aroma. Chapter 5 focuses on the impact of 3-mercaptohexanol for the flavour of Petite Arvine wine. In chapter 6 the release of 3-mercaptohexanol by Oenococcus oeni, the bacteria responsible for the malolactic fermentation, is discussed. In chapter 7 the impact of different fermentation parameters on the characteristic aroma of Petite Arvine wine using an experimental model design are presented. The subject of chapter 8 is the elucidation of the transformation of precursor into the aroma active free thiol by radioactive labelling. Finally in chapter 9, a general discussion, conclusions and an outlook can be found.

Chapter 3, 4, 5 have been published in peer reviewed journals. Chapter 7 has been accepted for publication and chapter 6 and 8 are prepared to be submitted for publication in peer-reviewed journals. Therefore, these chapters were written as independent papers with the consequence that overlapping, especially in the introductory parts and in the materials and methods section, were unavoidable.
Chapter 2

LITERATURE REVIEW

2.1 The wine of Petite Arvine

The climatic conditions in the Valais region in Switzerland allow the cultivation of a large number of vine varieties. Among these, several local varieties grow exclusively in this region. Petite Arvine belongs to this group [1].

From the 19th century on, grape varieties as Chasselas, Sylvaner and Pinot noir replaced the local varieties in the Valais. In the last years, this trend was reversed and the autochthonous wines became more popular. From 1960 to 2003 the harvested amount of Petite Arvine grapes was more than quintupled, to approx. 7000 hl in 2003 [2].

Because of the popularity of the old wine varieties, a program was established in the Valais to ensure the genetic diversity of the local varieties and to provide virus free plant material [3-4]. Studies of the DNA by the technique of microsatellite markers, have found no parent of Petite Arvine among the 1500 vine varieties tested (J. Vouillamoz, personal communication).

The vine of Petite Arvine is described as robust and sensitive at the same time. It has regular yields but needs ideal exposure and does not support dryness or too rich soils. The berries of the fruit are small, and at ripeness their juice is sweet and has a pleasant acidity. The berries' colour is green yellow, the skin browned by the sun. The grapes are late harvested, usually in the end of October [1].

Saccharomyces cerevisiae strains are responsible for the alcoholic fermentation of the must. Usually commercial yeast strains are used in order to optimize fermentation. Because these Saccharomyces yeast strains grow quickly, they inhibit the growth of other non-Saccharomyces yeast strains [5].

In addition to yeast strains, different bacterial strains are present in the must. The increasing ethanol content during alcoholic fermentation and the low pH of the must inhibits most of
them. Among the lactic acid bacteria, only \textit{Oenococcus oeni} has a technological importance. It is responsible for the so-called malolactic fermentation, the transformation of malic acid into lactic acid and CO$_2$ with a decrease in the total acidity [6]. The malolactic fermentation is generally applied in the wine making of Petite Arvine wines.

2.2 Aroma

2.2.1 Introduction to aroma

Flavour compounds are volatiles that are perceived by the olfactory receptors in the nose and trigger an odorous sensation. It has been estimated that humans are responsive to thousands of aromatic compounds [7]. Flavour compounds occur in low concentrations in foods, the compound with the lowest odour threshold known (0.02 ng/l), is 1-$p$-menthene-8-thiol [8], which has a reminiscent of grapefruit. Usually hundreds of flavour active compounds can be isolated from a foodstuff. The characteristic aroma, however, is mostly caused by a few substances, called flavour impact compounds. Many flavours, especially those in fruits and vegetables, are secondary metabolites. Others are generated by chemical or enzyme catalysed reactions during food processing [7].

2.2.2 Wine aroma

The wine flavour is very complex since its development is influenced by many biological, biochemical and technological parameters. Flavour compounds can either have its origin from the raisin, or they can develop during the alcoholic fermentation, other technological interactions or during the ripening process [9].

Only few varieties have aromatic must: Monoterpenes are the key flavour compounds for varieties as Riesling, Gewürztraminer and Muscat. The terpenes are mainly linalool, geraniol, nerol and $\alpha$-terpineol [10]. Pyrazines are characteristic for wine varieties of the "Cabernet Sauvignon" family, where they positively correlate with notes as green pepper [11]. However, the must of Petite Arvine does not exhibit a distinct aroma.

Non-aromatic musts contain non-volatile flavour precursors that are transformed to odorous compounds by enzyme action. Many different flavour precursors have been described in wine [9]. Glycosidically bound terpenes, norisoprenoids and other compounds play an important role as aroma precursors, they have been largely described by different authors, e.g. [12-14]. Another important group of precursors are $S$-cysteine conjugates, identified in Sauvignon
blanc and other grape musts [15-16]. Carotenoids, fatty acids and amino acids can act as flavour precursors as well [9].

Between the harvest of the grapes and the start of fermentation, the so-called pre-fermentative aromas are formed. They consist mainly of C6 components issued from the oxidation and enzymatic degradation of fatty acids [9, 17].

The alcoholic fermentation is essential for the aroma of wine; most of the volatile compounds are formed in this winemaking step. Apart from ethanol and higher alcohols, numerous other wine constituents are formed by the yeast metabolism, particularly acids, esters, aldehydes, ketones and S-compounds [18]. The non-volatile precursors are transformed to aroma active compounds by yeast enzymes during alcoholic fermentation [15, 19]. The applied yeast strain can have an influence on wine flavour [20]. In a critical review Thorngate [21] doubted about the importance of the yeast strain on wine flavour, since the results of some studies are contradictory.

Furthermore, the malolactic fermentation can have an influence on wine aroma. In this step, carbonyl compounds as diacetyl and acetoine are produced by Oenococcus oeni. These compounds induce flavour notes as lactic and buttery [22]. In a recent study, Oenococcus oeni was found to be able to hydrolyse glycosidically bound flavour compounds as well and to influence the wine aroma considerably [23].

The composition of the volatiles changes during the ripening process. The fermentative esters are hydrolyzed [24] and monoterpenes are modified by different reactions [25], both phenomena cause a loss in fruity aromas. The concentration of norisoprenic compounds as vitispirane and damascenone can increase during storage, what causes a change in the wine flavour [9].

2.2.3 The role of thiols for wine aroma

Sulphur containing substances generally have a very low threshold value and are therefore powerful flavour compounds. Few of them are considered to have a positive influence on wine aroma; their occurrence is more often correlated with off-flavours [26]. The sulphurous off-flavour (Böckser) in wine is caused by numerous compounds [27]. The formation can be due to a) improper addition of SO2, b) problems with the alcoholic fermentation, c) a lack of available nitrogen for the yeast metabolism, d) incorrect storage of the wine or e) traces of pesticides [27, 28].
Since approx. 15 years, the working group of Professor Dubourdieu at the University of Bordeaux is working on the flavour compounds of the wine of Sauvignon blanc and other wine varieties. Thiols have been identified to be responsible for the typicity of Sauvignon blanc [29-31], and some of these thiols also contribute to the fruity flavour of rosé and Merlot wines [16, 32], wine from the Canary islands [33], and Muscadet [34]. Table 1 gives an overview of the thiols identified in Sauvignon blanc.

Table 1: Thiols identified in the wine of Sauvignon blanc with their descriptors, the quantities found and their threshold values in a wine like ethanol solution (12 vol. %) containing 5 g/l tartaric acid [31].

<table>
<thead>
<tr>
<th>Thiol</th>
<th>Odour</th>
<th>Quantity (ng/l)</th>
<th>Odour threshold (ng/l)</th>
</tr>
</thead>
<tbody>
<tr>
<td>4-Mercapto-4-methylpentan-2-one</td>
<td>Box tree, cat urine</td>
<td>0-40</td>
<td>0.8</td>
</tr>
<tr>
<td>3-Mercaptohexanol</td>
<td>Rhubarb, grapefruit, passion fruit</td>
<td>200-12000</td>
<td>60</td>
</tr>
<tr>
<td>3-Mercapto-3-methylbutan-1-ol</td>
<td>Cooked leak</td>
<td>1500</td>
<td>&lt;=1500</td>
</tr>
<tr>
<td>4-Mercapto-4-methylpentan-2-ol</td>
<td>Citrus peel</td>
<td>0-150</td>
<td>55</td>
</tr>
<tr>
<td>3-Mercaptohexanol acetate</td>
<td>Box tree, passion fruit, grapefruit</td>
<td>0-1000</td>
<td>4</td>
</tr>
</tbody>
</table>

3-Mercaptohexanol is the most abundant of the identified thiols. The concentrations of 3-mercaptohexanol found in different wine varieties are listed in Table 2.

Table 2: Concentration of 3-mercaptohexanol (3-MH) found in different wine varieties.

<table>
<thead>
<tr>
<th>Wine variety</th>
<th>Concentration of 3-MH (ng/l)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Champagne wines</td>
<td>250-640</td>
</tr>
<tr>
<td>Gewürztraminer</td>
<td>1'336-3'278</td>
</tr>
<tr>
<td>Merlot</td>
<td>120-4'560</td>
</tr>
<tr>
<td>Muscadet</td>
<td>63-445</td>
</tr>
<tr>
<td>Muscat</td>
<td>124-898</td>
</tr>
<tr>
<td>Pinot blanc</td>
<td>89-248</td>
</tr>
<tr>
<td>Pinot gris</td>
<td>312-1'042</td>
</tr>
<tr>
<td>Riesling</td>
<td>407-970</td>
</tr>
<tr>
<td>Rosé wines produced of Merlot/ Cabernet Sauvignon</td>
<td>0-7'000</td>
</tr>
<tr>
<td>Sauvignon blanc</td>
<td>200-12'000</td>
</tr>
<tr>
<td>Sylvaner</td>
<td>58-146</td>
</tr>
<tr>
<td>Wine varieties from the Canary islands</td>
<td>108-2'640</td>
</tr>
</tbody>
</table>

3-Mercaptohexanol contains a chiral C-atom and exists therefore in two enantiomers (Figure 1).
Very often, enantiomers have different sensory properties [37]. R- and S-3-mercaptohexanol have the same sulphurous note [38], however, their esters have different odours [39]. In yellow passion fruit, the S-isomer is predominant (60-80 %) [40]. According to Tominaga [31], the isomers are equally distributed (46-57 % S and 43-54 % R) in Sauvignon blanc. The yeast strain used for fermentation did not play a role in the distribution of the two enantiomers.

Darriet et al. [41] showed that the use of copper ions in viticulture had a negative influence on the concentration of volatile thiols in the wines. Thiols can react with quinones to their mercapto derivatives [42]. In red wine, 3-mercaptohexanol reacts in the presence of oxygen with oxidised catechine to its mercapto derivative [43]. Tominaga [31] reported 3-mercaptohexanol to have a good stability in white wine: after completion of the alcoholic fermentation, the concentration of 3-mercaptohexanol remained stable during 150 days. In red wine, however, 3-mercaptohexanol is less stable and its concentration decreases considerably during storage [42].

It is known that components of the matrix can influence the stability of aroma active thiols. Components present in wine such as cysteine or ribose [44] and anthocyanes have a stabilizing effect on thiols [45]. On the other hand, sulphur dioxide can act as reducing agent [46]. The presence of oxygen is therewhile negative for the stability: The higher the oxygen dissolved in the wine, the lower the stability of the thiols [43].

The precursors of the described thiols are S-cysteine conjugates [15, 47-48]. The transformation of the precursor into the flavour active molecule by a β-lyase of the yeast occurs during alcoholic fermentation [15]. Cysteine-S-conjugate β-lyases (EC 4.4.1.13) catalyze the cleavage of C-S bonds thus releasing the flavour active thiols. They have shown to be present in plants [49], animals [50] and in intestinal microorganisms [51]. In vitro these enzymes showed only a low degree of enantioselectivity [52], the degree of enantioselectivity depending on the source of the enzyme [53].
The proposed pathway of formation of 3-mercaptohexanol from its precursor during alcoholic fermentation is shown in Figure 2.

![Pathway of 3-mercaptohexanol formation during alcoholic fermentation](image)

Figure 2: Pathway of 3-mercaptohexanol formation during alcoholic fermentation [47]

Only a few percent (2-10 %) of the precursor found in the must is actually transformed into the corresponding thiol during fermentation [31, 48]. Between 29 and 90 % of the precursor "disappear", thus could not be detected in the fermented must [48]. The reasons for this phenomenon are unknown.

The flavour precursor of 3-mercaptohexanol is located in the pulp (46 %) and the grape skin (54 %), whereas more than 80 % of the precursors of the other identified thiols are located in the pulp. That's why the maceration of the grape berries are favourable for the concentration of the precursor [48].

In the must of Sauvignon blanc, the S-glutathione conjugate of 3-mercaptohexanol was identified as well. During fermentation, only a small percentage (0.1-0.3 %) of this precursor was transformed into 3-mercaptohexanol; it does not seem to play an important role as flavour precursor [48, 54].

### 2.3 Wine aroma analysis

Both sensorial evaluation as well as chemical and instrumental analysis should be applied for aroma analysis. The characterisation of aroma compounds demands the description of their sensorial properties as well as the identification of their chemical structure.

#### 2.3.1 Sensory evaluation

Sensory evaluation is a scientific discipline used to evoke, quantify, analyze and interpret reactions to the characteristics of foods as perceived by the senses of sight, smell, taste, and touch. Because of the extraordinary number of interferences that can bias sensory evaluation, it is conducted under controlled conditions using trained subjects [55].
In sensory evaluation several different tests are known to describe, distinguish or determine the preferences of the panellists for the samples [56].

Historically the evaluation of wine flavour focused on the presence or absence of defects. This frequently involved the use of "expert" tasters who evaluated appearance, colour, odour, taste, and mouthfeel of a wine to obtain an overall impression of wine "quality" based on the absence of defects and the overall "balance" of the sensory properties of a wine [57]. Several types of scorecards have been developed to measure the wine quality by tasting [55, 58]. These scorecards are highly subjective and since sensory evaluation has developed into a scientific discipline in the recent years, the use of trained panellists in controlled environment has largely replaced the "quality" judgment by individual "experts" [57]. The problem of the subjectivity of the panellists has led to the development of standardized systems of terminology of wine description with corresponding standard references [59-62]. Flavour terminology has been developed for alcoholic beverages as beer [63, 64], whisky [65] and cider [66]. For wine, several approaches have been made. Jaubert et al. [59] developed a "field of odours", where each of 45 odours are defined by a chemical, as i.e. campher or eugenol. The "nose of wine" by Lenoir [60] uses concentrated aroma essences in small flasks for comparison. Noble et al. [61, 62] developed the "wine aroma wheel", where the terms are hierarchically structured in three levels. For every descriptor of the third level, the most detailed terms, a reference was created in order to train the panellists and to establish wine description analyses. The references are generally made of a wine with a neutral aroma and a piece of fruit or spice. Unlike other concepts where one single compound is used as reference for a descriptor, this approach takes into account the complexity of natural flavours.

2.3.2 Extraction of aroma active compounds

Most of the analytical instruments used for aroma analysis cannot handle sample matrices directly, but involve a sample preparation. The first step in aroma analysis consists in the isolation and concentration of the flavour active compounds by extraction, distillation or by adsorption/desorption.

Liquid extraction with an organic solvent is the classic method to isolate volatile compounds. The principle of this method lays in the low polarity of volatiles and the better solubility in organic solvents than in water. So this extraction is convenient for aqueous samples as alcoholic beverages [67]. This method has to be applied when large sample volumes are
needed for the identification of the chemical structure of a flavour compound. The most
important disadvantages are the waste of solvent and the time consuming handling.
Distillation takes advantage of the differences between the volatility of flavour components
and the non-volatility of the major food constituents. Steam-distillation with simultaneous
extraction (i.e. with a Linkens-Nickerson apparatus) is a widely used technique to isolate and
concentrate aroma compounds [67].
Direct analysis of the headspace vapours above a food product is the ideal method to isolate
flavours, but the primary problem is the low concentration of flavour compounds in the
headspace. Different techniques were developed to concentrate the flavour compounds in the
headspace as cryogenic traps or adsorption columns (for example charcoal, Tenax or Porapak
Q) [7].
The extraction of aroma compounds by solid phase microextraction (SPME) was developed in
the 1990'ies by Pawliszyn and co-workers [68]. The flavour compounds in the headspace of a
sample are adsorbed at a silica coated fibre and consequently desorbed in the injector of a GC.
The property of the coating of the fibre can be chosen in function of the analyte. The
advantages of this method are the fast sample preparation and the avoidance of organic
solvents. On the other hand, the sensitivity is limited, and this method is therefore not suitable
for trace analyses. Recently several studies have been published where wine aroma
compounds were extracted and analysed by SPME [69-72].

2.3.3 Instrumental analysis

Qualitative and quantitative determinations of aroma compounds require the use of separation
techniques as gas chromatography (GC) and GC- mass spectrometry (MS). After the
separation of the organic compounds in the GC-column, they are traced by a detector chosen
in function of the analytes. The quantification can be made by use of internal or external
standards. If a complex sample preparation was used, the use of an internal standard is
recommended. In GC-MS, the compounds are fragmented by ionisation after being separated
and the fragment masses detected. The mass spectrum is characteristic for each compound
and identification can be made either by comparing the spectrum to a library or to the
spectrum of a reference substance.

GC-Olfactometry (GC-O), sometimes referred to as "GC sniffing", is an important tool in
flavour research. The human nose is the detector for evaluating the effluent of the GC column
[73]. Olfactometry allows the identification of aroma components that contribute significantly
to the flavour of a wine among the hundreds of chemical substances present [74]. The most important disadvantage of GC-O is the need of a human subject as a detector, which implies psychological (concentration, fatigue) and physical (health) factors that are difficult to handle. A critical review has found a lack of reproducibility of sniffing analyses [75].

Different techniques as aroma extract dilution analysis (AEDA) [76-78] and Charm analysis [79] have been developed in order to systemise olfactometry and to produce more reliable and reproducible results. In the past decades, a large number of studies concerning olfactometry in wine analysis have been carried out [80]. Quantification is usually done by GC and GC-MS. Quantification by GC-O is less evident because of the mentioned "human factor". Nevertheless, Darriet [81] could measure the concentration of an olfactometrically detected peak with a variation coefficient of only 3 %.

### 2.3.4 Analysis of thiols

The analysis of sulphur compounds can be fastidious because of their low concentrations in food and their instability. Artefacts can easily occur through heat, sunlight and oxygen. The solvent has a major impact on stability; the most appropriate in thiols analysis seems to be dichloromethane [82].

Ellman [83] developed a fast method to determine the overall concentration of thiols. Thiols react with the "Ellman reagent" (dithiobis-(2-nitrobenzoic acid)) liberating a coloured anion. The concentration of the free thiols is proportional to the concentration of this anion and can be determined by spectrophotometry.

Tominaga [31, 84, 85] developed a selective method to extract and concentrate thiols. The thiols are complexed with an organic mercury compound (p-hydroxymercurio-benzoic acid, pHMB). This complex is reversible; the thiols can be liberated by the addition of an excess of cysteine.

Thiols have also been concentrated by covalent chromatography where pHMB is covalently bound to a gel [86]. The principle how the thiols are complexed and liberated is the same as described above.

Schneider et al. developed a method using a stable isotope dilution assay with deuterated analogues as internal standards [34, 87]. The assay was coupled to gas chromatography with atomic emission detection (GC-AED).

Methods to measure the concentration of the S-cysteine conjugates, precursors of thiols in wine, have also been developed. In the method proposed by Peyrot des Gachons et al. [88]
deuterated synthetic precursors are added to the grape must which was percolated on a column containing immobilized tryptophanase. The ratio between the liberated deuterated and non deuterated thiols was used to calculate the concentration of S-cysteinylated flavour precursor in the must. Murat et al. [16] proposed a faster and simpler method to measure the concentration of the precursor of 3-mercaptohexanol. The precursors were isolated by percolation of grape must containing synthesised $^{15}$N-precursor as internal standard, on a Sephadex column. The precursors were trimethylsilylated and analysed by GC-MS.

All the methods to analyse thiols and their precursors have one disadvantage: They are time-consuming and need trained staff to carry out the analyses.

### 2.4 References


[2] Annual report of the cantonal laboratory of the Valais (2003), Rue Pré d’Amédée 6, CH-1950 Sion


Chapter 2


Literature review


Chapter 3

SENSORY CHARACTERISATION OF PETITE ARVINE WINES

Abstract

The wine of Petite Arvine is an local white wine specialty of the Canton of Valais/Switzerland. This wine has become very popular, local white wine specialty of the Canton of Valais/Switzerland. The characteristic aroma of Petite Arvine wine is described as fruity and flowery. Up to now, no scientific sensorial evaluation has been performed on this wine. In this study, a descriptive sensorial analysis was carried out. The intensities of the descriptors were correlated to the typicality of the wine samples. The descriptors "cooked rhubarb", "fresh rhubarb", "grapefruit" and "quince" are correlated positively and can be considered as important for the characteristic flavour for this wine variety.

3.1 Introduction

Petite Arvine is an autochthonous grape cultivar used for wines unique to the Canton of Valais in Switzerland. This wine has become very popular, the acreage increased considerably from 1960 to 2000 and the production more than quintupled, to approximately 700 tons in 2003 [1]. The flavour of Petite Arvine is described as fruity and floral (grapefruit, wisteria, exotic fruit, rhubarb, etc.), however, the compounds responsible for the characteristic flavour are mostly unknown. The knowledge of these compounds would allow the study of the biogenesis of the wine aroma during the vinification and the ability to enhance the formation of typical flavour compounds, and to prevent the formation of off-flavours.

Sensory evaluation of wines has been used for several purposes. On one hand sensory studies have been carried out to explore the typical flavour of specific cultivars, e.g. Chardonnay [2-4]. On the other hand, sensory evaluation has been used to evaluate the influence of different

steps in wine making [5], and the influence of malolactic fermentation on the wine aroma [6, 7]. It has further been used to discriminate wines regarding their origin, site, vintage and quality [4, 8].

Many factors can influence wine attributes and therefore the sensory evaluation of wine. Even by using a trained panel and a strict procedure, it is necessary to develop a precise terminology in order to correctly describe the samples. Noble and co-workers [9, 10] developed the "wine aroma wheel", where the terms are hierarchically structured in three levels. For every descriptor of the third level, containing the most detailed terms, a reference made of a neutral wine and a piece of fruit or spice was created in order to train the panellists and to establish wine description analyses.

Prior to this study, no scientific sensorial evaluation has been carried out on Petite Arvine wines. Reliable results on the typicality of a Petite Arvine wine and the intensities of certain descriptors were needed to assist in the identification the flavour impact compounds of this wine cultivar.

3.2 Material and methods

3.2.1 Selection of experimental wines

The Petite Arvine wines (vintage 2000) were collected all over the Canton of Valais from different producers producing a minimum of 1500 litres. The grape must of the samples had an average content of 23.3 °Brix (sd 0.9) at harvest. These prerequisites helped to avoid outliers due to too low production volumes, specific viticultural conditions or simply too low or too high sugar content. None of the wines was stored in wooden barrels because the woody notes might have been too dominant and may have masked the typical flavours of Petite Arvine wines.

For the first sensory session (February/March 2001), the young wines were taken directly from the storage tanks of the producers, transferred to bottles under argon and stored at 12 °C. For the second sensory session (March/April 2002), bottled wines were purchased at the wine cellars. The bottled wines were 100 % Petite Arvine, not blended with other wines. Eventually some tanks of the same cellar were mixed before bottling.
3.2.2 Sensory analysis

3.2.2.1 Panels

Sensory evaluations were carried out with two panels. The main panel consisted of a dozen local wine experts (local enologists and wine producers). The wine experts were experienced tasters due to their daily work with the vinification of Petite Arvine and their regular participation in official wine tastings. Their ages were between 35-65 years, mainly male, only three females participated.

The second panel consisted of 15 students of enology at the University of Applied Science in Changins (ages around 25). The second panel was only employed once, to verify the found descriptors by the first panel.

3.2.2.2 Tasting conditions

All wines were tasted at a temperature of 12 °C and were evaluated by sniffing and tasting. The sessions took place in a specially designed room for sensory evaluation, making communication between the tasters impossible, white light was used. A volume of 40 ml of wine was presented in clear, tulip shaped tasting glasses. The wines were presented in random order, coded with three digit numbers. The tasting took place on different days to prevent fatigue of the panellists.

3.2.3 Creation of a list of descriptors

In the first session, taking place in spring 2001, the panellists described the wines without any given guidelines. After elimination of imprecise and hedonic terms, the panellists had to agree on a list of descriptors to describe Petite Arvine wines. The list of the descriptors was verified by a second panel of 15 panellists who were asked to describe the same samples of Petite Arvine, without being informed about the nature of the samples or the aims of the sensory experiment. A total of 11 wines were tasted; whereof one of the samples was eliminated because of an estery off-flavour that made the tasting impossible.

3.2.4 Attribute intensities to typicality

In the second session, taking place in spring 2002, the list of descriptors was shortened to the 12 descriptors showing the highest intensities in the samples tasted in the first session. A total of 7 wines were tasted, whereof one of the samples was eliminated because of a sulphurous off-flavour that made the tasting impossible.
Wine standards according to Noble et al. [9, 10] were used to facilitate an agreement between the panellists. The proposed standard references were either adjusted or newly developed for the purpose of being perfectly adapted to the tasting of Petite Arvine wines. In contrast to the references described by Noble et al. [9, 10], only fresh fruit or peel and no juice or canned fruit were used. The results with juice and canned fruit were unsatisfactory due to the "cooked" flavour of pasteurised (juice) or sterilised food (canned fruit).

The references were smelled by the panellists prior to wine evaluation. The recipes for the preparation of the odour reference standards are described in Table 1. The standards were freshly prepared on the days of the sensory evaluation (1-2 h before tasting).

<table>
<thead>
<tr>
<th>Descriptor</th>
<th>Recipe</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cooked rhubarb</td>
<td>1 teaspoon of warm rhubarb compote</td>
</tr>
<tr>
<td>Fresh rhubarb</td>
<td>slice of 1 cm of fresh fruit, cut up into 5 pieces</td>
</tr>
<tr>
<td>Grapefruit</td>
<td>piece of peel of yellow grapefruit (2 cm²)</td>
</tr>
<tr>
<td>Honey</td>
<td>1 teaspoon, dissolved</td>
</tr>
<tr>
<td>Lemon</td>
<td>piece of peel of lemon (1 cm²)</td>
</tr>
<tr>
<td>Mango</td>
<td>10 g fresh fruit, cut up into small pieces</td>
</tr>
<tr>
<td>Passion fruit</td>
<td>6 pips of fresh fruit</td>
</tr>
<tr>
<td>Pear William's</td>
<td>5 g of fresh fruit, cut up into small pieces</td>
</tr>
<tr>
<td>Pineapple</td>
<td>5 g of fresh pineapple, cut up into small pieces</td>
</tr>
<tr>
<td>Violet</td>
<td>1 ml of a solution of essential oil (1 drop /l)</td>
</tr>
</tbody>
</table>

These references were prepared in 40 ml of white wine (Vin blanc, Provins, Sion, Switzerland) and served in wine tasting glasses.

<table>
<thead>
<tr>
<th>Descriptor</th>
<th>Recipe</th>
</tr>
</thead>
<tbody>
<tr>
<td>Box tree</td>
<td>crushed leaves of fresh plant</td>
</tr>
<tr>
<td>Quince</td>
<td>quince jelly</td>
</tr>
</tbody>
</table>

These references were served in small bowls.

3.2.4.1 Categories

In both sensory sessions (spring 2001 and 2002), the panellists classified the wines according to their typicality into 7 categories (between 0 for no typicality and 6 for high typicality). The typicality was defined as the ease of identification of a sample as being a Petite Arvine wine. Wines, which could not be assigned to this variety, were considered as untypical.

The intensities of the descriptors were quantified on a non-structured scale (10 cm, divided into 100 units for interpretation) with a - and a + on the ends, indicating the minimum and maximum of intensity. Only the data of the second session were used as results.
3.2.5 Data analysis

The collected data were analysed by the calculation program Excel (Office 2000 for Windows/Microsoft), and were tested by an analysis of variance ANOVA (two-factor without replication). Further, the least square difference (LSD) was calculated in order to be able to compare the samples. A confidence level of $p < 0.05$ was chosen [11].

The correlations of two variables were calculated by linear least-squares regression.

3.3 Results and Discussion

3.3.1 Creation of the list of descriptors

The objective of the first session was to establish a list with the appropriate descriptors for Petite Arvine wines. The panellists described the wines, and the hereby-used terms were grouped into “families” and “subfamilies”.

The tasting experiment with the uninformed panellists confirmed the descriptors introduced by the experts and added only a few new ones. The descriptors are listed in Table 2. All these terms were found to be useful to describe the tasted Petite Arvine wine samples.
Table 2: Descriptors for Petite Arvine wine grouped into "families" and "subfamilies"

<table>
<thead>
<tr>
<th>Box tree like</th>
<th>Flowers</th>
<th>Rhubarb</th>
</tr>
</thead>
<tbody>
<tr>
<td>Blackcurrant leaves</td>
<td>Wisteria</td>
<td>Fresh rhubarb</td>
</tr>
<tr>
<td>Cat urine</td>
<td>Rose</td>
<td>Cooked rhubarb</td>
</tr>
<tr>
<td>Box tree</td>
<td>Lime tree blossom</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Violet</td>
<td></td>
</tr>
<tr>
<td>Citrus</td>
<td>&quot;Heavy&quot; fruits</td>
<td>Roast-flavours</td>
</tr>
<tr>
<td>Grapefruit</td>
<td>Blackberries</td>
<td>Toasted bread</td>
</tr>
<tr>
<td>Lemon</td>
<td>Blackcurrant</td>
<td>Roasted meat</td>
</tr>
<tr>
<td>Lime</td>
<td>Elderberries</td>
<td>Woody</td>
</tr>
<tr>
<td>Lemon peel</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Tangerine</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ester</td>
<td>Honey</td>
<td></td>
</tr>
<tr>
<td>Apple</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Quince</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pear (William’s variety)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Banana</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Exotic fruits</td>
<td>Red fruits</td>
<td></td>
</tr>
<tr>
<td>Pineapple</td>
<td>Strawberry</td>
<td></td>
</tr>
<tr>
<td>Passion fruit</td>
<td>Raspberry</td>
<td></td>
</tr>
<tr>
<td>Melon</td>
<td>Cherry</td>
<td></td>
</tr>
<tr>
<td>Lychee</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

The results of the classification of the wines according to their typicality and of the intensities of the descriptors (see Table 2) were not satisfactory at the early stage of wine making. The results from the tasting of the 5-6 month old wines revealed no significant differences among the wines in scoring neither typicality nor the intensities of descriptors. The typicality of the wines and intensities of most of the descriptors were low (data not shown). Apparently the wines were too young and the estery flavours masked the typical flavours. This was in agreement with the experience of wine experts who recommend drinking Petite Arvine wine no earlier than one year after vintage. Esters formed during the fermentation process are subject to hydrolysis during wine maturation, thus diminishing their concentration [12]. The descriptor families “heavy” fruits, red fruits and roast flavours seemed to be unnecessary, since the intensities of these descriptors were very low. Also some sub-families were obviously less important.
3.3.2 Correlation of typicality and intensity of descriptors

Using the results of the first session, the list of the descriptors to taste was shortened. All descriptors with low intensities in the tasting during the first session were eliminated. The final choice was discussed with the panellist in order to assure that no important descriptor was eliminated. The descriptors used in the second session were (in alphabetic order): box tree, grapefruit, honey, lemon, mango, passion fruit, pear, pineapple, quince, rhubarb (cooked/fresh), and violet. The results are shown in Table 3.

Table 3: Results of the sensory evaluation of Petite Arvine wine, mean values of the tasted descriptors of each wine, the results of the ANOVA of the results of the sensorial evaluation of Petite Arvine wine; confidence level and least square difference (LSD).

<table>
<thead>
<tr>
<th></th>
<th>Vétroz</th>
<th>Sion</th>
<th>Sierre</th>
<th>Leytron</th>
<th>Sion</th>
<th>Sierre</th>
<th>p-value</th>
<th>LSD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Box tree</td>
<td>27.44</td>
<td>29.00</td>
<td>21.44</td>
<td>46.56</td>
<td>33.56</td>
<td>6.33</td>
<td>0.038</td>
<td>23.4</td>
</tr>
<tr>
<td>Grapefruit</td>
<td>8.25</td>
<td>43.25</td>
<td>16.00</td>
<td>52.00</td>
<td>29.50</td>
<td>42.25</td>
<td>0.014</td>
<td>26.7</td>
</tr>
<tr>
<td>Honey</td>
<td>12.33</td>
<td>20.00</td>
<td>32.22</td>
<td>30.22</td>
<td>33.11</td>
<td>19.44</td>
<td>0.364*</td>
<td>-</td>
</tr>
<tr>
<td>Lemon</td>
<td>13.11</td>
<td>46.00</td>
<td>21.78</td>
<td>30.33</td>
<td>16.67</td>
<td>51.11</td>
<td>0.002</td>
<td>20.7</td>
</tr>
<tr>
<td>Mango</td>
<td>15.89</td>
<td>19.33</td>
<td>20.89</td>
<td>12.89</td>
<td>14.00</td>
<td>26.67</td>
<td>0.728*</td>
<td>-</td>
</tr>
<tr>
<td>Passion Fruit</td>
<td>23.11</td>
<td>27.44</td>
<td>34.22</td>
<td>28.22</td>
<td>32.33</td>
<td>17.44</td>
<td>0.782*</td>
<td>-</td>
</tr>
<tr>
<td>Pear William's</td>
<td>15.41</td>
<td>31.52</td>
<td>22.47</td>
<td>34.40</td>
<td>25.37</td>
<td>29.16</td>
<td>0.152*</td>
<td>-</td>
</tr>
<tr>
<td>Pineapple</td>
<td>31.25</td>
<td>19.13</td>
<td>69.38</td>
<td>36.75</td>
<td>29.00</td>
<td>27.125</td>
<td>0.022</td>
<td>28.9</td>
</tr>
<tr>
<td>Quince fruit</td>
<td>8.11</td>
<td>22.56</td>
<td>38.67</td>
<td>43.33</td>
<td>17.78</td>
<td>40.00</td>
<td>0.014</td>
<td>22.6</td>
</tr>
<tr>
<td>Rhubarb cooked</td>
<td>3.50</td>
<td>34.50</td>
<td>39.38</td>
<td>47.00</td>
<td>27.00</td>
<td>36.50</td>
<td>0.038</td>
<td>26.6</td>
</tr>
<tr>
<td>Rhubarb fresh</td>
<td>13.86</td>
<td>34.29</td>
<td>16.43</td>
<td>70.00</td>
<td>26.29</td>
<td>18.14</td>
<td>0.013</td>
<td>29.8</td>
</tr>
<tr>
<td>Violet</td>
<td>25.00</td>
<td>35.67</td>
<td>31.44</td>
<td>33.78</td>
<td>21.89</td>
<td>34.56</td>
<td>0.801*</td>
<td>-</td>
</tr>
<tr>
<td>Typicality</td>
<td>2.1</td>
<td>4</td>
<td>4.2</td>
<td>5.3</td>
<td>3.3</td>
<td>3.5</td>
<td>0.00002</td>
<td>1.07</td>
</tr>
</tbody>
</table>

*p > 0.05, results statistically non confident (the taster did not determine any difference between the intensities of that descriptor), and therefore no LSD could be calculated. Range 0-100 for the descriptors and 0-6 for the typicality.

The results of the descriptors honey, mango, passion fruit, pear Williams, and violet were not statistically reliable and could therefore not be taken into consideration. For illustration, the most and the least typical wines are compared in Figure 1.
Figure 1: Descriptive analysis aroma profile of a typical and an untypical Petite Arvine wine.

The most and the least typical wines had significantly different intensities in grapefruit, fresh and cooked rhubarb, as well as quince aroma, where the typical wine had higher intensities. These facts were affirmed by the correlation coefficients of the correlation between the intensities of the descriptors and the typicality. In our case (six samples tasted) the value of had to exceed 0.811 to statistically confirm a correlation between the variables with a confidence level of 0.95 (p=0.05), or 0.729 with a confidence level of 0.90 (p=0.10) [11]. The descriptors showing high correlations are highlighted (Table 4).

Table 4: Correlations between the typicality and the intensities of the descriptors p=0.05 (dark) and p=0.10 (light)

<table>
<thead>
<tr>
<th>Descriptor</th>
<th>Correlation coefficient (r)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rhubarb cooked</td>
<td>0.925</td>
</tr>
<tr>
<td>Rhubarb fresh</td>
<td>0.820</td>
</tr>
<tr>
<td>Grapefruit</td>
<td>0.769</td>
</tr>
<tr>
<td>Quince</td>
<td>0.769</td>
</tr>
<tr>
<td>Box tree</td>
<td>0.443</td>
</tr>
<tr>
<td>Lemon</td>
<td>0.410</td>
</tr>
<tr>
<td>Pineapple</td>
<td>0.161</td>
</tr>
</tbody>
</table>

The intensities of fresh and cooked rhubarb, grapefruit and quince correlated well with the typicality of the Petite Arvine wine. The wine was considered as typical if it showed high
intensities in the descriptors mentioned. The typicality was less dependent upon the intensity of the descriptors lemon and box tree. The typicality of Petite Arvine wine was independent on the intensity of pineapple, even if this descriptor was often mentioned as an important flavour for Petite Arvine. The correlations between the single descriptors were also calculated. The correlation coefficients are shown in Tables 5; the descriptors showing high correlations are highlighted.

Table 5: Dependences between the seven tasted descriptors of Petite Arvine wines (correlation coefficient r)
Significant correlations are highlighted, p=0.05 (dark) and p=0.10 (light)

<table>
<thead>
<tr>
<th></th>
<th>Grapefruit</th>
<th>Lemon</th>
<th>Fresh rhubarb</th>
<th>Cooked rhubarb</th>
<th>Box tree</th>
<th>Quince</th>
<th>Pineapple</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lemon</td>
<td>0.723</td>
<td></td>
<td>0.16</td>
<td>0.733</td>
<td>0.577</td>
<td>0.446</td>
<td>0.464</td>
</tr>
<tr>
<td>Fresh rhubarb</td>
<td>0.743</td>
<td>0.16</td>
<td>0.578</td>
<td>0.496</td>
<td>0.439</td>
<td>0.082</td>
<td>0.059</td>
</tr>
<tr>
<td>Cooked rhubarb</td>
<td>0.733</td>
<td>0.232</td>
<td>0.771</td>
<td>0.12</td>
<td>0.0099</td>
<td>0.407</td>
<td></td>
</tr>
<tr>
<td>Box tree</td>
<td>0.232</td>
<td></td>
<td>0.082</td>
<td>0.059</td>
<td>0.407</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Quince</td>
<td>0.577</td>
<td></td>
<td>0.898</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pineapple</td>
<td>0.446</td>
<td></td>
<td>0.156</td>
<td>0.082</td>
<td>0.407</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

The intensities of certain descriptors were highly correlated. The wines that were intense in grapefruit were also intense in lemon, fresh rhubarb and cooked rhubarb. Box tree is correlated with fresh rhubarb and quince with cooked rhubarb.

Two possible reasons for the correlation of two descriptors can be accounted for. One possibility is, that the panellists were not able to distinguish properly between two of them. This effect was tried to be minimized by using the wine references and a trained panel. The other possibility is more likely: The correlated aromas are caused by the same or by chemically related compounds. The compounds responsible for the descriptors in Petite Arvine wine are still partly unknown. Some of the compounds in the aroma of Petite Arvine have been identified (see chapter 4), however, the study of the volatile compounds of Petite Arvine wine has to be the subject of further studies.

3.4 Conclusions

The present study showed that Petite Arvine wines considered as typical show high intensities in rhubarb, grapefruit and quince aroma.

The sensory evaluation revealed that the wines of Petite Arvine could not be tasted and classified before an age of 12 month since the estery notes resulting from the alcoholic fermentation mask the typical flavours.

The use of reference standards has proofed to be an effective tool to improve the reliability of descriptive sensorial analyses.
The flavour of Petite Arvine is often described as flowery. However, in this sensorial evaluation, the data of the flowery compounds were statistically not interpretable. In a further session, these aspects should be studied in detail. For this reason, a panel specially trained on flowery aromas, should be considered.

3.5 References


Chapter 4

ANALYSIS OF VOLATILE COMPONENTS OF PETITE ARVINE WINE

Abstract

Petite Arvine is a white grape variety that grows exclusively in the canton of Valais in Switzerland and is used to produce a typical regional wine. In order to elucidate the nature of the flavour compounds that contribute to the characteristic aroma of this wine, the organic extracts were analysed by gas chromatography and olfactometry. The olfactometrically detected zones were further compared with the odour of extracts of vegetal material. 3-Mercaptohexanol, ß-ionone and other compounds were identified as main contributors to the characteristic aroma of Petite Arvine wine.

4.1 Introduction

Petite Arvine is an autochthonous grape variety grown in the canton of Valais in Switzerland, which is exclusively used to produce the regional white wine Petite Arvine. This wine is becoming very popular, the acreage of the vineyards and the wine production have more than quintupled in the last few years [1], and it is unlikely that this trend will change in the coming years because of the high demand and consequently the recent increase in the planting of Petite Arvine vines. The characteristic flavour of Petite Arvine wine is described as fruity (grapefruit, wisteria, exotic fruit, rhubarb, quince, etc.). The compounds responsible for the characteristic aroma are still unknown; up to now, their identification has not been the subject of a scientific study. Knowledge of these compounds would allow the study of the genesis of the typical aroma during the wine making and would allow it to be influenced in order to favour the formation of the desired flavour and to prevent the formation of off-flavours. Making Petite Arvine wine is considered to be rather challenging; therefore, the analysis of the characteristic flavour compounds is especially interesting.

Olfactometry is an often-used tool in flavour research of all kinds of foodstuffs. The sniffing of gas chromatography (GC) effluents allows the association of flavour descriptors to chemical constituents as well as the identification of the chemicals that contribute significantly to the overall flavour of a food. The technique has been developed and optimized in parallel to the development of GC [2–8] and has also been applied to alcoholic beverages [9–13]. The olfactometric studies on wine were reviewed by Ferreira et al. [14].

4.2 Materials and methods

4.2.1 Wine samples
The analyses were carried out on 14 samples of Petite Arvine wines. The wines were produced by various winegrowers at different locations in the canton of Valais and were from vintages 2000–2003. The Chasselas and Sylvaner wine samples were also from the canton of Valais, whereas the samples of Chardonnay were partly from the canton of Valais, and partly from other places all over the world.

4.2.2 Extraction of the organic compounds
Wine samples (350 ml) were extracted twice with portions of 100 ml dichloromethane (LiChrosolv, Merck, Darmstadt, Germany) in a 1 l Erlenmeyer flask under magnetic stirring for 5 min. The aqueous and the organic phases were divided in a separating funnel. The combined organic phases were centrifuged at 3,000 g (Suprafuge 22, Heraeus Sepatech, Osterode, Germany) to break the emulsion. The two phases were again divided in a separating funnel. The resulting organic phase was dried over Na2SO4 (Fluka, Buchs, Switzerland) and concentrated to a final volume of 0.350 ml by rotatory evaporation under reduced pressure and nitrogen. For the olfactometric and GC quantification analyses, 2-octanol (Fluka) was added to the wine samples as an internal standard. For the analysis of the organic compounds, five different wines of Petite Arvine were used, and all the analyses were done in duplicate.

4.2.3 Extraction of thiol compounds
The extraction was carried out according to the method developed and optimized by Tominaga et al. [15, 16]. In this method, the thiols are isolated by a specific complexation with p-hydroxymercuric benzoic acid from an organic extract of wine.
4.2.4 Extraction of organic compounds of quince jelly

Homemade quince jelly (250 g) was extracted with 200 ml dichloromethane (LiChrosolv, Merck) under continuous stirring overnight at room temperature. The organic phase was separated by decanting, filtered through cotton wool and dried over Na$_2$SO$_4$. The volume was reduced by rotatory evaporation under reduced pressure and a nitrogen stream to approximately 50 l.

4.2.5 Extraction of organic compounds of wisteria flowers

Wisteria flowers were picked from a home garden, the blossoms separated from the stalk and leaves and analysed the same day. The blossoms (10 g) were soaked in 250 ml diethyl ether (Siegfried, Zofingen, Switzerland) and extracted at room temperature under gentle stirring for 10 min. The organic phase was separated by decanting, filtered through cotton wool and dried over Na$_2$SO$_4$. The volume was reduced by rotatory evaporation under reduced pressure and a nitrogen stream to approximately 50 l.

4.2.6 Analysis of the volatile compounds by GC-flame ionization detection and olfactometry

The organic extracts were injected into a GC system (HRGC 5300 Mega Series, Carlo Erba Instruments, Milan, Italy), equipped with a BP 20 column (50 m × 0.22 mm × 0.25 m; SGE, Melbourne, Australia) and operated under the following conditions: initial temperature 40 °C for 1 min, temperature program at a rate of 3 °C/min to 230 °C and 230 °C for 10 min. The injector port was heated to 240 °C. Helium was used as a carrier gas at a pressure of 100 kPa. The injection volume was 2 µl. A flame ionization detector at 300 °C and supplemented with air (100 kPa) and hydrogen (80 kPa) was used. The data were acquired using ChromCard, version 1.07 (Thermo Quest, Milan, Italy). For olfactometric analyses the GC system was extended with a sniffer port (Sniffer 9000, Brechbühler, Schlieren, Switzerland). The intensities of the olfactometrically detected zones were registered on an integrator (HP 3394 A Integrator, Hewlett-Packard, Palo Alto, USA). The surfaces of the peaks were corrected by the surface of the internal standard (2-octanol) detected by the flame ionization detector to prevent biases due to extraction and injection differences. Nitrogen was used as a make-up gas at 50 kPa and the sniffer port was supplemented with humidified air at 100 kPa.
4.2.7 Analysis of thiol compounds by GC-flame photometric detection

The thiol compounds were analysed using the same GC system as described earlier. An aliquot of 3 µl was injected in the splitless mode. A flame photometer detector was used supplemented with air (70 kPa) and hydrogen (150 kPa), at a top temperature of 160 °C; the bottom temperature being 300 °C.

4.2.8 Identification and quantification of the volatile compounds by GC-MS

The volatile compounds were analysed by GC–mass spectrometry (MS) using the following conditions: GC HP Series II 5890 (Hewlett-Packard), supplemented with a mass-selective detector, HP 5971A (Hewlett-Packard). The gas chromatograph was equipped with a HP-WAX column (25 m × 0.22 mm × 0.4 m; Hewlett-Packard). This column corresponds in its properties to the BP 20 column used for GC–flame ionization detection (FID) and olfactometry (see the chapter 4.2.6). Helium was used as the carrier gas at 85 kPa. The injector and the detector had temperatures of 250 and 300 °C, respectively. The same temperature profile was used as for the GC–FID/flame photometric detection and GC–olfactometry analyses.

The sample volume injected into the GC was 0.2 µl. For a semi-quantitative estimation of the amounts of the compounds identified the area of the peaks was compared with the area of the internal standard, and the concentrations were calculated using a response factor of 1. The samples were analysed in duplicate, and the mean value was calculated.

4.2.9 Quantification of phenylethanol and phenylethyl acetate

Phenylethanol and phenylethyl acetate were quantified after solid-phase microextraction and GC analysis as described by Rodriguez-Benecomo et al. [17] but with slight modifications. The wine sample (10 ml) and NaCl (2.9 g, Fluka) were placed in a 20 ml vial (BGB-Analytik, Adliswil, Switzerland). The volatiles present in the headspace of the vial were adsorbed by a polydimethylsiloxane 100 fibre (Supelco, Bellefonte, USA) at a sample temperature of 30 °C for 40 min. The volatiles were analysed by GC–FID after desorption of the fibre in the splitless injector of the gas chromatograph at 300 °C for 2 min. The GC (6890 N, Agilent Technologies, Palo Alto, USA) was equipped with a DB-Wax column (30 m×0.25 mm×0.25 m, J&W Scientific, Folsom, USA) and operated at the following conditions. The initial temperature was 40 °C for 1 min. Then, the temperature was raised to 230 °C at a rate of 5 °C/min. Helium was used as the carrier gas at a flow rate of 1 ml/min. The flame ionization
detector (300 °C) was supplemented with 40 ml/min hydrogen and 400 ml/min air. Quantification was done by using 2-phenylethyl acetate and 2-phenylethanol (both Fluka) as external standards and a calibration curve. All analyses were carried out in duplicate and the mean values were used for calculation.

4.3 Results and discussion

4.3.1 Compounds present in the organic extract of Petite Arvine wine

The gas chromatogram of an organic extract of Petite Arvine wine is shown in Figure 1. The corresponding substances are listed in Table 1. The retention times correspond to the conditions used for the GC-MS analyses.
Figure 1: Gas-chromatogram of an organic extract of Petite Arvine wine, the numbers correspond to the peak numbers in Table 1, for reasons of legibility, not all peaks are numbered (for experimental details see text).
Table 1: Identification and amounts of compounds present in Petite Arvine wines, a total of 5 wines were analysed \( (n=2) \).

<table>
<thead>
<tr>
<th>Peak number</th>
<th>Retention time</th>
<th>Compound</th>
<th>Concentration (mg/l)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>6.73</td>
<td>Ethyl butyrate (^{a, c})</td>
<td>0.4 - 1.2</td>
</tr>
<tr>
<td>2</td>
<td>8.45</td>
<td>2-Methyl-1-propanol (^{a, c})</td>
<td>2.3 - 10.2</td>
</tr>
<tr>
<td>3</td>
<td>9.69</td>
<td>Isoamylacetate (^{a, c})</td>
<td>0.5 - 3.0</td>
</tr>
<tr>
<td>4</td>
<td>10.42</td>
<td>Butanol (^{b})</td>
<td>0.09 - 0.3</td>
</tr>
<tr>
<td>5</td>
<td>13.46</td>
<td>3-Methyl butanol (^{a, c})</td>
<td>55 - 75</td>
</tr>
<tr>
<td>6</td>
<td>14.32</td>
<td>Ethyl hexanoate (^{c})</td>
<td>0.079 - 1.8</td>
</tr>
<tr>
<td>7</td>
<td>15.90</td>
<td>Hexyl acetate (^{b})</td>
<td>1.0 - 1.78</td>
</tr>
<tr>
<td>8</td>
<td>16.04</td>
<td>3-Hydroxy-2-butanone (^{b})</td>
<td>0.5 - 4.5</td>
</tr>
<tr>
<td>9</td>
<td>16.39</td>
<td>2-Octanone (^{b})</td>
<td>0.83 - 0.9</td>
</tr>
<tr>
<td>10</td>
<td>18.88</td>
<td>Ethyl lactate (^{b})</td>
<td>11.4 - 40</td>
</tr>
<tr>
<td>11</td>
<td>19.44</td>
<td>1-Hexanol (^{b})</td>
<td>0.8 - 2.5</td>
</tr>
<tr>
<td>12</td>
<td>22.94</td>
<td>Ethyl octanoate (^{b})</td>
<td>1.3 - 7.2</td>
</tr>
<tr>
<td>13</td>
<td>23.29</td>
<td>Acetic acid (^{b, c})</td>
<td>2.5 - 4.3</td>
</tr>
<tr>
<td>14</td>
<td>23.81</td>
<td>Furfural (^{b})</td>
<td>0.29</td>
</tr>
<tr>
<td>15</td>
<td>23.89</td>
<td>Heptan-1-ol (^{b})</td>
<td>3.1 - 3.7</td>
</tr>
<tr>
<td>16</td>
<td>24.10</td>
<td>n.i.</td>
<td>0.14 - 0.51</td>
</tr>
<tr>
<td>17</td>
<td>26.39</td>
<td>3-Hydroxy butanoic acid, ethyl ester (^{b})</td>
<td>0.17 - 0.58</td>
</tr>
<tr>
<td>18</td>
<td>27.08</td>
<td>2H Thiopyran-3-(4H)-one dihydro (^{b})</td>
<td>n.n. - 0.13</td>
</tr>
<tr>
<td>19</td>
<td>27.72</td>
<td>2-(Methylthio) ethanol (^{b})</td>
<td>0.05 - 0.31</td>
</tr>
<tr>
<td>20</td>
<td>28.39</td>
<td>1,3 Butandiol (^{b})</td>
<td>1.3 - 4.7</td>
</tr>
<tr>
<td>21</td>
<td>28.51</td>
<td>1-Octanol (^{b})</td>
<td>0.06 - 0.14</td>
</tr>
<tr>
<td>22</td>
<td>28.71</td>
<td>n.i.</td>
<td>0.09 - 0.17</td>
</tr>
<tr>
<td>23</td>
<td>28.95</td>
<td>Dimethyl Propandioic acid (^{b})</td>
<td>0.34 - 0.98</td>
</tr>
<tr>
<td>24</td>
<td>29.84</td>
<td>2,3 Butandiol (^{b})</td>
<td>0.13 - 1.1</td>
</tr>
<tr>
<td>25</td>
<td>30.00</td>
<td>1,2 Propaniol (^{b})</td>
<td>0.07 - 0.1</td>
</tr>
<tr>
<td>26</td>
<td>30.74</td>
<td>Butyro lactone (^{b})</td>
<td>1.0 - 4.6</td>
</tr>
<tr>
<td>27</td>
<td>31.42</td>
<td>Butanoic acid (^{b})</td>
<td>n.n. - 0.27</td>
</tr>
<tr>
<td>28</td>
<td>31.68</td>
<td>Decanoic acid, ethyl ester (^{b})</td>
<td>0.64 - 1.44</td>
</tr>
<tr>
<td>29</td>
<td>32.56</td>
<td>n.i.</td>
<td>0.84 - 0.24</td>
</tr>
<tr>
<td>30</td>
<td>33.08</td>
<td>3-Methylbutanoate (^{b})</td>
<td>0.08 - 0.52</td>
</tr>
<tr>
<td>31</td>
<td>33.23</td>
<td>Diethylsuccinate (^{b})</td>
<td>0.57 - 15</td>
</tr>
<tr>
<td>32</td>
<td>34.43</td>
<td>3-Methylthiopropanol (^{a, c})</td>
<td>0.23 - 0.65</td>
</tr>
<tr>
<td>33</td>
<td>35.25</td>
<td>n.i.</td>
<td>0.44 - 1.6</td>
</tr>
<tr>
<td>34</td>
<td>37.85</td>
<td>Methyl 2 tetrahydro thiophene (^{b})</td>
<td>0.67 - 7.1</td>
</tr>
<tr>
<td>35</td>
<td>38.25</td>
<td>2-Phenylethyl acetate (^{a, c})</td>
<td>0.24 - 1.6</td>
</tr>
<tr>
<td>36</td>
<td>39.30</td>
<td>Hexanoic acid (^{a, c})</td>
<td>0.63</td>
</tr>
<tr>
<td>37</td>
<td>39.67</td>
<td>Ethyl dodecanoate (^{b})</td>
<td>0.0 - 0.24</td>
</tr>
<tr>
<td>38</td>
<td>40.22</td>
<td>n.i.</td>
<td>0.19 - 0.86</td>
</tr>
<tr>
<td>39</td>
<td>40.33</td>
<td>Phenylmethanol (^{b})</td>
<td>0.27 - 0.53</td>
</tr>
<tr>
<td>40</td>
<td>40.50</td>
<td>4-Hydroxy, 3-methyl 2-butanone (^{b})</td>
<td>0.15 - 0.23</td>
</tr>
<tr>
<td>41</td>
<td>41.79</td>
<td>Phenylethanol (^{a, c})</td>
<td>17 - 58</td>
</tr>
<tr>
<td>42</td>
<td>46.33</td>
<td>Diethyl malate (^{b})</td>
<td>3.9 - 7.2</td>
</tr>
<tr>
<td>43</td>
<td>47.00</td>
<td>Octanoic acid (^{a, b})</td>
<td>0.07 - 1.92</td>
</tr>
<tr>
<td>44</td>
<td>54.23</td>
<td>Decanoic acid (^{b})</td>
<td>0.16 - 3.2</td>
</tr>
</tbody>
</table>

\( a\): identification by comparing reference substances
\( b\): identification by GC-MS, comparing the spectrum with the nbs library
\( c\): identification by olfactometry
n.i. not identified

A total of 44 compounds were detected by GC-MS. All identified compounds are well known constituents of the organic fraction of wine and have been described in numerous publications and reviews, for example [18-21]. These compounds are mainly formed during alcoholic
fermentation. The concentrations found in Petite Arvine extracts are in the same order of magnitude of the values found in other white wines [20-24].

4.3.2 Compounds present in the thiol extract

Thiols have been identified to be important for the characteristic aroma of Sauvignon blanc and other wines [15, 23, 25]. They have a low odour threshold and occur only in traces in wine. In the thiol-extract of Petite Arvine, four thiol compounds could be detected, whereof three could be identified by injecting reference substances: Mercaptoethyl acetate (retention time 36.3 min), 3-mercapto propanyl acetate (41.9 min), 3-mercapto hexanol (53.3 min) and one unidentified compound (54.5 min).

Mercaptoethyl acetate and 3-mercapto propanyl acetate have been previously identified in Sauvignon blanc, where they are responsible for the "grilled" and "roasted meat" aroma note [26]. In the present study these two substances were not detected by olfactometry (see later), and can therefore be considered as not important for the characteristic aroma of Petite Arvine wine. The olfactometric zone of 3-mercapto hexanol, however, was very strong, with a reminiscent of rhubarb. 3-Mercapto hexanol has been identified first in passion fruit [27], later in Sauvignon blanc [15] and other wines [23, 25]. Comparing the chromatograms of the thiol extract of Petite Arvine to the one of Sauvignon blanc, less substances could be detected, and the amounts of different thiols are considerably lower in Petite Arvine than in Sauvignon blanc (results not shown).

4.3.3 Olfactometry

Olfactometry is an excellent tool to associate the substances detected in the form of peaks in gas chromatograms with their odour. In this way, the aroma-relevant substances of a foodstuff can be distinguished from the irrelevant ones. The most important peaks detected by GC-olfactometry of organic extracts of Petite Arvine wine are shown in Figure 2 and Table 2. Only zones that could be detected in several samples are reported. The retention times correspond to the conditions used in GC-FID coupled to olfactometry.
### Table 2: Olfactometrically perceived odours in an organic extract of a Petite Arvine wine.

<table>
<thead>
<tr>
<th>Peak number</th>
<th>Retention time</th>
<th>Odour perceived</th>
<th>Compound</th>
<th>Intensity</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>14.5</td>
<td>sweet caramel</td>
<td>Ethyl butyrate(^{a,b,c})</td>
<td>m</td>
</tr>
<tr>
<td>2</td>
<td>15.32</td>
<td>sweets</td>
<td>n.i.</td>
<td>w</td>
</tr>
<tr>
<td>3</td>
<td>16.00</td>
<td>alcoholic, fruity</td>
<td>Isobutanol(^{a,b})</td>
<td>m</td>
</tr>
<tr>
<td>4</td>
<td>16.75</td>
<td>alcoholic</td>
<td>n.i.</td>
<td>w</td>
</tr>
<tr>
<td>5</td>
<td>18.2</td>
<td>banana, ester</td>
<td>Isoamyl acetate(^{a,b,c})</td>
<td>s</td>
</tr>
<tr>
<td>6</td>
<td>20.2</td>
<td>flowery, wisteria, burnt</td>
<td>n.i.</td>
<td>w</td>
</tr>
<tr>
<td>7</td>
<td>22.5</td>
<td>rancid almonds, pungent</td>
<td>3-Methyl butanol(^{a,b})</td>
<td>s</td>
</tr>
<tr>
<td>8</td>
<td>23.90</td>
<td>pear, estery</td>
<td>Ethyl hexanoate(^{b,c})</td>
<td>m</td>
</tr>
<tr>
<td>9</td>
<td>28.9</td>
<td>flowery, wisteria</td>
<td>n.i.</td>
<td>w</td>
</tr>
<tr>
<td>10</td>
<td>32.8</td>
<td>vinegar</td>
<td>Acetic acid(^{b,c})</td>
<td>s</td>
</tr>
<tr>
<td>11</td>
<td>33.2</td>
<td>exotic fruit, passion fruit</td>
<td>Ethyl octanoate(^b)</td>
<td>m</td>
</tr>
<tr>
<td>12</td>
<td>43.17</td>
<td>sourish, stinky</td>
<td>n.i.</td>
<td>s</td>
</tr>
<tr>
<td>13</td>
<td>45.19</td>
<td>sweat, rubber, stinky</td>
<td>n.i.</td>
<td>s</td>
</tr>
<tr>
<td>14</td>
<td>48.68</td>
<td>jelly, quince</td>
<td>n.i.</td>
<td>Q</td>
</tr>
<tr>
<td>15</td>
<td>50.25</td>
<td>flowery</td>
<td>n.i.</td>
<td>w</td>
</tr>
<tr>
<td>16</td>
<td>50.57</td>
<td>slightly pungent, rancid</td>
<td>n.i.</td>
<td>w</td>
</tr>
<tr>
<td>17</td>
<td>51.26</td>
<td>rose, flowery, honey</td>
<td>Phenylethyl acetate(^{a,b,c})</td>
<td>s</td>
</tr>
<tr>
<td>18</td>
<td>52.86</td>
<td>rancid</td>
<td>Hexanoic acid(^{a,b,c})</td>
<td>m</td>
</tr>
<tr>
<td>19</td>
<td>53.22</td>
<td>thiolic, burnt</td>
<td>n.i.</td>
<td>w</td>
</tr>
<tr>
<td>20</td>
<td>53.3</td>
<td>rhubarb, fruity</td>
<td>3-Mercaptohexanol(^{a,c})</td>
<td>s</td>
</tr>
<tr>
<td>21</td>
<td>56.15</td>
<td>rose</td>
<td>Phenylethanol(^{a,b,c})</td>
<td>s</td>
</tr>
<tr>
<td>22</td>
<td>57.39</td>
<td>violet</td>
<td>β-Ionone(^{a,c})</td>
<td>s</td>
</tr>
</tbody>
</table>

Intensity: w: weak, m: medium, s: strong  
W: Olfactometric zone also detected in an organic wisteria flower extract, Q: Olfactometric zone also detected in an organic quince jelly extract

a: identification by injecting reference substances  
b: identification by GC-MS, comparing the spectrum with the nbs library  
c: identification by olfactometry  
n.i. not identified
Figure 2: Chromatogram of an organic extract of Petite Arvine wine, with the olfactometric zones, numbers correspond to Table 2, for conditions of the GC-FID analysis see experimental part.
About 50% of the detected olfactometric zones could not be attributed to an identified flavour compound. Furthermore, some of the olfactometric zones seem not to make an important contribution to the typical flavour of Petite Arvine wine. Either the odour is unpleasant (Peaks 4, 12, 13, 16) or the zone was only perceived weakly (Peaks 2, 6, 9, 15, 19). Peak 14, however, was very strong and had an aroma (quince jelly note) that had been described in sensory evaluation of Petite Arvine wines and was correlated positively with the typicity (results not shown). In addition the peaks 20 and 22 seem to contribute to the typical aroma of Petite Arvine wine as well. These zones could not be detected in organic extracts of other wine varieties, such as Chasselas, and Chardonnay (data not shown).

4.3.4 Identification and quantification of some aroma compounds which contribute to the characteristic flavour of Petite Arvine

Olfactometric analyses of the wine extracts revealed an intense zone at a retention time of 48 min (peak 14, Figure 2, Table 2). The zone exhibited a typical quince (Cydonia oblonga) jelly aroma. To identify the chemical nature of this compound, quince jelly was extracted and analysed under the same conditions. In the organic extract of quince jelly a peak exhibiting the same olfactometric properties was detected at the same retention time as in the wine extract. However, the substance could not be identified up to now. Tsuneya et al. [28, 29] identified the so-called marmelo lactones [(+)- and (-)-2,7-dimethyl-4-hydroxy-5,7-octadienoic acid lactone] as important aroma compounds in quince extracts. However, these substances were identified at another retention time in our organic quince extract. Schreyen et al. [30] found ethyl-2-methyl-2-butenoate to be an important contributor to the quince aroma. Since the concentration determined in the quince extract was high [30], and the odour threshold of 65 μl/l is relatively high [31], it is improbable that this ester is responsible for the olfactometric zone in which peak 14 was included. Furthermore, it can be excluded that peak 14 corresponds to a thiol, since no peak was detected at a retention time of 48 min in the thiol extract (see chapter 4.3.2). Additional work has therefore to be invested in the identification of the substance exhibiting a strong quince jelly aroma.

In tastings of Petite Arvine wine the aroma of wisteria flower (Wisteria sinensis) is often mentioned. To check the nature of the substances responsible for this association, an organic extract of wisteria flower was prepared and analysed by GC–olfactometry and compared with the wine extract. Several olfactometric zones were identical to zones perceived in the organic extract of Petite Arvine wine. Some of the substances responsible for these odours were
identified. The intensities of the unidentified olfactometric zones are weak and therefore not of interest.

Phenylethanol and phenylethyl acetate are well-known products of alcoholic fermentation. Both substances are degradation products of the amino acid phenylalanine [26] and have a strong flowery characteristic reminiscent of rose. Phenylethanol has been isolated from rose oil, the essential oil of other flowers and from alcoholic beverages [30]. Both compounds have been described to be present in wisteria flower as well [18].

Phenylethyl acetate and phenylethanol were shown to be present in considerable quantities in Petite Arvine wine and in two other wine varieties (Chardonnay and Sylvaner) as well (Table 3).

Table 3: Concentration of phenylethyl acetate and phenylethanol in different samples of Petite Arvine, Chardonnay, and Sylvaner wines (n=2).

<table>
<thead>
<tr>
<th>Wine sample, origin and vintage</th>
<th>Phenylethyl acetate (µg/l)</th>
<th>Phenylethanol (mg/l)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chardonnay, Chamoson, 2001</td>
<td>72</td>
<td>9.85</td>
</tr>
<tr>
<td>Chardonnay, Fully, 2000</td>
<td>54</td>
<td>9.85</td>
</tr>
<tr>
<td>Chardonnay, Saillon, 2001</td>
<td>147</td>
<td>12.0</td>
</tr>
<tr>
<td>Chardonnay, Sierre, 2001</td>
<td>139</td>
<td>16.7</td>
</tr>
<tr>
<td>Chardonnay, Saillon, 2001</td>
<td>71</td>
<td>9.81</td>
</tr>
<tr>
<td>Chardonnay, Leytron, 2001</td>
<td>97</td>
<td>11.9</td>
</tr>
<tr>
<td><strong>Mean value</strong></td>
<td><strong>96.7 (rsd 39.8)</strong></td>
<td><strong>11.7 (rsd 22.8)</strong></td>
</tr>
<tr>
<td>Petite Arvine, Corin/Sierre, 2002</td>
<td>131</td>
<td>12.6</td>
</tr>
<tr>
<td>Petite Arvine, Saillon, 2002</td>
<td>169</td>
<td>10.5</td>
</tr>
<tr>
<td>Petite Arvine, Fully, 2000</td>
<td>47</td>
<td>11.1</td>
</tr>
<tr>
<td>Petite Arvine, Muraz/Sierre, 2002</td>
<td>181</td>
<td>18.0</td>
</tr>
<tr>
<td>Petite Arvine, Sierre, 2000</td>
<td>179</td>
<td>19.9</td>
</tr>
<tr>
<td>Petite Arvine, Oillon, 2001</td>
<td>87</td>
<td>13.2</td>
</tr>
<tr>
<td>Petite Arvine, Chamoson, 2000</td>
<td>139</td>
<td>14.5</td>
</tr>
<tr>
<td>Petite Arvine, Leytron, 2000</td>
<td>53</td>
<td>11.8</td>
</tr>
<tr>
<td>Petite Arvine, Vétroz, 2000</td>
<td>46</td>
<td>14.5</td>
</tr>
<tr>
<td>Petite Arvine, Sion, 2000</td>
<td>109</td>
<td>11.2</td>
</tr>
<tr>
<td>Petite Arvine, Loèche, 2000</td>
<td>116</td>
<td>13.3</td>
</tr>
<tr>
<td><strong>Mean value</strong></td>
<td><strong>114 (rsd 44.8)</strong></td>
<td><strong>13.7 (rsd 21.5)</strong></td>
</tr>
<tr>
<td>Sylvaner, Chamoson, 2001</td>
<td>287</td>
<td>21.0</td>
</tr>
<tr>
<td>Sylvaner, Chamoson, 2002</td>
<td>143</td>
<td>11.9</td>
</tr>
<tr>
<td>Sylvaner, Sierre, 2001</td>
<td>142</td>
<td>17.2</td>
</tr>
<tr>
<td>Sylvaner, Chamoson, 2002</td>
<td>185</td>
<td>14.7</td>
</tr>
<tr>
<td><strong>Mean value</strong></td>
<td><strong>189 (rsd 36.0)</strong></td>
<td><strong>16.2 (rsd 23.8)</strong></td>
</tr>
</tbody>
</table>

rsd: relative standard deviation

The concentrations of both compounds are not significantly different between the three wine varieties. The relative standards are high, especially for the concentrations of phenylethyl acetate.
The concentrations of both compounds are not significantly different between the three wine varieties. The relative standards are high, especially for the concentrations of phenylethyl acetate.

This result indicates neither phenylethyl acetate nor phenylethanol plays a role in the typical aroma of Petite Arvine wine. The concentration of phenylethyl acetate in Petite Arvine is in all samples below the threshold value of 250 g/l [22], whereas the concentration of phenylethanol is consistently above the threshold value of 10 mg/l [22]. Phenylethanol probably plays a role in the overall flowery aroma of wines. According to Rapp and Güntert [33] the concentrations of both compounds are considerably higher in very young wines. This may explain the differences in the concentrations of phenylethanol found in the wines shown in Table 1, where one wine sample was very young. In microvinification experiments samples with very high concentrations up to 940 µg/l and 21 mg/l of phenylethyl acetate and phenylethanol, respectively, were measured (data not shown). According to the literature the concentrations of esters and fusel alcohols are high immediately after alcoholic fermentation and decrease over time [34].

β-ionone was perceived olfactometrically in the extract of Petite Arvine wine as well as in the organic extract of wisteria flowers. The presence of β-ionone in wisteria flowers has been described previously [32]. This compound has a very low threshold value of 30 ng/l in water [35] and of 90 ng/l in wine [36, 37]. The compound has also been identified in other food items, such as tomatoes [38], rhubarb [39] and grapefruit oil [40] as well as in red [36, 37, 41–43] and white wines [44]. The concentrations found in red wine reached 337 ng/l [37].

In the present study β-ionone was identified by olfactometry (strong intensity) and by injection of a reference substance; however, the concentration of β-ionone in the organic extract of Petite Arvine was too low to be detected by GC-MS.

4.4 Conclusions

The organic extract of Petite Arvine wine was analysed by GC–olfactometry and GC–MS. Most of the olfactometrically detected zones could be attributed to an identified flavour compound. 3-Mercaptohexanol and β-ionone seem to contribute to the typical aroma of this wine variety together with some hitherto unidentified compounds. The contribution of
phenylethanol and phenylethyl acetate to the typical Petite Arvine aroma was disproved. These two aroma compounds have been shown to be present in two other wine varieties in similar concentrations.

The identification of the as yet unidentified compounds should be pursued further in order to elucidate the overall typicity of the Petite Arvine wine aroma.

Acknowledgements

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4.5 References

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Analysis of volatile compounds of Petite Arvine wine


Chapter 5

3-MERCAPTOHEXANOL, AN AROMA IMPACT COMPOUND OF A WINE PRODUCED FROM AN AUTOCHTHONOUS GRAPE VARIETY

Abstract

The characteristic aroma of Petite Arvine, a local white wine specialty prepared from the autochthonous grape variety Petite Arvine in the Canton of Valais/Switzerland, is described as intense in grapefruit and rhubarb flavours. In sensory evaluation by a triangle-test, the impact of thiol compounds on the wine aroma was demonstrated. In gas-chromatography-olfactometric and gas-chromatography-mass spectrometric analyses, 3-mercaptohexanol was identified as one of the key aroma compounds for the wine aroma. The concentration of 3-mercaptohexanol in 11 Petite Arvine wines was in the range between 210 and 6100 ng/l; all values being above the odour threshold value in aqueous ethanol solutions for this compound.

5.1 Introduction

Thiols have been found to be responsible for the typicity of Sauvignon blanc [1-4]. Some of the thiols contribute to the fruity flavour of rosé wines and Merlot wines [5, 6] as well. They have also been identified in young white wines from the Canary islands [7], and in white wines from the Alsace [8].

The precursors of the aroma active thiols are S-cysteinyl conjugates. The transformation of the precursor into the flavour compound occurs during alcoholic fermentation, and is catalyzed by a yeast β-lyase [9, 10]. Original methods to analyze thiols at low concentrations were developed by Tominaga and co-workers [11, 12].

3-Mercaptohexanol is one of the above mentioned identified aroma active thiols in different wines. This thiol has also been identified in yellow passion fruit [13], grapefruit, guava,

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tomato leaves and rhubarb [3]. The odour threshold was determined to be 60 ng/l in a 12 % (v/v) aqueous ethanolic solution [4].

The concentrations of 3-mercaptohexanol found in different wine varieties and in vegetal material are listed in Table 1.

Table 1: Concentration of 3-mercaptohexanol in different wine varieties [ng/l] and in vegetal material [ng/g], for grapefruit juice [ng/l] and yellow passion fruit [μg/l].

<table>
<thead>
<tr>
<th>Wine variety / Fruit</th>
<th>Concentration range</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Wine variety</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Champagne wines</td>
<td>250 - 640</td>
<td>[14]</td>
</tr>
<tr>
<td>Gewürztraminer</td>
<td>1'336 - 3'278</td>
<td>[8]</td>
</tr>
<tr>
<td>Merlot</td>
<td>120 - 4'560</td>
<td>[6]</td>
</tr>
<tr>
<td>Muscadet</td>
<td>63 - 445</td>
<td>[15]</td>
</tr>
<tr>
<td>Muscat</td>
<td>124 - 898</td>
<td>[8]</td>
</tr>
<tr>
<td>Pinot blanc</td>
<td>89 - 248</td>
<td>[8]</td>
</tr>
<tr>
<td>Pinot gris</td>
<td>312 - 1'042</td>
<td>[8]</td>
</tr>
<tr>
<td>Riesling</td>
<td>407 - 970</td>
<td>[8]</td>
</tr>
<tr>
<td>Rosé wines made of Merlot/Cabernet Sauvignon</td>
<td>0 - 7'000</td>
<td>[5]</td>
</tr>
<tr>
<td>Sauvignon blanc</td>
<td>600 - 12'822</td>
<td>[11]</td>
</tr>
<tr>
<td>Sylvaner</td>
<td>58 - 146</td>
<td>[8]</td>
</tr>
<tr>
<td>Different white varieties from the canary islands</td>
<td>108 - 2'640</td>
<td>[7]</td>
</tr>
<tr>
<td><strong>Fruit</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Guava</td>
<td>25</td>
<td>[3]</td>
</tr>
<tr>
<td>Grapefruit juice</td>
<td>150</td>
<td>[3]</td>
</tr>
<tr>
<td>Rhubarb</td>
<td>2.5</td>
<td>[3]</td>
</tr>
<tr>
<td>Tomato leaf</td>
<td>10</td>
<td>[3]</td>
</tr>
<tr>
<td>Yellow passion fruit</td>
<td>1 - 195</td>
<td>[13]</td>
</tr>
</tbody>
</table>

Petite Arvine is an autochthon grape variety grown in the Canton of Valais/Switzerland, from which the Petite Arvine white wine specialty is produced. Sensory evaluation of Petite Arvine wine has shown that the intensities of grapefruit and rhubarb flavour positively correlated with the typicity of the wine (see chapter 3). In the present study sensorial, gas-chromatographic-olfactometric and gas-chromatographic mass spectrometric methods were applied to verify that 3-mercaptohexanol is one of the flavour impact compounds of Petite Arvine wine and contributes significantly to the characteristic rhubarb and grapefruit aroma of the wine.
5.2 Materials and methods

5.2.1 Selection of experimental wines

The wines for sensory analysis (vintage 2000) were collected from different cellars producing a minimum of 1500 l of Petite Arvine wine and located all over the Canton of Valais. The samples had an average sugar content of 23.3 °Brix (sd 0.9). None of the wines was elevated in wooden barrels because the woody notes would be too dominant and mask the typical flavour notes of Petite Arvine wines. The same wines were used for all sensorial and gas chromatographic-olfactometric analyses. For the identification of flavour compounds and quantification analyses Petite Arvine wines from other vintages and from cellars with a smaller production volume were also included. For the comparison by olfactometry, samples of Chardonnay and Chasselas wines from different producers and vintages were used in addition to the samples of Petite Arvine.

5.2.2 Sensorial analysis

The experienced panellists, 8 local enologists and wine producers, classified the wines according to their typicity into 7 categories. The typicity was defined as the ease of identification of a sample as being a Petite Arvine wine. Wines that could not be easily assigned to this variety were considered as untypical.

The intensities of the descriptors "rhubarb" and "grapefruit" were quantified on a non-structured scale (10 cm, divided into 100 units for interpretation) with a - and a + on the ends, indicating the minimum and maximum of intensity.

The impact of thiols on the wine flavour was established in a triangle test. The panellists were asked to distinguish between wine samples, some of which were fortified with copper sulphate. Copper ions and thiols form stable odourless metal complexes and the typical thiol odour disappears.

All wines were tasted at a temperature of 12°C and were evaluated by sniffing and tasting. The sessions took place in a specially designed room to make communication between the tasters impossible. A volume of 35 ml wine with different typicity (high, average and low typicity) was presented in tasting glasses. Either one or two of the presented samples were fortified with 1 mg CuSO₄ per glass (waterfree, Fluka AG, Buchs, Switzerland). The tasters had to determine which of the three presented samples were different. The order of the presented samples was randomized as proposed by Stone and Sidel [16].

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5.2.3 Extraction of organic compounds

Wine samples (350 ml) were extracted twice with portions of 100 ml of dichloromethane (LiChrosolv, Merck, Darmstadt, Germany) in a 1 l Erlenmeyer flask under magnetic stirring for 5 minutes. The aqueous and organic phases were divided in a separating funnel. The combined organic phases were centrifuged at 3000 g (Suprafuge 22, Heraeus Sepatech, Osterode, Germany) to break the emulsion and then divided again in a separating funnel. The organic phase obtained was dried over Na₂SO₄ (waterfree, Fluka) and concentrated to a final volume of 0.350 ml by rotatory evaporation under reduced pressure and nitrogen. For the gas-chromatographic-olfactometric and the quantification analyses 2-octanol was added to the wine samples as internal standard.

5.2.4 GC-FID and olfactometry

The GC system (HRGC Carlo Erba Instruments, 5300 Mega Series, Milano, Italy) equipped with a BP 20 column (50m x 0.22 mm x 0.25 μm; SGE, Melbourne, Australia) was operated at the following conditions: Initial temperature 40°C for 1 min heating, rate 3°C/min and 230°C during 10 min. The injector port was heated to 240°C. An FID detector supplemented with air (100 kPa) and hydrogen (80 kPa) was used, its temperature was 300°C. Helium was used as carrier gas at a pressure of 100 kPa. The injection volume was 2 μl. The chromatographic data were acquired by using ChromCard, Version 1.07 (Thermo Quest, Milano, Italy).

The GC system was equipped with an olfactometry tool to sniff the GC effluents (Sniffer 9000, Brechbühler AG, Schlieren, Switzerland). The intensities of the olfactometrically perceived zones were registered on an integrator (HP 3394 A Integrator, Hewlett Packard, Palo Alto, USA). The peak areas were corrected by the area detected by FID for the internal standard (2-octanol) to prevent biases due to extraction and injection differences. Nitrogen was used as make-up gas at a pressure of 50 kPa and the sniffer port was supplemented with humidified air at a pressure of 100 kPa. The effluent of the column was split in the ration 1:1 on the FID and the sniffer port.

5.2.5 Analysis of thiols

The thiols were analyzed by the gas-chromatographic method developed and optimized by Tominaga et al. [11, 12]. The GC was operated as described in the prior section (2.4), with the exception, that a volume of 3 μl was injected in the splitless mode. An FPD detector was used.
supplemented with air (70 kPa) and hydrogen (150 kPa), at a temperature of 160°C, the bottom temperature being 300°C. The wines were extracted and analyzed in duplicate.

5.2.6 Identification by GC-MS

The gas-chromatography-mass spectrometric analyses were performed on a GC HP Series II 5890 (Hewlett Packard), supplemented with a mass selective detector, HP 5971A (Hewlett Packard). Gas chromatographic separation was performed on a HP-WAX column (25m x 0.22mm x 0.4μm; Hewlett Packard). This column corresponds in its properties to a BP 20 column used for GC-FID and GC-olfactometry studies. Helium was used as carrier gas at a pressure of 85kPa. The injector and the detector were set at a temperature of 250°C and 300°C respectively. The same temperature profile was used as in the GC- FPD and GC-olfactometry analysis.

5.3 Results and discussion

5.3.1 Sensorial analysis

The wines had different degrees of typicity: Wine A: high typicity (score: 5.3 out of 6); wine B: average typicity (score: 3.5 out of 6); wine C: low typicity (score: 2.1 out of 6). The scores were determined in a prior session of sensory analyses (see chapter 3). The panellists were asked to distinguish wine samples fortified with copper sulphate from genuine wine samples in a triangle test. All panelists could distinguish the most typical wine from its copper sulfate fortified samples. Wines with average or low typicity could not be distinguished. Only 5 out of 8 and 2 out of 8 panelists were able to differentiate wine B and wine C, respectively, which is not statistically significant (p> 0.05) (Stone and Sidel 2004).

Wines with a high typicity are more sensitive to copper sulfate addition; this indicates that thiols may play an important role for the characteristic aroma of Petite Arvine. Wine C (untypical) probably contains less thiols, and could therefore not be discriminated. For the wine of average typicity, the panelists disagreed about the preference for the copper sulfate fortified or genuine sample. This result suggests that besides thiols, other aroma compounds could play a role for the typicity of Petite Arvine wine.
5.3.2 Olfactometry

In previous studies a strong rhubarb odour was perceived in olfactometric analyses at the retention time of 53 min when using the described GC-FID conditions (see chapter 4). The thiol 3-mercaptohexanol showed the same retention time when injected as reference substance (3-mercaptohexanol, Interchim, Montluçon, France). The intensities of the olfactometrically perceived "rhubarb" zone were registered. Wines made of other wine varieties were also analyzed by olfactometry in order to estimate the importance for the typicity of this zone. Neither in the organic extracts of Chasselas wine nor of Chardonnay wine a similar zone could be detected. In our experiments, it has been shown that 3-mercaptohexanol changes its odour in function of concentration, at low concentration it has a reminiscent of grapefruit, at moderate concentration of passion fruit and rhubarb and at high concentration an unpleasant sulphur note. The concentration which occurred in the olfactometrically analyzed extract, 3-mercaptohexanol had a smell of rhubarb. In sensorial analyses, the intensities of grapefruit and rhubarb correlated positively (data not shown).

The intensities of the rhubarb aroma note correlated positively with the detected olfactometric zones (Figure 1).
3-Mercaptohexanol, an aroma impact compound of a wine produced from an autochthonous grape variety

![Graph](image)

The correlation coefficients $R^2$ for fresh and cooked rhubarb were 0.687 ($F=8.78$) and 0.604 ($F=6.1$) respectively, whereas for the grapefruit flavour $R^2$ corresponded to 0.520 ($F=5.63$). The required $F$-values are 7.71 for a confidence level of 0.95, resp. 4.54 for a confidence level of 0.9 [17].

The data of the sensory evaluation were tested by variance analysis and were found to have a significance of $p<0.05$. However, the relative standard deviations were high (0-70 %), but comparable to the relative standard deviations for the sensorial evaluation (data not shown). The "detector" in olfactometry being the human nose, factors such as fatigue, concentration, physical and psychological conditions influence the reliability of the results.
5.3.3 Quantitative analyses

The concentration of 3-mercaptohexanol in several Petite Arvine wines was determined by GC-FPD analysis (Table 2). The odour activity value (OAV) is defined as the ratio between the actual concentration of a chemical in a sample to its odour threshold. It helps to estimate the importance of a flavour compound to the aroma of a foodstuff.

Table 2: Concentration of 3-mercaptohexanol in Petite Arvine wines and Odour Activity Value (OAV=concentration/odour threshold) (n=1).

<table>
<thead>
<tr>
<th>Wine sample, year of vintage</th>
<th>Concentration (ng/l)</th>
<th>OAV</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vétroz, 2000</td>
<td>212</td>
<td>3.53</td>
</tr>
<tr>
<td>Sierre, 2000</td>
<td>375</td>
<td>6.24</td>
</tr>
<tr>
<td>Saillon, 2002</td>
<td>406</td>
<td>6.76</td>
</tr>
<tr>
<td>Corin/Sierre, 2002</td>
<td>508</td>
<td>8.47</td>
</tr>
<tr>
<td>Sion, 2000</td>
<td>614</td>
<td>10.2</td>
</tr>
<tr>
<td>Flanthey, 2002</td>
<td>1'351</td>
<td>22.5</td>
</tr>
<tr>
<td>Ollon, 2001</td>
<td>1'897</td>
<td>31.6</td>
</tr>
<tr>
<td>Sierre, 2002</td>
<td>1'900</td>
<td>31.7</td>
</tr>
<tr>
<td>Conthey, 2002</td>
<td>1'908</td>
<td>31.8</td>
</tr>
<tr>
<td>Leytron, 2000</td>
<td>6'112</td>
<td>101.9</td>
</tr>
</tbody>
</table>

The concentrations of 3-mercaptohexanol found in Petite Arvine wine vary between 200 and 6100 ng/l. The high typicity wine (A) and the low typicity wine (C) tasted in the triangle test are the wines with the highest and lowest concentration of 3-mercaptohexanol.

A large variation in the concentration was also observed in the Sauvignon blanc wine [11]. The reasons for this large variation remain unknown. A concentration of a few 100 ng/l could be found in every white wine analyzed [7, 8, 11]. This phenomenon could be explained as follows: At low concentration levels (up to ≈ 500 ng/l) 3-mercaptohexanol could contribute to the overall fruity flavour of white wines. In wines like Sauvignon blanc and Petite Arvine the concentration of 3-mercaptohexanol is usually considerably higher and this thiol is perceived as a distinct key aroma compound. No other powerful flavour compounds such as terpenes dominate the aroma of Petite Arvine and Sauvignon blanc, as it is the case for Gewürztraminer and Muscat wines.

5.3.4 Identification by GC-MS

The presence of 3-mercaptohexanol in the extract of Petite Arvine was verified by GC-MS. In the GC-MS mode scan, 3-mercaptohexanol could not be detected since its peak was over-
3-Mercaptohexanol, an aroma impact compound of a wine produced from an autochthonous grape variety

lacking with the one of hexanoic acid. However, using the SIM mode, the characteristic fragments of 3-mercaptohexanol (m/z 134 and 100) could be detected at the retention time of 3-mercaptohexanol, verified by injecting a reference substance.

5.4 Conclusion

The addition of copper sulfate to a typical Petite Arvine wine changes its sensorial properties, so it can be concluded that thiols contribute significantly to the characteristic aroma of this wine variety. Analyses by GC-olfactometry, GC-FPD and GC-MS could identify 3-mercaptohexanol as important contributor to the typical aroma of Petite Arvine wine. It has a reminiscent of rhubarb or grapefruit depending on the concentration. The concentrations found were between 200 and 6100 ng/l, in all samples, the concentration was above the threshold value.

Acknowledgements

The financing of the research project by the Haute École Spécialisée – Suisse Occidentale (HES-SO) is gratefully acknowledged.

The authors thank Dr. Jean-Claude Villettaz and Alexandre Béné for their preliminary studies in this field. Additionally the authors express their gratitude to Prof. Denis Dubourdieu University of Bordeaux II, France, for his interest in and support of the research on Petite Arvine wine and for valuable scientific discussions.

5.5 References


3-Mercaptohexanol, an aroma impact compound of a wine produced from an autochthone grape variety


Chapter 6

DEGRADATION OF AN S-CYSTEINYLATED FLAVOUR PRECURSOR DURING MALOLACTIC FERMENTATION IN A MODEL SYSTEM

Abstract

The ability of *Oenococcus oeni* to liberate 3-mercaptohexanol from its S-cysteinylated flavour precursor was studied by fermenting a model milieu under different conditions. Under optimal growing conditions for *Oenococcus oeni* (pH 4.8, 30°C), the cysteine conjugate was transformed into the corresponding thiol, whereas under conditions similar to winemaking (pH 3.5, 20°C), no 3-mercaptohexanol could be measured. The transformation rate was poor (0.06 %), but taking into account the instability of 3-mercaptohexanol in the used growth medium, the transformation rate corresponded to approximately 0.5 %. Compared to the transformation rates of flavour precursors by yeast cells, it can be deduced that the impact of the malolactic fermentation on the release of 3-mercaptohexanol is neglectable. The chemical stability of the S-cysteinylated flavour precursor was studied as well. Over a period of 10 weeks at room temperature (pH 4-9), no flavour development could be observed. Apparently the aroma compound is only liberated enzymatically.

6.1 Introduction

Must of non-aromatic wine varieties contain non-volatile flavour precursors that are transformed into aroma compounds during wine making. Different types of non-volatile precursors have been identified in wine; S-cysteine conjugates have been identified to be precursors for different flavour active thiol compounds [1, 2]. Such thiols have been shown to contribute essentially to the characteristic aroma of Sauvignon Blanc [3, 4, 5], Petite Arvine (see chapter 5) and other wine varieties [6, 7, 8]. 3-Mercaptohexanol is particularly interesting

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*Fretz, C., Hafner, V., Hauri, R., Luisier, J.-L., Amadò, R. (prepared to be submitted)
since it has been found in all of the above mentioned wine varieties at levels above its threshold value. It has a reminiscent of grapefruit, rhubarb and passion fruit.

During alcoholic fermentation, the S-cysteine conjugate 1 is transformed into 3-mercaptonehexanol 2 by a β-lyase of the yeast Saccharomyces cerevisiae [1]. The proposed pathway is shown in Figure 1.

\[
\begin{align*}
&\text{NH}_2 \\
\text{S} & \text{OH} \\
\text{SH} & \text{S} \text{OH} \\
\beta-\text{lyase} & \text{SH} \\
\text{OH} & \text{SH} \\
1 & 2
\end{align*}
\]

Figure 1: Proposed pathway of 3-mercaptonehexanol formation during alcoholic fermentation. Transformation of the non-volatile precursor (3-S-hexan-1-ol-L-cysteine) 1 into the free thiol (3-mercaptonehexanol) 2, under the action of a β-lyase [1].

β-Lyases (E.C. 4.4.1.13) catalyze the cleavage of C-S bonds in S-conjugates. These enzymes have also been identified in gut bacteria [9, 10, 11], in mammal renal, lung as well as in hepatic tissues [12] and in plants [13].

In wine making, the malolactic fermentation is one of the most difficult steps to control. It generally takes place after completion of the alcoholic fermentation. It is implemented by lactic acid bacteria, preferably by Oenococcus oeni, which deacidify the wine by converting malic acid into lactic acid, leading to a wine with a softer mouth feeling [14]. Malolactic fermentation has an impact on the wine flavour and wine quality. Aromas as buttery, creamy, smokey-roasted or grapefruit are more intense, the overall quality higher and the product more balanced and complex if the wine has undergone malolactic fermentation [15-17]. Oenococcus oeni bacteria were found to be able to release terpenic aroma compounds from their non-volatile glycosylated precursors during malolactic fermentation. The amount of the transformed precursors can significantly contribute to the overall flavour of a wine [18].

To our knowledge the ability of Oenococcus oeni to release thiols from the corresponding S-cysteine conjugates has not been studied yet. According to Peyrot des Gachon [19] up to 60% of the initial flavour precursor can still be present after the alcoholic fermentation in Sauvignon blanc wines. The aim of this study was therefore to investigate the ability of the lactic acid bacteria responsible for the malolactic fermentation to release the flavour active
thiols, thus contribute to the characteristic aroma of wines in which thiols are known to act as flavour impact compounds, such as Sauvignon blanc, Petite Arvine and others.

Up to now the cleavage of S-cysteine conjugates was described to occur uniquely by an enzymatic reaction [1, 20, 21]. Under wine making conditions without any yeast activity, no release of free thiol from the precursor could be observed over a period of 10 days [2]. After completion of the alcoholic fermentation the concentration of the precursor remained stable over a period of 4 month, provided the wine was ripened on lees [18]. In this study, the chemical stability of the precursor was examined in a wine-like model solution at different pH values and over a period of 10 weeks.

6.2 Materials and methods

6.2.1 Stability of 3-mercaptohexanol in MRS broth

To evaluate the effective formation rate of free thiol from the S-cysteine flavour precursor, the stability of 3-mercaptohexanol was tested in the growth medium used for malolactic fermentation by Oenococcus oeni, MRS (De Man, Rogosa and Shape) broth with tween 80 (Biolife, Milano, Italy). The MRS broth was prepared according to the instructions of the producer. In order to optimize the malolactic fermentation, 2 g/l of L-malic acid (Fluka, Buchs, Switzerland) was added to the medium. The pH was adjusted to 3.5, and 4.8, respectively with 1M HCl (SDS, Peypin, France). The medium was sterilized at 121°C during 15 min. 3-Mercaptohexanol (Interchim, Monluçon, France) was added after sterilization by sterile filtration. The concentration of 3-mercaptohexanol was measured over time.

6.2.2 Extraction and analysis of 3-mercaptohexanol

The 3-mercaptohexanol was extracted and analyzed according to the method developed and optimized by Tominaga et al. [5, 22]. In this method, the thiols are isolated by a specific complexation with para-hydroxymercuric benzoic acid (pHMB) from an organic extract of wine. 4-Methoxy-2-methyl-2-mercaptobutane (Oxford chemicals, Brackley, UK) was used as internal standard.

The following chromatographic conditions were used:
Gaschromatograph: HRGC, 5300 Mega Series, (Carlo Erba Instruments, Milano, Italy)
Column: BP 20 column (SGE, Melbourne, Australia, 50 m x 0.22 mm, id 0.25 μm)
Temperature program: 40°C, 1 min; rate 3°C/min; and 230°C, 10 min
Injection volume: 3 μl (splitless; split opened after 2 min)
Injector temperature: 240°C
Detector: FPD, supplemented with air (70 kPa) and hydrogen (150 kPa); 160°C, bottom temperature 300°C
Carrier gas: Helium (100 kPa).
Data acquisition: ChromCard, Version 1.07 (Thermo Quest, Milano, Italy).

6.2.3 Fermentation with *Oenococcus oeni*

6.2.3.1 Fermentation

A commercial *Oenococcus oeni* strain (EQ 54, Lalvin, Lallemand S.A., Blagnac, France) was used for the fermentation experiments. Synthesized precursor (0.6 mg/l) was added to sterilized MRS broth by sterile filtration. The precursor was synthesized according to Luisier *et al.* [23].

The bacterial population was counted under the microscope using a counter plate type Neubauer (Assistant, Sondheim, Germany). The inoculation volume was determined as a function of the bacterial density in the preculture to inoculate 10^6 CFU per ml.

All aqueous solutions were prepared using millipore quality water (Milli-Q A 10, Millipore, Billerica, USA).

The fermentations were carried out in 2 l Erlenmeyer flasks, closed with glycerine-filled fermentation caps, allowing gas to escape but not to enter into the system. After inoculation, the flasks were swept with Argon 5.0 (Pangas AG, Luzern, Switzerland). The samples were kept in incubators at constant temperature conditions (20°C; 30°C).

6.2.3.2 Verification of the malolactic fermentation course

A 1 ml sample of the culture was centrifuged at 20,000 g during 5 min (Hettich 32 R, Rotor E 1547, Hettich, Tuttingen, Germany). The supernatant was sterile filtered and diluted. The concentrations of glucose, malic and lactic acid were determined by HPLC (1100 Series,
Agilent Technologies, Palo Alto, USA) equipped with an Aminex, HPX-87H column (3000 mm x 7.8 mm). Elution was performed with 5 mM H₂SO₄, at a flow rate of 0.6 ml/min with refraction index monitoring for glucose and UV monitoring at 210 nm for the organic acids. The column and detector temperature was kept at 35°C, the injection volume was 20 μl. All samples were analyzed in duplicate.

6.2.1 Chemical stability of the precursor of 3-Mercaptohexanol

6.2.3.3 Model medium and procedure

The following model medium was used: Tartaric acid (5g, Fluka) and sodium sulfite (0.05 g, Fluka) were added to 1000 ml of a 12 % (v/v) aqueous ethanol solution (absolute ethanol, Amtech-Chemie, Kölliken, Switzerland). The pH was adjusted to 3.0, 5.0 and 9.0 with 10 M NaOH. The medium was sterilised at 121°C during 15 min. After sterilization, 20 mg/l of the synthesized precursor (S-3-hexanol-L-cysteine) was added. The samples were kept at room temperature (23°C ± 3°C) for a period of up to 10 weeks.

6.2.3.4 Extraction and analysis of the thiols

Since high concentrations of 3-mercaptopentanol could be expected, the procedure to extract and analyze thiols was simplified as follows: A sample volume of 100 ml was extracted twice with 20 ml dichloromethane. The organic phase was dried over Na₂SO₄ (Fluka), and concentrated by rotatory evaporation in a nitrogen stream to an end volume of 0.1 ml. As internal standard 2-octanol (Merck, Darmstadt, Germany) was used. The thiols were analyzed by GC as described in the previous paragraph, with the exceptions that no splitless injection mode was applied, and a FID was used to monitor the peaks.

6.3 Results and discussion

6.3.1 Stability of 3-mercaptopentanol in the MRS broth

Thiols are known to be very unstable mainly because of their sensitivity to oxidation and their reactivity with heavy metals [24]. The influence of Oenococcus oeni on the stability of 3-mercaptopentanol was tested in MRS broth; the initial concentration being approximately 5 μg/l and 10 μg/l respectively. Several culture media have been described for Oenococcus, but the MRS medium is the most widely used. It is often called 'Lactobaccilli medium' broth but it also meets the Oenococcus requirements. The development of the concentration of 3-mercaptopentanol over time is shown in Figure 2.
The 3-mercaptohexanol decreased sharply during the first hours of incubation. After about 50 h the concentration of 3-mercaptohexanol in the solution stabilizes to approximately 10 % of the initial value under all experimental conditions chosen. After approx. 10 days, 91 % and 86 % of the 3-mercaptohexanol had disappeared at pH 4.8, 30°C when incubated without and with Oenococcus oeni respectively. At conditions similar to wine making (pH 3.5, 20°C) 88 % disappeared after a period of 7 days. Tominaga [25] reported 3-mercaptohexanol to have a better stability in wine. After completion of the alcoholic fermentation, the concentration of 3-mercaptohexanol remained stable during 150 days. It is known that components of the matrix can influence the stability of flavour thiols. Components present in wine such as cysteine or ribose [26] as well as anthocyanes can stabilize thiols [27]. Other compounds, as sulphur dioxide, can act as reducing agents [28]. The reasons for the instability observed in the present investigation are not evident.

It could be that 3-mercaptohexanol reacted with constituents of the MRS broth or oxidized to disulfides and possibly also to other oxidation products. The conditions being anaerobic because of the large production of CO₂ during the fermentation, an oxidation caused by oxygen can be excluded or its effects neglected. The MRS broth contains catalysts of oxidative reactions or possible reaction partners, as metal ions, sugars, proteins and amino acids [24]. Thiols can react with the sulphydryl groups of proteins to form disulfides [29]. The interaction of thiols and disulfides with food proteins was also proved [30]. The concentration of these possible reaction products stabilized after a time of approx. 50 h, independent of the initial concentration of the 3-mercaptohexanol and of the presence of Oenococcus oeni. This
result led to the hypothesis that these reactions do not proceed to completion, but are stabilized in equilibrium. The possible reactions of 3-mercaptohexanol in MRS broth and reaction products formed has not been elucidated; this should be subject of a future study.

Neither the presence of the bacteria nor the pH influenced the stability of 3-mercaptohexanol. The application of a malolactic fermentation in wine making should therefore not have negative influence on the content of 3-mercaptohexanol. But this has to be verified in vinification experiments with grape must.

The instability of 3-mercaptohexanol in MRS broth had to be taken into account when the results of its possible formation during the malolactic fermentation step is evaluated.

### 6.3.2 Degradation of the S-cysteine conjugate by Oenococcus oeni

Before the inoculation, a preculture was made in order to optimize growth. *Oenococcus oeni* was inoculated in MRS broth and incubated at 30°C during 24 h. Kinetic studies showed the bacteria to grow exponentially after a lag phase of 24 h at pH 4.8 (data not shown). *Oenococcus oeni* was incubated together with 0.6 mg synthetic precursor per litre medium. Since the molar masses have to be considered, this concentration corresponds to 0.36 mg/l of potentially liberated 3-mercaptohexanol. After an incubation period of 15 days the concentration of 3-mercaptohexanol was measured. Additionally, after 11 days, the concentrations of glucose, malic acid and lactic acid were determined by HPLC in order to estimate the course of the malolactic fermentation.

![Figure 2](image.png)

**Figure 2:** Concentration of glucose (■), malic (●) and lactic acid (▲) before and after 15 days of malolactic fermentation under different conditions.
Usually, the malolactic fermentation is completed, when malic acid has been completely consumed by the lactic acid bacteria to form lactic acid and carbon dioxide. In this study, even after an incubation period of 11 days some of the initial malic acid was still present. In the samples incubated at optimal conditions for bacterial growth (pH 4.8, 30°C) the glucose present in the MRS broth was completely metabolized. This was not the case for the samples incubated at conditions similar to wine making (pH 3.5, 20°C); 15.1 g/l of the initial 17.4 g/l were still present. Under real wine making conditions, no glucose is available for the malolactic fermentation, because it has been consumed by the yeasts during the previous alcoholic fermentation step. Nevertheless, lactic acid was produced in both trials, so the bacteria had metabolized substrate and had grown, so the fact that the fermentation is not completed should not influence the test of evidence of the degradation of cysteinylated flavour precursors, because a metabolic activity was proved.

Under optimal conditions for bacterial growth (pH 4.8, 30°C), the S-cysteine conjugate was transformed into the corresponding thiol, whereas under similar conditions as in wine making (pH 3.5, 20°C), no 3-mercaptohexanol could be measured; the detected peaks were below the limit of quantification. The time dependency of the formation of 3-mercaptohexanol at pH 4.8 and 30°C is shown in Figure 3.

![Figure 3](image)

**Figure 3**: Development of 3-mercaptohexanol from its precursor during the malolactic fermentation in MRS broth at pH 4.8 and 30°C. The fermentations were carried out in duplicate: ■ batch 1; □ batch 2.

After approx. 13 days of malolactic fermentation, 230 ng/l 3-mercaptohexanol were still present, which would correspond to a transformation rate of 0.06 % of the added precursor. The effective liberation of 3-mercaptohexanol is actually higher; because in the stability tests
Degradation of an S-cysteinylated flavour precursor during malolactic fermentation in a model system

(see above), 86% of the 3-mercaptotetrol disappeared under the same conditions (pH 4.8, with *Oenococcus oeni*, 30°C). If this instability is taken into account, the expected transformation rate was at 0.43%, which would correspond to 1543 ng/l. The value measured after 75 h (approx. 1250 ng/l), indicated that the development of 3-mercaptotetrol took place during the first days of malolactic fermentation; followed by a degradation and stabilization at a low concentration during the remaining fermentation time.

The formation rate of 3-mercaptotetrol during alcoholic fermentation of grape must by yeast is considerably higher (2-10%) [19], the formation in a fermenting wine-like model solution was in the same order of magnitude (data not shown). In our experiments, under wine-making conditions no 3-mercaptotetrol formation could be measured. Therefore it can be assumed that despite of the ability of *Oenococcus oeni* to release 3-mercaptotetrol under certain conditions, the impact of the malolactic fermentation on the concentration of 3-mercaptotetrol in the final product is neglectable.

Ugliano et al. [18] reported different starter cultures of *Oenococcus oeni* to exhibit different glucosidase activity. It could be possible, that different strains also have different β-lyase activity. In our study, only one *Oenococcus oeni* strain was used. It would be interesting to test more bacterial strains. Furthermore, the experiments were carried out in a model medium (MRS), where 3-mercaptotetrol showed poor stability, whereas it has been shown to be stable in a real wine [25]. Future experiments need to be carried out using fermenting grape musts and under real wine making conditions in order to estimate the influence of lactic acid bacteria on the release of free thiols known to contribute to the typical aroma of different wine varieties.

6.3.3 Chemical stability of the flavour precursor

After 10 weeks of incubation of S-cysteine conjugates, no thiols could be detected. So it can be concluded that no 3-mercaptotetrol was released from the corresponding S-cysteine conjugate under our experimental conditions. However, it can not be excluded that other products were formed during this period, but if so, these substances are not aroma relevant. This result suggests that the non-volatile aroma precursors are only degraded by the aid of specific enzymes.

Our results confirm findings in literature. Peyrot des Gâchons [19] showed the concentration of precursors to be stable during the wine ripening on lees over a period of 4 month. Wayakabashi et al. [2] studied the stability of an S-cysteine conjugate of pulgeone in different
solvents. The liberation of pulgeone as consequence of retro-Michael reaction was observed, but no cleavage of the C-S bond was described.

Acknowledgements

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6.4 References


Degradation of an S-cysteinylated flavour precursor during malolactic fermentation in a model system


Chapter 7

USE OF AN EXPERIMENTAL DESIGN MODEL TO DETERMINE THE IMPACT OF DIFFERENT FERMENTATION PARAMETERS ON THE DEVELOPMENT OF FLAVOUR COMPOUNDS IN WINE*

Abstract

An experimental design developed by Youden and Steiner was successfully applied to micro-fermentation experiments with two different grape musts. This tool allowed the verification of the impact of several fermentation parameters on the fermentation course and on flavour development with a restricted number of experiments. The positive effects of a higher fermentation temperature on the development of 3-mercaptohexanol, an important contributor to the characteristic aroma of the Petite Arvine wine, could be demonstrated.

7.1 Introduction

Thiol compounds, particularly 3-mercaptohexanol, have been shown to be of outstanding importance for the flavour of Sauvignon blanc wine [1-3]. 3-Mercaptohexanol was also identified in Merlot and rosé wines made from Merlot grapes [4, 5], white wines from the Alsace [6] and Petite Arvine, an autochthonous wine specialty produced in the Canton of Valais, Switzerland (see chapter 5). The precursors of the flavour active thiols are $S$-cysteine conjugates that are transformed into the free thiols during alcoholic fermentation by a yeast $\beta$-lyase [7, 8]. The transformation rates from the precursors have been shown to be low [9].

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Peyrot des Gâchons [9, 10] studied the influence of pre-fermentation procedures on the content of the precursors of thiols that are important for the characteristic aroma of Sauvignon blanc. Maceration of grape berries led to a higher concentration of the cysteinyalted precursor of the aroma active thiols, thereof the precursor of 3-mercaptohexanol increased by 50%. This phenomenon is the consequence of the localization of the precursor in the skin of the berries [10]. The application of pectinolytic enzymes, however, did not increase the concentration of precursors, but decreased the concentration of thiols in the finished wines [9]. Schneider et al. [11] showed that wine aging "sur lies" increased the content of 3-mercaptohexanol, probably because of the reducing effects and the oxygen consumption of the yeast lees.

Youden and Steiner [12] proposed a statistical method to verify the ruggedness of analytical methods and procedures based on experimental design. This method allows the estimation of the degree of influence of chosen parameters on the result of the analysis. This method has been applied e.g. by Mirza and Tan [13] to verify the robustness of newly developed analytical methods.

In this study, the “Youden procedure” was applied to study the influence of some vinification parameters on the transformation rate of the flavour precursor (5-cysteine conjugate) into 3-mercaptohexanol and on the formation of two typical fermentation products (2-phenylethanol and 2-phenylethyl acetate) during alcoholic fermentation. 3-Mercaptohexanol was determined because of the above mentioned importance as flavour impact compound of Petite Arvine and many other wine varieties. 2-Phenylethanol and 2-phenylethyl acetate are not impact compounds of Petite Arvine wine but general constituents of wine aroma and have been identified in numerous alcoholic beverages [14-16]. These two substances were determined to verify the usefulness of the experimental model because of their general interest.

7.2 Materials and methods

7.2.1 Experimental model

“Youden’s procedure” uses a combination of 7 parameters in 8 batches. To fulfil the prerequisites of this approach, each parameter had to be used in two variants were selected to have a high and a low value for each parameter in the model. If possible, two extreme values were taken (e.g. temperature of 16 °C and 25 °C respectively); the variants did not need to be realistic in practice.
Use of an experimental design model to determine the impact of different fermentation parameters on the development of flavour compounds in wine

The following 7 parameters were studied: Turbidity of the must, yeast strain, temperature of the alcoholic fermentation, de-acidification of the must, suspension of the lees, presence of oxygen and nitrogen supplementation (Table 1).

Table 1: The experimental design, with the two chosen variants.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Variant 1</th>
<th>Variant 2</th>
</tr>
</thead>
<tbody>
<tr>
<td>Turbidity</td>
<td>A NTU 11</td>
<td>a NTU 740-765</td>
</tr>
<tr>
<td>Yeast strain</td>
<td>B S. cerevisiae VL1</td>
<td>b S. cerevisiae CEPO 20</td>
</tr>
<tr>
<td>Fermentation temperature</td>
<td>C T = 16 °C</td>
<td>c T = 25 °C</td>
</tr>
<tr>
<td>De-acidification</td>
<td>D Total acidity 3.8 g/l*</td>
<td>d Total acidity 4.2 g/l*</td>
</tr>
<tr>
<td>Suspension of the lees</td>
<td>E With stirring</td>
<td>e Without stirring</td>
</tr>
<tr>
<td>Oxidation</td>
<td>F Headspace filled with air</td>
<td>f Headspace filled with nitrogen</td>
</tr>
<tr>
<td>Nitrogen supplementation</td>
<td>G No supplementation of ammonium sulfate</td>
<td>g Supplementation of ammonium sulfate (0.3g/l)</td>
</tr>
</tbody>
</table>

* calculated as tartaric acid

In the 8 fermentation batches, 4 times the high variant and 4 times the low variant of the parameter have to be applied. After fermentation, the concentrations of 3-mercaptohexanol, 2-phenylethanol and 2-phenylethyl acetate were determined. The results of the different batches were assigned to the letters s-z (Table 2).

Table 2: Combination of the parameter variants in the different batches.

<table>
<thead>
<tr>
<th>Batch</th>
<th>Parameter</th>
<th>Results</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>A B C D E F G</td>
<td>s</td>
</tr>
<tr>
<td>2</td>
<td>A B c D e f g</td>
<td>t</td>
</tr>
<tr>
<td>3</td>
<td>A b C d E f g</td>
<td>u</td>
</tr>
<tr>
<td>4</td>
<td>A b c d e F G</td>
<td>v</td>
</tr>
<tr>
<td>5</td>
<td>a B C d e F g</td>
<td>w</td>
</tr>
<tr>
<td>6</td>
<td>a B c d E f G</td>
<td>x</td>
</tr>
<tr>
<td>7</td>
<td>a b C D e f G</td>
<td>y</td>
</tr>
<tr>
<td>8</td>
<td>a b c D E F g</td>
<td>z</td>
</tr>
</tbody>
</table>

The influence of each parameter on the fermentation process is determined by calculating the mean value and the absolute differences between the two variants (Table 3). The higher the absolute difference, the higher the influence of this parameter on the result, e.g. on the amount of flavour compound developed during the fermentation. The algebraic sign (+/-) before calculating the absolute value indicates whether the variant with the capital letter (A-G) or with the lower case-letter (a-g) influences the result positively or negatively.
Table 3: Calculation of the mean value and the absolute difference of each parameter

<table>
<thead>
<tr>
<th>Mean value</th>
<th>Absolute difference</th>
</tr>
</thead>
<tbody>
<tr>
<td>( \bar{A} = \frac{(s+t+u+v)}{4} )</td>
<td>( \bar{a} = \frac{(w+x+y+z)}{4} )</td>
</tr>
<tr>
<td>( \bar{B} = \frac{(s+t+w+x)}{4} )</td>
<td>( \bar{b} = \frac{(u+v+y+z)}{4} )</td>
</tr>
<tr>
<td>( \bar{C} = \frac{(s+u+w+y)}{4} )</td>
<td>( \bar{c} = \frac{(t+v+x+z)}{4} )</td>
</tr>
<tr>
<td>( \bar{D} = \frac{(s+t+y+z)}{4} )</td>
<td>( \bar{d} = \frac{(u+v+w+x)}{4} )</td>
</tr>
<tr>
<td>( \bar{E} = \frac{(s+u+x+z)}{4} )</td>
<td>( \bar{e} = \frac{(t+v+w+y)}{4} )</td>
</tr>
<tr>
<td>( \bar{F} = \frac{(s+v+w+z)}{4} )</td>
<td>( \bar{f} = \frac{(t+u+x+y)}{4} )</td>
</tr>
<tr>
<td>( \bar{G} = \frac{(s+v+x+y)}{4} )</td>
<td>( \bar{g} = \frac{(t+u+w+z)}{4} )</td>
</tr>
</tbody>
</table>

The critical difference varies upon the assay; according to the Swiss Food Manual differences < 10 % of the mean value of the analysis results (s-z) have a minor influence [17].

In order to estimate the natural variability between the concentrations of flavour compounds of two identically fermented batches, two batches were fermented under realistic conditions (20 °C, untreated must).

### 7.2.2 Parameters

The turbidity of the different fermentation batches was measured in NTU (Nephelometric Turbidity Units) by comparing the turbidity of the must before inoculation of the yeast with a reference solution of formazine (solution of hexamethylen tetramin and hydrazine sulphate (Fluka, Buchs, Switzerland) using the turbidity photometer LPT 4 (B. Lange, Berlin, Germany). The method was carried out as described in the Swiss Food Manual [17]. The turbidity of the must was adjusted with its own suspending matter deposited on the bottom of the containers. The used variants were NTU of 11 and NTU of 740-765.
Use of an experimental design model to determine the impact of different fermentation parameters on the development of flavour compounds in wine

Two different commercial *Saccharomyces cerevisiae* yeast strains were used in this study: *S. cerevisiae* Zymaflore VL1 (Laffort, Bordeaux, France) and *S. cerevisiae* CEPPO 20 (Littorale Oenologie, Langon, France). The yeast strains were re-hydrated and inoculated following the manufacturer’s instructions.

The fermentations were carried out at 16 °C and 25 °C respectively. In order to keep the temperatures constant, the fermentation took place in incubation stoves.

The total acidity of the must was lowered by addition of CaCO₃ to form insoluble tartaric acid complexes. The addition of 1 g/l of CaCO₃ decreased the total acidity by 1.5 g/l (calculated as tartaric acid). The deacidified must was separated from the insoluble particles by manual decanting. The total acidity of the must was determined by titration with 0.1 M NaOH, and calculated as tartaric acid [17]. The acidity of the batches was adjusted to 3.8 g/l and 4.2 g/l respectively. The acidity was low because of the prior refrigeration of the must, and the consequent precipitation of tartaric acid.

The yeast lees were kept in suspension by stirring the fermentation flasks daily.

The contact of the grape must with oxygen was minimized by flushing the flasks with nitrogen (Pangas, Dagmersellen, Switzerland, quality 4.0) before filling and the headspace after filling. The other variant was not treated specially.

Nitrogen supplementation was made according to the Swiss Food Legislation [18] which allows the addition of 0.3 g/l ammonium sulphate (Fluka).

### 7.2.3 Fermentation

The fermentations were carried out in 2 l Erlenmeyer flasks with specially designed seals filled with glycerine in order to allow gas to escape but no gas to enter. Each batch was prepared, inoculated and incubated at the same time. The fermentation course was followed by measuring the weight loss due to CO₂ production. The sugar content was determined semi-quantitatively using the “Diabur-Test”, which indicates the sugar concentration by coloration of a test strip (Roche Diagnostics, Rotkreuz, Switzerland).

Must of Chasselas grapes was used to study on the development of 3-mercaptohexanol; the must was supplemented with 100.1 µg/l of synthetic flavour precursor (S-3-(hexan-1-ol)-L-cysteine) [19]. For the studies on the development of 2-phenylethanol and 2-phenylethyl acetate, must of Petite Arvine grapes was used. The grape must was sulphated (50 mg/l) and stored at -20 °C until use.
7.2.4 Analysis of 3-mercaptohexanol

3-Mercaptohexanol was extracted and its concentration determined according the method developed and optimized by Tominaga *et al.* [20, 21]. In this method, the thiols are selectively extracted by a complexation with *para*-hydroxymercuric benzoic acid (pHMB). The thiol compounds were analyzed by GC-FPD.

The following chromatographic conditions were used:

- **Gaschromatograph:** HRGC, 5300 Mega Series, (Carlo Erba Instruments, Milano, Italy)
- **Column:** BP 20 column (SGE, Melbourne, Australia; 50 m x 0.22 mm, id 0.25 μm)
- **Temperature program:** 40°C, 1 min; rate 3°C/min; and 230°C, 10 min
- **Injection volume:** 3 μl (spiltless; split opened after 2 min)
- **Injector temperature:** 240°C
- **Detector:** FPD, supplemented with air (70 kPa) and hydrogen (150 kPa); 160°C, bottom temperature 300°C
- **Carrier gas:** Helium (100 kPa).
- **Data acquisition:** ChromCard, Version 1.07 (Thermo Quest, Milano, Italy).

All analyses were carried out in duplicate and the mean value was calculated.

7.2.5 Analysis of 2-phenylethanol and 2-phenylethyl acetate

The fermentation products 2-phenylethanol and 2-phenylethyl acetate were quantified by SPME extraction and GC analysis as described by Rodriguez *et al.* [22], but slightly modified: The wine sample (10 ml) and NaCl (2.9 g, Fluka) were placed in a 20 ml vial (BGB-Analytik, Adliswil, Switzerland). The volatiles present in the headspace of the vial were adsorbed by a PDMS 100 fibre (Supelco, Bellefonte, USA) at a sample temperature of 30 °C for 40 min. The volatiles were analyzed by GC-FID after desorption of the fibre in the splitless injector of the GC at 300°C during 2 min. The GC (Agilent Technologies 6890 N, Palo Alto, USA) equipped with a DB-Wax column (30 m x 0.25 mm x 0.25 μm; J&W Scientific, Folsom, USA) was operated at the following conditions: Initial temperature 40° C, 1 min. The temperature was raised to 230°C at a rate of 5°C/min. Helium was used as carrier gas at a pressure of 1 ml/min. The FID detector (300°C) was supplemented with 40 ml/min hydrogen and 400 ml/min air. Quantification was done by using solutions of 2-phenylethanol (concentration range 5-200 mg/l) and 2-phenylethyl acetate (20-1000 μg/l) as external
standards and a calibration curve (both substances from Fluka). All analyses were carried out in duplicate and the mean values were used for calculation.

### 7.3 Results and discussion

#### 7.3.1 Choice of the vinification parameters

The fermentation parameters were chosen as a function of their potential influence on yeast growth (fermentation course) and formation of aromatic compounds. Extreme variants of each parameter were chosen as proposed by Youden and Steiner [12]. The variants did not need to be realistic in practice, rather they were chosen as extremes in order to better estimate their influence on the investigated value.

All experiments were made in must ready to ferment, so the influence of parameters prior to that stage e.g. ripeness of the berries, maceration and pressing of the grapes could not be taken into consideration.

Insoluble grape material present in grape must is mainly composed of insoluble polysaccharides, unsaturated lipids, metal ions and proteins [23]. Wines that were made from very turbid must have a “heavier” aroma, are green and bitter in taste, also they are richer in phenols and more sensitive to oxidation [16]. Phenols can react with thiols to form odourless complexes [24]. On the other hand, the suspending matter is important for yeast growth, due to nutritional elements and due to a mechanical support of the yeast cells. Turbidity values between 60 and 200 NTU (Nephelometric Turbidity Units) are recommended in order to ensure optimal fermentation course and to avoid aroma alteration [16]. The applied yeast strain can have an influence on wine flavour [25]. A critical review [26], however, doubted about the importance of the yeast strain on wine flavour, since the results of the some studies are contradictory. The yeast strain \( S. \text{cerevisiae} \) Zymaflore VL1 is often used to ferment Petite Arvine grapes and according to the wine producers favours the formation of fruity flavours. \( S. \text{cerevisiae} \) CEPPO 20 is used to ferment Chasselas wines and is reported to form aromatic neutral wines. High temperatures are favourable for the growth of yeast cells [16], but the fermentation temperature also influences the formation of aroma: Temperatures above 20 °C are correlated with low ester and high fusel alcohol production [27], whereas fermentation temperatures below 18 °C are correlated with a high and unwanted ester development. The fermentation of Petite Arvine wine is usually conducted at temperatures of about 20°C according to several producers from the Canton of Valais. Deacidification of the must is usually carried out for sensorial reasons, however yeast and bacterial growth is also dependent
on the acidity of the must. If the lees are in suspension the contact surface of the organisms and the substrate is larger and higher yields in metabolites can be expected. The re-suspension ("batonage") is often applied for fermentation and ripening in oak barrels. Thiols are sensitive to oxidation. Dubourdieu and Lavigne [28] found a significant decrease in the intensity of odours caused by thiols when the must was hyperoxygenated or even in presence of oxygen. The addition of nitrogen in form of ammonium salts promotes yeast growth [16].

### 7.3.2 Fermentation course

All the batches were weighted daily in order to estimate the fermentation course. The total weight loss is listed in Table 4.

<table>
<thead>
<tr>
<th>Batch</th>
<th>Denomination of results</th>
<th>Chasselas (g/batch)</th>
<th>Petite Arvine (g/batch)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>s</td>
<td>111.1</td>
<td>197.5</td>
</tr>
<tr>
<td>2</td>
<td>t</td>
<td>121.7</td>
<td>197.3</td>
</tr>
<tr>
<td>3</td>
<td>u</td>
<td>107.6</td>
<td>203.8</td>
</tr>
<tr>
<td>4</td>
<td>v</td>
<td>119.5</td>
<td>202.5</td>
</tr>
<tr>
<td>5</td>
<td>w</td>
<td>122.7</td>
<td>207.9</td>
</tr>
<tr>
<td>6</td>
<td>x</td>
<td>133.4</td>
<td>210.0</td>
</tr>
<tr>
<td>7</td>
<td>y</td>
<td>121.5</td>
<td>209.5</td>
</tr>
<tr>
<td>8</td>
<td>z</td>
<td>141.4</td>
<td>211.6</td>
</tr>
<tr>
<td>mean value</td>
<td></td>
<td>122.4</td>
<td>205.0</td>
</tr>
<tr>
<td>standard deviation</td>
<td></td>
<td>10.9</td>
<td>5.61</td>
</tr>
<tr>
<td>relative standard deviation</td>
<td></td>
<td>8.94</td>
<td>2.74</td>
</tr>
<tr>
<td>10 % of the mean value</td>
<td></td>
<td>12.2</td>
<td>20.5</td>
</tr>
</tbody>
</table>

The results of the weight loss were also evaluated according to the "Youden procedure". With the results s-z, the absolute differences were calculated as described in the experimental part; the values are listed in Table 5.

Table 5: Absolute differences of the parameters, calculated on weight loss during fermentation.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Petite Arvine</th>
<th>Chasselas</th>
</tr>
</thead>
<tbody>
<tr>
<td>Da Turbidity</td>
<td>9.5</td>
<td>14.8</td>
</tr>
<tr>
<td>Dc Temperature</td>
<td>0.7</td>
<td>13.3</td>
</tr>
<tr>
<td>Dd Desacidification</td>
<td>2.0</td>
<td>3</td>
</tr>
<tr>
<td>Df Oxygen</td>
<td>0.3</td>
<td>2.6</td>
</tr>
<tr>
<td>De Suspension of the lees</td>
<td>1.4</td>
<td>2</td>
</tr>
<tr>
<td>Dg Nitrogen supplementation</td>
<td>0.3</td>
<td>1.9</td>
</tr>
<tr>
<td>Db Yeast strain</td>
<td>3.7</td>
<td>0.3</td>
</tr>
</tbody>
</table>

* number was positive before taking the absolute values; highlighted values exceed 10 % of the mean value
Use of an experimental design model to determine the impact of different fermentation parameters on the development of flavour compounds in wine

The fermentation course of the experiment with Chasselas must was only significantly influenced by the turbidity and the incubation temperature; the other parameters were shown to play a minor role. At high NTUs and at 25°C the sugar was metabolized faster and the weight decreased more rapidly. During fermentation of Petite Arvine, the batches did not differ significantly in weight loss.

Batches with a high turbidity lost more weight than those with low turbidity. Must with high turbidity have an additional supply of nutrients, the solid particles enhance the distribution of the yeast and they help to eliminate CO₂ [29].

The positive effect of higher fermentation temperatures on yeast growth are well known [16], the closer the fermentation temperature to the ideal growth temperature (30 °C), the higher the growth rate.

With regard to the total weight loss of Petite Arvine wine no parameter caused a difference exceeding 10 % of the mean value. This could be explained by the different duration of the fermentation. The must of Petite Arvine was fermented within 7 days. After that period, the sugar content was in the range of 0-2 g/l, except for batches 1 and 3, where 10 g/l were left. Chasselas was subsequently fermented during 12 days, in order to assure the completion of the fermentation of all batches. The reason for the incomplete fermentation of batches 1 and 3 of the Petite Arvine essay could have been the fermentation temperature of 16°C and in addition the variant with low turbidity.

Rapp [30] has shown that the formation of 2-phenylethyl acetate is completed after 4-5 days, e.g. before the completion of alcoholic fermentation. The formation of 2-phenylethyl acetate and phenylethanol are highly correlated [31], and the same behaviour can be expected for the 2-phenylethanol. The incomplete fermentations should therefore not query the study.

The impact of temperature on the fermentation course of Chasselas must is illustrated in Figure 1.
7.3.3 Influence of the fermentation parameters on the concentration of 3-mercaptohexanol

The concentration of 3-mercaptohexanol after 12 days of fermentation of Chasselas must was between 226 ng/l and 430 ng/l (Table 6), the highest and the lowest concentration differing by a factor of about 2. The concentrations of 3-mercaptohexanol are relatively small. Since 100.1 µg/l (approx. purity 80 %) were added, the transformation rate is between 0.37 and 0.71 % which is low comparing to the published data of 2-10 % [9]. The reasons remain to be elucidated.

Table 6: Concentration of 3-mercaptohexanol in the 8 fermentation batches.

<table>
<thead>
<tr>
<th>Batch</th>
<th>Denomination of results</th>
<th>3-mercaptohexanol after fermentation (ng/l)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>s</td>
<td>230</td>
</tr>
<tr>
<td>2</td>
<td>t</td>
<td>311</td>
</tr>
<tr>
<td>3</td>
<td>u</td>
<td>216</td>
</tr>
<tr>
<td>4</td>
<td>v</td>
<td>430</td>
</tr>
<tr>
<td>5</td>
<td>w</td>
<td>226</td>
</tr>
<tr>
<td>6</td>
<td>x</td>
<td>381</td>
</tr>
<tr>
<td>7</td>
<td>y</td>
<td>279</td>
</tr>
<tr>
<td>8</td>
<td>z</td>
<td>354</td>
</tr>
<tr>
<td>mean value</td>
<td></td>
<td>303.5</td>
</tr>
<tr>
<td>standard deviation</td>
<td></td>
<td>79.4</td>
</tr>
<tr>
<td>relative standard deviation</td>
<td></td>
<td>26.2</td>
</tr>
<tr>
<td>10 % of the mean value</td>
<td></td>
<td>30.4</td>
</tr>
</tbody>
</table>

3-Mercaptohexanol was also quantified following the "Youden procedure" (Table 7).
Use of an experimental design model to determine the impact of different fermentation parameters on the development of flavour compounds in wine

Table 7: Absolute differences of the parameters; calculation based on the measured concentration of 3-mercaptohexanol.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Absolute difference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dc Temperature</td>
<td>131</td>
</tr>
<tr>
<td>Dg Nitrogen supplementation</td>
<td>53.5*</td>
</tr>
<tr>
<td>Db Yeast strain</td>
<td>32.5</td>
</tr>
<tr>
<td>Dd Deacidification</td>
<td>19.5</td>
</tr>
<tr>
<td>De Suspension of the lees</td>
<td>16</td>
</tr>
<tr>
<td>Df Oxidation</td>
<td>13.5*</td>
</tr>
<tr>
<td>Da Turbidity</td>
<td>13</td>
</tr>
</tbody>
</table>

*number was positive before calculation the absolute values; highlighted values exceed 10 % of the mean value

Temperature has been shown to be the most important parameter with respect to the formation of 3-mercaptohexanol during fermentation, high fermentation temperatures turned out to be favourable. The batch without nitrogen supplementation and the one using the yeast strain CEPPO 20 affected the formation of 3-mercaptohexanol positively as well, but to a less important degree than fermentation temperature. All the other parameters do not play a role regarding the release of 3-mercaptohexanol. The fact that high fermentation temperature affects the release of the 3-mercaptohexanol positively is quite surprising. Lower fermentation temperatures are generally favourable for the fruity aroma of wines, mainly caused by alcohols and esters [29, 32]. It might also be expected that reactions of the thiol (e.g. oxidations) would be accelerated at higher temperatures. To our knowledge, the characteristics of β-lyase of yeast have not been investigated yet, so no data could be found on temperature optima. It could be that the enzyme activity is increased at higher temperatures to such an extent, that the negative effects are outweighed. Nitrogen supplementation has a negative effect on the final 3-mercaptohexanol concentration. The yeast strain CEPPO 20 is not used in practice for the fermentation of Petite Arvine musts, although our results showed a higher formation rate for 3-mercaptohexanol than with the strain VL1. This should be verified at a real wine making scale.

The relative standard deviation for two identically fermented samples (12.0 %, data not shown) was lower than the relative standard deviation of the 8 batches in this trial (26.2 %). This indicates that the different variants influenced the concentration of 3-mercaptohexanol during the alcoholic fermentation.
7.3.4 Influence of the fermentation parameters on the concentration of 2-phenylethanol and 2-phenylethyl acetate

The concentration of two typical fermentation compounds (2-phenylethanol and 2-phenylethyl acetate) was also determined in each batch in order to verify the method. (Table 8).

Table 8: Concentration of 2-phenylethanol and 2-phenylethyl acetate in the 8 fermentation batches.

<table>
<thead>
<tr>
<th>Batch</th>
<th>Denomination of results</th>
<th>2-Phenylethanol (mg/l)</th>
<th>2-Phenylethyl acetate (µg/l)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>s</td>
<td>12.6</td>
<td>653</td>
</tr>
<tr>
<td>2</td>
<td>t</td>
<td>15.5</td>
<td>646</td>
</tr>
<tr>
<td>3</td>
<td>u</td>
<td>19.7</td>
<td>685</td>
</tr>
<tr>
<td>4</td>
<td>v</td>
<td>18.2</td>
<td>727</td>
</tr>
<tr>
<td>5</td>
<td>w</td>
<td>15.2</td>
<td>624</td>
</tr>
<tr>
<td>6</td>
<td>x</td>
<td>19.8</td>
<td>938</td>
</tr>
<tr>
<td>7</td>
<td>y</td>
<td>16.7</td>
<td>577</td>
</tr>
<tr>
<td>8</td>
<td>z</td>
<td>21.3</td>
<td>823</td>
</tr>
<tr>
<td>mean value</td>
<td></td>
<td>17.4</td>
<td>709</td>
</tr>
<tr>
<td>standard deviation</td>
<td></td>
<td>2.9</td>
<td>118</td>
</tr>
<tr>
<td>variant coefficient</td>
<td></td>
<td>16.7</td>
<td>16.6</td>
</tr>
<tr>
<td>10 % of the mean value</td>
<td></td>
<td>1.74</td>
<td>70.9</td>
</tr>
</tbody>
</table>

The concentrations of 2-phenylethanol and particularly of 2-phenylethyl acetate are high compared to the usual contents in wines. However, it is known that the content of esters and alcohols are highest immediately after fermentation and are decreasing during ripening and storage of wine [33].

The relative standard deviations of two identically fermented samples were 9.2 % for 2-phenylethanol and 5.8 % for 2-phenylethyl acetate respectively (data not shown). This is lower than the relative standard deviation of the 8 samples (16.6, resp. 16.7 %), so it can be concluded that the different variants of the parameters had an influence on the concentrations.

The calculations according to Youden and Steiner [12] are shown in Table 9.

Table 9: Absolute differences of the parameters, calculation based on the concentrations of 2-phenylethanol and 2-phenylethyl acetate after fermentation.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>2-Phenylethanol</th>
<th>2-Phenylethyl acetate</th>
</tr>
</thead>
<tbody>
<tr>
<td>Db Yeast strain</td>
<td>3.2</td>
<td>12.25*</td>
</tr>
<tr>
<td>De Temperature</td>
<td>2.7</td>
<td>148.8</td>
</tr>
<tr>
<td>Da Turbidity</td>
<td>2.0*</td>
<td>131.3*</td>
</tr>
<tr>
<td>Db Deacidification</td>
<td>1.8</td>
<td>62.8</td>
</tr>
<tr>
<td>Df Oxidation</td>
<td>1.7</td>
<td>68.8</td>
</tr>
<tr>
<td>Dg Nitrogen supplementation</td>
<td>1.1</td>
<td>4.8</td>
</tr>
</tbody>
</table>

* number was positive before calculating the absolute values; highlighted values exceed 10 % of the average
The fermentation temperature influences the formation of 2-phenylethanol and 2-phenylethyl acetate, with yeast strain CEPO 20; more 2-phenylethanol was produced and the regular resuspending of the yeast lees favoured the formation of 2-phenylethyl acetate. Wagner and Rapp [34] also found a higher 2-phenylethanol production at higher fermentation temperatures. They also demonstrated that the yeast plays a role for formation of 2-phenylethanol. Turbidity did not influence the formation of these fermentation products; these results agree with those of Kanagiannis and Lanaridis [35], showing no relation between must turbidity and the formation of 2-phenylethyl acetate. In a recent study, Hernandez-Orte et al. [36] report a significant decrease in phenylethanol when ammonium was added to the must; this is in contradiction with our findings.

7.4 Conclusions and Outlook

Using the experimental design procedure for micro-vinification it was clearly demonstrated that the formation of 3-mercaptohexanol is more sensitive to the fermentation parameters than 2-phenylethanol and 2-phenylethyl acetate. High fermentation temperatures are particularly favourable for the release of 3-mercaptohexanol. The results obtained with the vinification trials confirm previous studies, thus proving the correctness of the experimental design. The method proposed by Youden and Steiner [12] is not only a powerful tool for studying the influence of a fermentation parameter on distinct quality aspects, as e.g. the concentration of a flavour compound, but also for the relevance of the parameter.

In the present study the results of the “Youden analysis” do not deliver absolute results but tendencies, since extreme values of parameters were chosen. In practice, the development of one single aroma compound should be put in relation to the influence of other quality aspects, and a compromise has to be found. Besides aroma analysis, other applications of the micro-vinification procedure can be imagined. Using the same experimental design a scale up to the dimensions of a wine cellar should be carried out.

Acknowledgement

The financing of the research project by the Haute Ecole Spécialisée – Suisse Occidentale (HES-SO) is gratefully acknowledged. We also thank the cooperative Provins Valais for providing the grape musts.
7.5 References


Use of an experimental design model to determine the impact of different fermentation parameters on the development of flavour compounds in wine


Use of an experimental design model to determine the impact of different fermentation parameters on the development of flavour compounds in wine


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Chapter 8

STUDIES OF THE TRANSFORMATION OF CYSTEINYLATED FLAVOUR PRECURSORS BY RADIOACTIVE LABELLING

Abstract

The transformation of an S-cysteinylated flavour precursor during alcoholic fermentation of a Petite Arvine must was studied by radioactive labelling. The S-3-(hexan-1-ol)-L-cysteine has been synthesized using radioactive $^{35}$S-cysteine and added to a fermenting must. The fermented must was subsequently separated into different water-soluble and organic solvent-soluble fractions and the radioactivity measured by liquid scintillation. The results showed that most of the $^{35}$S is incorporated in water-soluble compounds, no loss of sulphur containing substances took place by evaporation and only a small loss by the removal of the yeast lees.

8.1 Introduction

Volatile thiols contribute to the characteristic aroma of wine varieties as Sauvignon blanc [1-3], Petite Arvine (see chapter 4 and 5) and other wines [4-7].

S-cysteine-conjugates have been shown to be flavour precursors of the aroma active thiol compounds found in wine [8-12]. S-cysteinylated compounds can also act as precursors of flavour active thiols in different food [13-15]. S-Cysteine-conjugates are intermediate products of the detoxification metabolism in microorganisms, plants and animals [16]. In the must of Sauvignon blanc, also the S-glutathionyl conjugate of 3-mercaptohexanol was found, but it does not seem to play an important role as flavour precursor [17].

In wine making, the transformation from the S-cysteine-conjugate to the corresponding thiol is catalyzed by a β-lyase of the yeast [8, 9]. β-Lyase activity has first been described in the gastrointestinal bacteria Eubacterium limnosum [18] and was also found in Oenococcus oeni,

*Fretz, C., Jaquenod, F., Luisier, J.-L., Amadò, R. (prepared to be submitted)*
the bacteria responsible for the malolactic fermentation often applied in winemaking (see chapter 6).

Studies of the precursors and the transformation products showed a low transformation rate of precursor in grape must and in model media. The rates found were below 0.5 % for model solutions [19] and between 2-10 % in grape must [20]. Interestingly up to 70 % of the initial $S$-cysteine-conjugates "disappeared" and were not measurable after alcoholic fermentation [20]. This behaviour is poorly explored.

There are two main hypotheses: Either the thiol is generated from its precursor and reacts afterwards to derivatives as disulfides, thioesters or thioethers, or the precursor itself is derivatised on its functional groups and therefore no longer detectable in the wine by the applied methods [11, 12].

Thiols are generally unstable, very sensitive to oxidation and can react to many different products [21-23]. Lavigne and Dubourdieu [24] demonstrated the ability of yeast lees to bind free thiols on the mannoproteins of the cell walls. Other studies, however, found a stabilization effect of yeast lees for the thiols, due to their oxygen consumption [25]. Another study suggests the participation of metallic cations associated to the yeast lees on the reduction of thiol concentration in a synthetic medium [26]. The concentration of 3-mercaptohexanol has found to be stable over a period of 20 weeks, in wine elevated on yeast lees ("sur lies"). The concentration of 3-mercaptohexanol of wine stored in bottles during one year decreased only to a small extent (max. 20%), depending on the vintage and producer [27].

The objectives of this study are the elucidation of the transformation of the cysteinylated flavour precursor and the explanations in what form the precursor is present in the fermented must. The work reports on the attempts to explain the "disappearance" of the precursor, to follow up the chemical reactions of the precursor and its corresponding thiol during alcoholic fermentation and to elucidate under what form the reaction products are present. For this purpose, $^{35}S$-labelled synthesized precursor (S-3-(hexan-1-ol)-L-cysteine) was added in a fermenting grape must of Petite Arvine wine and the young wine subsequently fractionated in different aqueous and organic solvent phases, and the radioactivity was determined in each fraction.
8.2 Materials and methods

8.2.1 Synthesis of the $^{35}$S-Precursor

The synthesis was done according to Luisier et al. [28] with some modifications. Ethyl hexenoate was prepared from hexenoic acid and ethanol by azeotropic distillation of water with benzene. Trans-2-hexenoic acid (13.52 g, 0.1182 mol) (Acros, Basel, Switzerland) and 100 ml ethanol were introduced in a three necked round bottom flask of 250 ml equipped with a Dean Stark water trapping apparatus. Then 80 ml benzene and 2 ml concentrated sulfuric acid were added. The solution was heated to boiling during 4 h. The solution was then neutralized to pH 7 with 10 % (w/v) NaHCO$_3$ (Fluka, Buchs, Switzerland), the organic phase separated and the benzene evaporated. The residue was distilled under vacuum. The yield was 13.2 g (79.8 %) of a product with a refractive index of 1.432, which corresponds to literature [29].

$^{35}$S-Cysteine (Perkin-Elmer Life Sciences, Schwerzenbach, Switzerland) was dissolved in a 10 mM dithiothreitol (DTT, Perkin-Elmer Life Sciences) solution at pH 5, and extracted by adding ethyl acetate in order to remove the DTT for prevention of oxidation, as indicated by the manufacturer.

Cysteine-HCl (215.2 mg, 1.37 mM, Fluka, Buchs, Switzerland) and 5 ml water were given into a 25 ml round bottom flask, N$_2$ was bubbled through the solution. By adding 1.8 ml of a 1 M NaOH solution, the pH was adjusted to 7.5. Radioactive cysteine was added to this solution to obtain a radioactivity of about 50 kBq. An excess of ethyl hexenoate (1.2 g, 8.2 mM) was added and the solution was stirred for 3 h at 50°C. The reaction was stopped by adding 10 ml of a solution of sodium dihydrogen phosphate (NaH$_2$PO$_4$) (Fluka), the excess ethyl hexenoate extracted with petrolether (40-60 standard, Fluka) and the reaction solution was extracted three times with tetrahydrofuran (THF) (Fluka). The organic phase was evaporated and dried overnight under vacuum at room temperature. The yield was 223 mg (62 %).

The synthesised 223 mg S-3-(ethyl-hexenoate)-L-cysteine were given in 10 ml 1,2 dimethoxyethane (Fluka) and the solution was heated to reflux, then 1.2 g trimethoxy sodiumborohydride (NaB(OCH$_3$)$_3$H, Sigma-Aldrich, Buchs, Switzerland) suspended in 20 ml of 1,2 dimethoxyethane was slowly added to the solution and the reflux maintained for 2 h. The reaction was stopped by addition of 1 ml water and neutralized to pH 7 with 1M HCl. The precipitate formed was separated by decanting; the resulting clear solution was evaporated up to a few ml. The reaction product was chromatographically purified on a
silicagel column using a solution of isopropanol: ethyl acetate: water in the ratio of 1:1:0.5 as eluent. The main fractions, containing most of the radioactivity were collected and the organic phase was evaporated. The product was separated by thin layer chromatography and the radioactivity of the spots measured by a Geiger counter of type alpha/beta (Berthold LB 124, Berthold technologies, Bad Wildbad, Germany).

8.2.2 Fermentation

A volume of 1 l of grape must of Petite Arvine was fermented with a commercial yeast strain (Zymaflore VL1 (Laffort, Bordeaux, France)). The yeast was re-hydrated and inoculated (0.2 g/l) as recommended by the manufacturer. To assure completion of the fermentation, the sugar content was determined semi-quantitatively by using the “Clini-Test”, giving an indication of the sugar concentration by coloration of a test strip (Bayer, Düsseldorf, Germany). The fermentation was considered as completed when the sugar content was indicated as 0 g/l.

The fermentation was carried out in a 2 l Erlenmeyer flask; the formed gas was purged sequentially through a solution of CuSO₄ (1,25 g/l) and a solution of a BaCl₂ (2 g/l) in order to capture thiols and SO₂ respectively.

Before fermentation, synthesized precursor corresponding to 50kBq radioactivity (about 0.1 mg/l) was added to the must. The fermentation flask was held at room temperature (22.5 +/- 0.5°C) throughout the experiment. The reliability of the method was controlled by comparison of the results obtained in two different fermentations with subsequent separation of the fractions.

8.2.3 Separating scheme

The separation of the different organic- and water-soluble fraction was carried out after completion of the alcoholic fermentation.

Capture of the volatiles: The volatiles formed during the alcoholic fermentation were captured by purging the gas stream through a CuSO₄ and BaCl₂ solution as described above. A volume of 250 ml was retained for the following operations.

Separation of the yeast lees: The yeast lees were separated from the must by centrifugation, at 1200 g for 10 min (ECCO centrifuge, type B 2.5 S, Müller & Krempel, Bülach, Switzerland)

Extraction with organic solvent: The centrifuged must was extracted twice with 50 ml of dichloromethane (LiChrosolv, Merck, Darmstadt, Germany) under stirring during 5 minutes.
The organic phases were centrifuged at 1200 g (ECCO centrifuge) during 10 min to break the emulsion and divided in a separating funnel.

**Extraction of the thiols:** Thiols were extracted from the organic phase twice with 10 ml of a solution of p-hydroxymercuribenzoate (pHMB, 360 mg/l, Fluka) and Trizma base (24.2 g/l, Sigma-Aldrich) under stirring. The same pHMB/Trizma base solution was used for all thiol extraction steps.

**Reduction of the organic solvent-soluble disulfides:** A volume of 10 ml Na-citrate buffer (1 M, pH 8.0, Fluka) and NaBH₄ (100 mg/l, Merck) were added to the organic extract and incubated under stirring during 2 h at room temperature. The pH of the aqueous phase was subsequently adjusted to 3.5 with some drops of 10 % (w/v) HCl. The aqueous phase was separated and the thiols were extracted from the organic phase with 2 x 5 ml of the pHMB/Trizma base solution (see above) under stirring during 5 min.

**Reduction of the water-soluble disulfides:** The pH of the aqueous phase of the extraction with the organic solvent was adjusted to >8 by adding 10 M NaOH. As reductant, NaBH₄ (200 mg) was added and incubated for 2 h at room temperature. The pH was subsequently adjusted to 3.5 with 10 % (w/v) HCl and the volatiles were extracted twice with 25 ml of dichloromethane under stirring during 5 min. The phases were divided in a separating funnel and the organic phases were centrifuged at 1200 g (ECCO centrifuge) for 10 min to break the emulsion. The thiols were extracted from the organic phase twice with 10 ml of pHMB/Trizma solution under stirring during 5 min. The phases were divided in a separating funnel.

**Decomplexation of metal-thiol complexes:** The thiols were liberated by adding an excess of cysteine (7 g) to the aqueous phase after reduction and stirred for 5 min. The free thiols were extracted twice with 25 ml of dichloromethane under stirring during 5 min. The organic phases were centrifuged at 1200 g (ECCO centrifuge) during 10 min to break the emulsion and divided in a separating funnel.

8.2.4 **Radioactive analysis using liquid-scintillation technique**

Radioactivity measurements were performed using a Tri-Carb 2900TR Liquid Scintillation Counter (LSC) from Packard Instrument with Quanta-Smart version 1.1 software (Canberra Packard, Zurich, Switzerland). The spectra were acquired on a 4096 channel MCA (Multi Channel Analyzer, Canberra Packard S.A.).
8.2.4.1 $^{35}\text{S}$ radioactive tracing solution characteristics

The radioactive $^{35}\text{S}$ tracing solution is composed by L-cysteine. $^{35}\text{S}$ disintegrates to the ground state nuclide $^{35}\text{Cl}$ by $\beta^-$ pure emission. The $^{35}\text{S}$ has a half-life time of 87.32(16) days and the maximum energy of the emitted electron is 167.1 keV.

8.2.4.2 Sample preparation and experimental setup

The samples, composed by 1 ml of organic extract, yeast lees, must or aqueous phase were mixed with 15 ml Perkin Elmer Insta-Gel Plus liquid scintillator solution (Perkin-Elmer Life Sciences). The cocktails were sealed in 20 ml glass vials made with low potassium borosilicate concentration (Perkin-Elmer Life Sciences).

The quench level tSIE (transformed Spectral Index External) was measured for each sample, and the background was obtained using the measure of a 16 ml inactive cocktail during 24 h. The detection efficiency was obtained using the CIEMAT-NIST method [30, 31] with the help of a certified $^3\text{H}$ batch sample with different quench levels.

8.2.4.3 Measurements

After synthesis (see chapter 8.1), the precursor was measured to determine its $^{35}\text{S}$ radioactive concentration. Then a small quantity of the radioactive solution (50kBq) was added to 1 litre of Petite Arvine must. At each step of the procedure such as the inoculation in the must, the end of the fermentation process, the separation of the various fractions, etc., 1 ml of solution was added to 15 ml of liquid scintillator solution for radioactive concentration measurements.

The radioactive concentration uncertainty takes into account the statistical A-type component (> 1 %) and the B-type part that is of the order of 4 % for the efficiency calculation and 5 % for the weighting. A-types comprise statistical uncertainties, in this case the measure by liquid scintillation, because the count of a radioactive source is the square root of the average value.

In our case two B-type (measurement errors) uncertainties exist, the uncertainty of the determination of the weight of the fractions, and the uncertainty of the measurement by liquid scintillation.
8.3 Results and discussion

8.3.1 Purity of the synthesised $^{35}$S-Precursor

The purity of the synthesised product was checked by thin layer chromatography and the radioactivity of the spots measured by a Geiger counter of type alpha/beta. The product consisted of approximately 80 % as S-3-(hexan-1-ol)-L-cysteine 1, 8 % of the unreduced ester 2 and 12 % in another more polar product, probably the saponified ester 3 (Figure 1).

The impurity of the synthesised product added to the fermenting must (see 8.2) had to be taken into account for the interpretation of the results. The compounds 1-3 are expected to be degraded by the yeast $\beta$-lyase, since this enzyme has shown to degrade different S-cysteine-conjugates into the corresponding free thiols [11]. The same synthesis has been performed without labelling. Analysis of the final product by GC-MS and LC-MS, confirmed the presence of the three compounds from Figure 1 (results not shown).

8.3.2 Distribution of the radioactivity

The separation of the different organic solvent- and water-soluble fraction was carried out after completion of the alcoholic fermentation (9 days). The radioactivity was measured in the fermented must and in every fraction. The distribution of the radioactivity is shown in Figure 2.

The recovery of the radioactivity was between 93.4 % and 105.3 %. The overall recovery of the initial radioactivity and the radioactivity found in the final fractions for the two experiments was 94.2 % and 92.3 % respectively. These results prove that the fractionating system and the measuring method have an acceptable repeatability.
Figure 2: Distribution of the radioactivity in the different organic and aqueous fraction. The %S percentages correspond to the average value from two separate experiments.
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Volatile (A): Considerably high percentages of aroma compounds, mainly esters, were found to be carried along by fermentative CO$_2$ [32]. However, no sulphur containing volatiles have been captured, so it can be concluded that volatile thiols are not expelled by the generated CO$_2$ during the alcoholic fermentation.

Lees (B): Approximately 5% of the radioactivity was found in the isolated yeast lees. The $^{35}$S-compounds can either be covalently bound to the yeast lees, as proposed by Lavigne and Dubourdieu [24] and Vasserot et al. [26], or they can be present in the must that is unintentionally separated together with the yeast lees. However, this corresponds to practice of wine making, where the lees cannot be separated exactly from the must either. The loss of sulphur compounds due to the removal of the yeast lees is moderate.

Organic extract (C): The compounds extracted by dichloromethane are most probably degradation products of the precursor, mainly 3-mercaptohexanol and its reaction products.

Free thiols (D): The concentration of free thiols (5.4%) was in the range published by Peyrot des Gâchons [20] for 3-mercaptohexanol. There the author found a transformation rate of 2-10%. Using S-3-(hexanol)-L-cysteine as precursor, 3-mercaptohexanol and its derivatives can be expected in the free thiol fraction. 3-mercaptohexanol has been identified in Petite Arvine (see chapter 4 and 5), whereas the acetic ester of 3-mercaptohexanol although being identified in Sauvignon blanc [2], was not detected in Petite Arvine.

Organic phase (E): Sulphur compounds present in this fraction are soluble in organic solvent but do not possess a free thiol group. They can be divided into disulfides (F) and other, non-thiol S-compounds (G).

Disulfides and saponified esters (F): The compounds found in this fraction are free thiols issued from the reduction process by NaBH$_4$ in the organic extract. They were either present as disulfides and have been liberated by the reduction step or they were present as thio-esters, which have been saponified during the strong basic reducing conditions. The amount of thiols oxidized to disulfides is low. This result is surprising taking into account that thiols oxidize easily. The milieu of fermenting grape must seems to prevent oxidation to a large extent.

Non-thiol S-compounds (G): The compounds found in this fraction have to be non-thiol sulphur compounds. They could either be organic extractible oxathianes, thioesters, thioethers, or sulphoxides. The formation of oxathianes by addition of an aldehyde to thiols has been described [33], although the corresponding oxathianes have not been detected in Petite Arvine (data not shown). The formation of sulphoxides during wine making can be almost excluded, since the conditions in the fermenting must are not favourable for oxidation reactions; even the formation of disulfides has shown to be poor.
**Water-soluble compounds generated by the reduction process (H):** The composition of this fraction is not easily explainable; no radioactivity was expected since only the "waste" of the reduction step should be present. These compounds have to become water-soluble by the treatment of the reduction.

**Aqueous phase (I):** The majority of the radioactive labelled sulphur (approx. 64 %) is incorporated in not aroma-active water-soluble compounds. Un-degraded precursor, its derivatives or water-soluble derivatives of the free thiol could be present in this fraction.

**Disulfides (J):** Only a small amount (<1 %) of the radioactivity of the aqueous phase (I) was found as disulfides, or saponifiable thio-esters.

**Metal complexes (K):** The reactions of thiols and metal ions, especially copper, are well known [21]; 3.6 % of the initial radioactive labelled sulphur are present as metal complexes. This shows the importance of avoiding any contamination of must with metal ions.

**Other water-soluble S-compounds (L):** Almost half of the initial radioactivity is found in water-soluble compounds that are neither disulfides nor metal complexes. The compounds present in this fraction could comprise non-hydrolyzed precursor, derivatives thereof or oxidation products such as sulfoxides or strong metal complexes, in which the ligand could not be exchanged by a surplus of cysteine. The presence of cysteine liberated by retro-Michael addition can be excluded since the precursor has shown to be stable in water (results not shown). Reactions between free thiol and wine constituents as chinones and phenols have been described [21, 34], the possible reaction products would also be present in this fraction. Further work should be carried out to identify the substances present in this fraction.

**Organic solvent-soluble compounds from the reduction (M):** Interestingly, the compounds of this fraction were water-soluble before the reduction, however, they are not reduced disulfides, i.e. they are not extractable with a pHMB solution. Since this fraction is quantitatively important (9.2 %), it would be interesting to elucidate its composition. Only few hypotheses about the possible components of this fraction can be developed, for example saponified esters, where the ester but not the S-containing partner is water-soluble, could be present.

Overall the results of the distribution of the radioactively labelled S-compounds in the different fractions reveal that the reaction pathways of the S-cysteinyalted flavour precursor are very complex.
8.4 Complementary experiments

Reduction of the disulfide bonds in both, the water-soluble and the organic solvent-soluble fraction led to results, which were difficult to interpret: The treatment turned water-soluble compounds into compounds soluble in organic solvent and vice-versa. The reduction was therefore studied in more detail. The application of sodium borhydride needs an adjustment of pH since the reaction takes place only at alkaline conditions (pH > 8), whereas a representative extraction of thiols by dichloromethane needs wine-like, sour conditions (pH 3.5). In an additional experiment, samples of fractions E and I (see Figure 2) were divided in two parts, the pH of both parts was adjusted to 8-9. With one part a reduction by NaBH₄ was carried out as usual. To the other part no NaBH₄ was added, but its headspace was filled with argon in order to avoid oxidation. The results for the reduction of the fraction I are shown in Figure 3, the result of the reduction of fraction E were comparable (results not shown).

![Diagram of non-thiol sulfur]

Figure 3: Distribution of the radioactivity after treatment with or without NaBH₄ under the same conditions.

The presence of NaBH₄ did hardly influence the distribution of radioactivity in the fractions. The basic conditions favour the hydrolysis of esters (saponification), but also the hydrolysis of thiol hemi-acetales and the oxidation of thiols. The latter was avoided by applying an argon atmosphere in the flask. Thus on the basis of our data, we can assume that few or no disulfides are present in the must, and that an important part of the sulphur compounds is present as thiol hemi-acetales or as oxathianes.

8.5 Conclusions

The release of a thiol from the corresponding S-cysteinylated precursor during alcoholic fermentation has been described in numerous publications [8, 9, 13]. The reasons for the poor transformation rate have been explored by adding a radioactively labelled precursor to a fermenting must. During alcoholic fermentation, no radioactivity was lost by evaporation. The
yeast lees retained only about 5% of the total radioactivity. Approximately one third of the radioactive sulphur issued from the precursor could be found as organic solvent-soluble compounds, which are most probably free thiols as well as their reaction products. The most abundant part of the radioactivity was found in an water-soluble form that is not elucidated in details. Even if it is assumed that all sulphur found in the aqueous phase is un-hydrolyzed precursor, still 45% of the radioactivity is found as neither thiol no precursor.

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8.6 References


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GENERAL DISCUSSION AND OUTLOOK

The aims of the study were to identify the contributors to the characteristic aroma of Petite Arvine wine, to identify their precursors and to describe the transformation of precursor into flavour active compounds during wine making. Before this study was carried out, the wine of Petite Arvine had not been examined scientifically; only empiric statements of wine makers and wine tasters were available.

Sensory analyses of Petite Arvine wine were carried out in order to determine the most important aroma descriptors for the characteristic aroma of Petite Arvine. An expert panel was asked to describe samples of Petite Arvine wine, and to indicate the typicity of the samples as well as the intensity of the most important descriptors. By sensory evaluation a positive correlation between the typicity of this wine specialty and the intensity of grapefruit, rhubarb and quince aroma notes could be demonstrated. Other often named descriptors for Petite Arvine wine as pineapple and box-tree were shown not to play a role for its typicity. Unfortunately, the panellists were not able to obtain statistically reliable results on flowery descriptors. According to wine experts, these are important for the characterisation of the overall aroma of Petite Arvine wine as well; additional sensory evaluation with a panel especially trained for flowery flavours should be carried out in order to complete the aroma profile of Petite Arvine wine. Although the sensory panel consisted of professional wine makers and wine tasters, the application of wine reference standards was proven to be necessary to generate reliable results. The standards according to Noble et al. [1] were found to be perfectly suited for this purpose. In addition, the results of sensory analysis showed that Petite Arvine wine develops its typicity only about 12 month after vintage because during this time, fermentative esters mask the typical flavours.
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The organic extracts of Petite Arvine wines were analyzed by GC-olfactometry and GC-mass spectrometry. Most of the olfactometrically detected zones could be attributed to an identified flavour compound. The most powerful olfactometric zones were caused by isoamyl acetate, 3-methylbutanol, acetic acid, phenylethyl acetate, 3-mercaptobenzaldehyde, phenylethanol and β-ionone. However, the compounds responsible for three intense olfactometric zones could not be identified in the course of this study. Since “quince” and “wisteria flower” flavour notes correlated positively to the characteristic aroma of Petite Arvine wine, the results of olfactometry of the organic extract of Petite Arvine wine were compared to the olfactometric zones of the organic extracts of quince jelly and wisteria flowers. The comparison between the organic extract of Petite Arvine wine and the vegetal material has indicated common olfactometric zones, of which an unknown compound and β-ionone showed high intensity in quince fruit and violet, respectively. The “quince”-zone is one of the mentioned unidentified zones. In wisteria flowers, three intense zones also found in Petite Arvine wine were perceived, and the corresponding compounds identified to be phenylethanol, phenylethyl acetate and β-ionone. Phenylethanol and phenylethyl acetate were quantified in Petite Arvine and other wines; the concentration did not differ significantly, so these two compounds do not belong to the flavour impact compounds of Petite Arvine wine. Further experiments are necessary to identify the unknown compound and to check the impact of β-ionone and the unknown substance on the aroma of Petite Arvine wine.

Using GC-O and GC-MS it was possible to identify 3-mercaptohexanol as one of the flavour impact compounds of Petite Arvine wine. This thiol has reminiscent of grapefruit and rhubarb flavours. The impact of 3-mercaptobenzaldehyde on the aroma of Petite Arvine wine was clearly demonstrated by a sensory triangle-test and by the determination of its concentration in the wine. The triangle-test confirmed the importance of thiol compounds on the aroma of Petite Arvine wine. The concentration of 3-mercaptobenzaldehyde was measured in 11 Petite Arvine wines; all samples had concentrations that exceeded the threshold value by far. Of the identified compounds, the impact of 3-mercaptobenzaldehyde on the characteristic aroma of Petite Arvine wine is the best investigated and confirmed. However, the results indicate that this substance is not the only contributor to the characteristic aroma; other non-thiols play an important role for the complex aroma. This statement is supported by the fact that adding copper sulphate to Petite Arvine wine, a fruity aroma is retained although the thiols are aroma-inactivated by complexation. Investigation should therefore be pushed further to
identify other aroma constituents, their precursors and the way of liberation during wine making.

The aroma neutral precursor of 3-mercaptohexanol is a S-cysteine conjugate that is transformed during the alcoholic fermentation by a ß-lyase of the yeast. The release of a free thiol from an S-cysteine conjugate has been described in numerous studies for a number of different wines, but nevertheless some aspects have not been elucidated up to now.

In our study, we showed that lactic acid bacteria (*Oenococcus oeni*) responsible for the malolactic fermentation are able to liberate the free thiol as well, when the bacteria are cultivated under ideal growth conditions. The transformation rate, however, is low, so that the contribution to the amount of 3-mercaptohexanol in wine probably plays a minor role in practice. The impact of *Oenococcus oeni* and the general application of the malolactic fermentation on the content of 3-mercaptohexanol and other flavour compounds should be verified under real wine making conditions. The cysteinylated precursor has been shown to resist transformation to the free thiol without enzymatic action. After the storage of the aroma precursor during nearly 10 weeks in an aqueous solution at wine similar conditions, no transformation into 3-mercaptohexanol could be observed.

The impact of different fermentation parameters on the release of 3-mercaptohexanol was tested by micro-fermentations, set up in an experimental model according to Youden [2]. To our knowledge, the experimental design used in this study was applied for the first time on micro-vinification. It turned out to be an effective tool to study the impact of different vinification parameters on wine quality as e.g. the aroma. Not only several parameters can be studied at the same time in a restricted number of experiments and analyses, but also the relevance of each parameter could be determined. Thus it could be demonstrated that the release of 3-mercaptohexanol from the precursor S-3-(hexan-1-ol)-L-cysteine is positively influenced by higher fermentation temperatures. Other fermentation parameters as nitrogen supplementation, absence of oxygen or the applied yeast strain do not influence the concentration of the liberated 3-mercaptohexanol. The results should be verified by fermentation experiments in larger scales and under real wine making conditions. The same experimental set-up could be applied to study other parameters that could influence quality aspects of wines, e.g. the aroma composition of Petite Arvine wine.

The transformation rate of the precursor into the free thiols is low, only a few percent of the precursor can be found as free thiol after alcoholic fermentation. However, from the literature
it is known that most of the precursors “disappear” during the wine making. Experiments with synthetic $^{35}$S-labelled precursor were carried out to elucidate the reasons for the disappearance of the precursor by studying the distribution of the radioactivity in different wine fractions. The labelled synthesized precursor was therefore added to a fermenting must. The gases formed during alcoholic fermentation were trapped and collected. After completion of the fermentation, the young wine was separated into different fractions to elucidate the chemical properties of the transformation products obtained from the S-cysteine conjugate. Our trials showed that no radioactivity was lost by evaporation during the alcoholic fermentation. The yeast lees retained less than 5 % of the total radioactivity. Approximately one third of the radioactive sulphur issued from the precursor could be found as organic solvent soluble compounds, most probably free thiols and their reaction products. The most abundant part of the radioactivity was found in the water-soluble fraction. The composition of this fraction remains to be studied. When the reaction products are identified, strategies to favour the transformation into flavour active free thiols could be developed. The studies on the degradation pathways during alcoholic fermentation clearly showed that the behaviour of cysteinyalted precursors is very complex.

In conclusion, the present study could define important descriptors for the characteristic aroma of Petite Arvine. One of its flavour impact compounds (3-mercaptohexanol) has been identified and quantified in several samples of Petite Arvine wine. Some new aspects of the release of 3-mercaptohexanol from the S-cysteinyalted precursor have been studied. The present work determined the ability of lactic acid bacteria (Oenococcus oeni) to release 3-mercaptohexanol from its precursor during the malolactic fermentation step. The impact on the wine’s aroma, however, seems to be minor. The pathways of the precursor transformation were partly elucidated by tracking the precursor by means of radioactive labelling. The experimental design model according to Youden was adapted to study the influence of fermentation parameters on the formation of aroma compounds. This method seems to produce reliable results and could be a powerful tool to determine the influence of technological parameters on quality aspects of wine. The present investigation on the typicity of Petite Arvine wine, particularly with respect to its characteristic flavour has led to several very interesting results. However, many items could not be addressed, leaving a whole bunch of important questions and problems to be solved in future research work.
The focus of this investigation was the description of the development of the characteristic aroma of Petite Arvine wine during wine making. Future studies should take into consideration the influence of different agronomic conditions on the aroma composition of the wine as well. The influence of the “terroir” (soil, climate, exposition etc.), of genetic aspects of the plants and of the treatment of the vines during maturation should be studied. A particular emphasis should be put on the influence of the clone on the concentration of flavour precursors. For this purpose, a reliable analytical method to determine the precursors has to be established.

Several other parameters could be studied as well in order to optimise the development of the characteristic aroma of the Petite Arvine wine:

- Cysteine conjugates are known to represent intermediate products of the detoxification metabolism. The sanitary state of the vines and the quality of the crop should therefore be put into relationship with the content of 3-mercaptohexanol and its precursor.
- 3-Mercaptohexanol showed very different stabilities in different media. Interactions with compounds of the media and the possible reaction products should be elucidated with the purpose to improve the stability of 3-mercaptohexanol in model solutions to simplify studies and interpretation.
- The must of Petite Arvine shows a very fast browning compared to the must of other white wine varieties. The reasons for this observation should be elucidated and put in relation to the aroma composition of the wine.
- Petite Arvine wine has a characteristic salty after taste. The reasons for this phenomenon are unknown; maybe salty peptides play a role. Investigations on the peptide composition of Petite Arvine wine could possibly explain the presence of the salty taste.

References:


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