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Characteristics of the sur lie vinification method applied to Grüner Veltliner

Submitted by
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Abstract

During sur lie ageing of wine, several changes take place thereby affecting both the sensory and the chemical characteristics of wines. The present study evaluates the effect of this vinification method on the quality of Grüner Veltliner. For this purpose, the ageing procedure was monitored during four vintage periods and with regard to the inclusion of different yeast strains used for fermentation as well as to structural variations in lees (fine lees and crude lees). In addition, the use of exogenous enzyme preparations, different durations of the ageing procedure, the addition of SO_2 and temperature variations were considered in the trials undertaken. In order to assess the effect of the various treatments, general wine composition, concentrations of amino acids and higher alcohols, biogenic amines and the identification of of lactic acid bacteria involved in spontaneous malolactic fermentation were monitored. Furthermore, sensory analyses of the wines were carried out.

Concerning the augmentation of amino acids it turned out that qualitative and quantitative differences are induced by individual yeast strains, ageing time and malolactic fermentation. Ageing on lees also slightly influenced the levels of higher alcohols, methanol and ethyl acetate. Although biogenic amines were found at low concentrations in Grüner Veltliner wines, putrescine levels increased in sur lie wines with spontaneous malolactic fermentation and stored under certain conditions.

The most important sensory changes observed during lees contact were related to an increase in body, harmony and complexity and a gain in autolytic character. Concerning the sur lie vinification without SO_2 addition a loss of intensity regarding the attributes fruity and fresh was noticed. Structural variations of the lees did not exert any effect on the sensory attributes. “Spicy and peppery”, which are well-known as being the most typical sensory attributes of Grüner Veltliner could be enhanced under certain conditions during ageing on lees. In conclusion, the elaborated results indicate that the procedure of sur lie ageing can be regarded as an appropriate tool for manufacturing Grüner Veltliner wines of a new style.

Zusammenfassung

Während der Lagerung auf der Hefe (frz. „Sur lie“) finden eine Reihe von Veränderungen im Wein statt, die sowohl die sensorischen als auch die chemischen Merkmale betreffen. Die vorliegende Studie untersucht die Auswirkung dieser Art von Vinifizierung hinsichtlich der Qualität des Grünen Veltliners. Dafür wurde der Reifeprozeß während vier Jahrgänge beobachtet. Berücksichtigt wurden verschiedene Hefestämme für die Gärung, sowie auch unterschiedliche Hefequalitäten (feine und grobe Hefe). Zusätzlich wurden bei den Versuchen exogene Enzymepräparate, verschiedene Zeitperioden, der Zusatz von SO_2 und unterschiedliche Temperaturlevels berücksichtigt. Um die Auswirkung der unterschiedlichen Behandlungen besser beurteilen zu können, wurden die allgemeine Weinzusammensetzung, die Konzentration von Aminosäuren und Höheren Alkoholen, biogene Amine und die Identifikation von Milchsäuerebakterien, die für den spontanen biologischen Säureabbau (BSA) zuständig sind, beobachtet. Außerdem wurden auch sensorische Untersuchungen unternommen.

Bezüglich der Vermehrung von Aminosäuren konnte festgestellt werden, dass qualitative und quantitative Unterschiede durch die jeweiligen Hefestämme, die Reifezeitperioden und den biologischen Säureabbau verursacht wurden. Die Hefelagerung hat ebenfalls den Anteil der Höheren Alkohole, Methanol und Ethylacetat leicht beeinflusst. Obwohl kleine Mengen biogener Amine in den Grüner Veltliner-Weinen gefunden wurden, ist der Anteil von Putrescine in „Sur lie“-Weinen mit spontanem BSA und einer Lagerung unter bestimmten Bedingungen gestiegen.

Die wichtigsten sensorischen Veränderungen, die während der Hefelagerung beobachtet wurden, war die Intensivierung der Attribute „Körper“, „Harmonie“ und „Komplexität“, sowie „autolytische Noten“. Bei der „Sur lie“-Lagerung ohne SO_2-Zusatz wurde ein Verlust der Eigenschaften „fruchtig“ und „frisch“ festgestellt. Die Hefequalität hat keinen negativen Einfluss auf die sensorischen Attribute. "Würzig und pfefferig" - die typischen sensorischen Eigenschaften des Grüner Veltliner-Weines, können mittels der Hefelagerung intensiviert werden. Zusammengefasst zeigen die Ergebnisse, dass sich die „Sur lie“-Methode als ein geeignetes Instrument für die Herstellung von Grüner Veltliner-Weinen mit einer neuen Stilisitk erweist.

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List of abbreviations

AABA	Alpha-amino butyric acid
ANOVA	Analysis of variance
ATP	Adenosine triphosphate
BLAST	Basic Local Alignment Search Tool
CO_2	Carbon dioxide
CTP	Cytidine triphosphate
DNA	Deoxyribonucleic acid
dNTP	Deoxyribonucleotide triphosphate
EDTA	Ethylenediaminetetraacetic acid
FTIR	Fourier transform infrared spectroscopy
LAB	Lactic acid bacteria
MLF	Malolactic fermentation
ns	not significant
NTU	Nephelometric Turbidity Units
PCA	Principal component analysis
PCR	Polymerase chain reaction
rpm	Rotations per minute
SO_2	Sulphur dioxide
TA	Tartaric acid
TAE	Tris-acetate-EDTA-buffer
UPLC	Ultra performance liquid chromatography

1. Introduction

1.1 The sur lie vinification method

Maturing wines in the presence of lees under controlled conditions is a method used in major wine producing countries (France, the United States, Australia, and South Africa). This procedure is carried out in 228 l oak barrels, or in vats and imparts the wine with new compositional and sensorial features, leading to a product of a new style as a consequence of complex changes associated with the autolysis of yeast cells. The most famous grape varieties aged in the presence of lees are Muscadet and Chardonnay from the Loire Valley and Burgundy, France respectively. Sur lie vinification is also used in the maturation of other grape varieties to a greater or lesser degree.

Grüner Veltliner is the most important grape variety in Austria. The soil, the climate and its particular varietal character are factors that give Austria identity in the wine world. About 20,000 ha of Grüner Veltliner vines are planted all over the world, of which 67% (13,520 ha) are planted in Austria (Statistik Austria 2009). Grüner Veltliner is a fascinating grape variety. It has many faces; from green, peppery and spicy to yellow, fruity and floral. It is also amazing how many different styles of wine can be produced from this variety, from dry sparkling to very sweet Eiswein.

Until now there has been no in-depth study or trial undertaken to establish the changes of Grüner Veltliner wines during ageing on lees, the factors which positively influence this process, and the ability of the variety to produce agreeable, competitive products of a new style. Applying the sur lie process on Grüner Veltliner will make the flavor spectrum even more spectacular.

1.1.1 Autolysis

The main process taking place during the ageing on lees is autolysis. The autolysis takes place at the end of the stationary phase of cell growth and is usually associated with cell death (Babayan and Berzukov, 1985). Several authors have defined the process of autolysis accordingly: the biological degradation of the yeast cell (Charpentier, 2010); the hydrolysis of intracellular biopolymers by yeast enzymes activated after cell death characterized the yeast autolysis. Hydrolytic enzymes release cytoplasmic peptides, fatty acids, nucleotides, amino acids and cell wall compounds (mannoproteins) into the wine (Alexandre & Guilloux-Benatier, 2006). Autolysis can be described as a loss of dry matter, a decrease in the percentage of protein and nucleic acids in this dry matter, as a result of intracellular proteolytic activity (Leroy, et al. 1990). When sugars and nutrients are consumed, yeast cells turn to their own internal energy reserves. When these are consumed, cells degeneration begins and the autolysis starts (Torresi, et al., 2011).

Proteolysis and degradation of the cell wall comprise the main parts of autolysis.

1.1.1.1 Proteolysis

The yeast cell possesses many enzymes. From the enzymes involved in the autolysis process, protease A is the one most involved in the breakdown of yeast cell components (Lurton, et al., 1989). Alexander (2001) reported late extracellular protease A activity which

suggests that protease A is not easily released and supported the idea that protease A activity is responsible for peptide degradation, yet there is no correlation between protease A activity, cell death and autolysis.

Charpentier (2010) described the five stages of proteolysis:

1. Liberation of hydrolytic enzymes in the cytoplasm by disorganization of the membranous system of the cell.
2. The proteolytic activation of these enzymes.
3. Enzymatic degradation of intracellular macromolecules
4. The porosity of the cell wall increases and the products of autolysis are liberated into the wine
5. Breaking down of substances released from the cell by yeast enzymes

1.1.1.2 Degradation of the cell wall

The cell wall is a flexible structure with different properties. The outer layer is composed of heavily glycosylated mannoproteins. The inner layer which gives the wall mechanical strength is composed of β1,3-glucan and chitin and represents about 50-60% of the wall dry weight. The β1,3-glucan chains are probably responsible for the elasticity of the wall (Klis, et al., 2002). Charpentier and Freyssinet (1989) summarized the steps of the cell wall degradation:

1. The glucans are hydrolysed by glucanases resulting the liberation of mannoproteins linked to the glucans;
2. The glucans are released due to the glucanase activity;
3. The mannoproteins are broken down by proteases enzymes of the yeast;

1.1.1.3 Autophagy

Autophagy is a process that takes place after the yeast autolysis. Autophagy is an exquisitely organized and regulated process. Autophagy occurs during starvation and it is a catabolic mechanism involving the trafficking of membranes and intracellular components (Carrascosa et al. 2011). Cebollero and Gonzalez (2006) provided the first demonstration of autophagy in industrial yeasts under enological conditions and proposed two ways in which the autophagy could be a potential target for genetically improving autolytic properties. First, the degradation of macromolecules would be expected to be greater if the yeast cells possess high rates of autophagy leading to accelerated cell death and autolysis. Second, cells defective in autophagy are known to die faster in the stationary phase, it can be anticipated that cells defective in autophagy would show accelerated autolysis.

A number of studies have been carried out on yeast autolysis in the past 20 years, but some aspects remain unknown, such as the molecular mechanism for the induction of yeast autolysis during wine ageing, the kinetics of glucanase activity and mechanism for the release of nucleotides, nucleosides and lipids (Torresi, et al., 2011).

At the end of alcoholic fermentation, if the wine remains in contact with the lees, the process of autolysis starts or is stimulated.

1.1.1.4 Products of autolysis

During autolysis the following substances are released in the extracellular medium: proteins, peptides, amino acids (Martinez-Rodriguez and Polo, 2000; Arizumi, et al., 1994; Sato, et al., 1997; Feuillat and Charpentier, 1982; Herraiz, et al., 1993; Babayan and Bezrukov, 1985; Alcaide-Hidalgo, et al. 2007), lipids (Ferrari, et al. 1987; Pueyo, et al. 2000), glucans, mannoproteins (Doco, et at., 2003; Rosi, et al. 1999; Feuillat, M., 2002) and nucleotides.

Free amino acids are the most utilized tool for monitoring the process of autolysis. Amino acids are involved in different reactions, such as the formation of higher alcohols, esters and ketonic acids. Therefore the concentration of amino acids can influence the sensory properties of wine. During wine ageing amino acids can form flavour compounds by reacting with carbonyl compounds (diacetyl, glyoxal) or with hydroxy ketones (acetoine and acetol) even at low temperatures and low pH values (Pripis-Nicolau, et al., 2000). The amount and the type of free amino acids release from naturally occurring proteins during fermentation can significantly improve the taste of food products in naturally occurring or intentionally added flavor potentiators (Wong, et al., 2008). Amino acids are necessary for biosynthesis of enzymes structural proteins. Essential amino acids cannot be synthesized by the human body therefore they must be provided with the diet (Arrieta & Prats-Moya, 2012). Wines matured on lees can be a source of such amino acids.

1.1.2 Advantages and disadvantages of ageing on lees

1.1.2.1 Advantages

The main changes produced during the maturation on lees are related to the autolysis of yeast cells.

- The wine gains in sensorial complexity, body, roundness and creaminess.
- Mannoproteins extracted from yeast cells improve the tartrate stability of wines (Moine-Ledoux and Dubourdieu, 2002; Lubbers, et al., 1993). The mannoproteins extracted by heat in alkaline buffers are different from those accompanying the enzymatic release during sur lie process. In model medium, the effect of mannoproteins extracted by physical processes in improving tartrate stability was not been established (Ribereau-Gayon, et al. 2006).
- Mannoproteins also improve protein stability in white wine by lowering the size of the haze particles (Waters, et al. 1993; Dupin, et al. 2000). The polysaccharide responsible for protein stability is a high mass mannoprotein with a molecular weight of 420 kDa.
- Mannoproteins from yeast lees are able to interact with phenolic compounds of wines (Feuillat, 2000).
- Lees are capable of absorbing different undesirable substances from wine. The level of ochratoxin A (OTA), a carcinogenic mycotoxin, is greatly reduced by the contact with the lees (Garcia-Moruno, et al. 2005; Caridi, et al. 2006).

- If the ageing on lees is carried out in wood barrels, the lees can bind different volatile compounds from oak, reducing the oak taste therefore diminish the impact of the aromatic substances from oak wood on wine aroma. Eugenol, 4-propylguaicol, 4-methylguaicol, furfural and 5-methylfurfural presented the highest affinity for the lees (Moreno and Azpilicueta, 2007).

- Ageing wine on lees is a technique employed to protect the wine against oxidation. This is really important when the maturing process is conducted without sulphur addition. Lipids of yeast lees react with dissolved oxygen. They undergo a mild oxidation process. Lipid peroxides and unknown end-products are compounds produced by oxidation (Salmon, et al. 2000).

- Nucleotides could influence the flavor of wines. There is a synergism between the different nucleotides and the presence of glutamic acid (Charpentier, et al. 2005). Nucleic acids (ADN and ARN) are known as flavour enhancers.

- Esters of fatty acids liberated with the cell content of dead yeasts display sweet and spicy aromas (ethyl hexanoate, ethyl octanoate).

1.1.2.2 Disadvantages

- Ageing on lees may induce off-flavours, such as hydrogen sulphide. The yeast lees possess the ability to reduce sulphur dioxide to hydrogen sulphide (H_2S). Sulphide overproduction can be a problem caused by the technique of prolonged contact of new wines with their lees deposit (Karagiannis and Lanaridis, 1999). In vats more hydrogen sulphide can be produced, as a result of reducing power of lees. In barrels the oxygen diffuses through the wood therefore the reducing power is counterbalanced (Charpentier, 2010).

- During the ageing on lees unwanted MLF can occur. The development of lactic bacteria is enhanced by the nutrients released by the yeasts into the wine. LAB can be involved in a great number of possible alterations of wine composition which go from slight flaws to heavy faults and alterations. Additionally, LAB can produce biogenic amines during malolactic fermentation.

- Biogenic amines are hazardous for human health; most countries have maximum limits for histamine concentration in wine: 6 mg/L Belgium, 10 mg/L in Switzerland and Austria, 2 mg/L in Germany, 8 mg/L in France and 4 mg/L in Holland (Busto, et al. 1996). In consequence the malolactic fermentation should be carried out in the proper method.

- The concentration of histamine and tyramine was found to be influenced by the weekly stirring. The concentration of those amines was higher in stirred wine at the end of the ageing process (Gonzalez, A.M and Azpilicueta, C. A., 2006). Some enological practices like storage on lees and skin maceration strongly influenced biogenic amine content. Factors like wine pH, characteristics of the vintage can also influence the biogenic amine concentration (Martin-Alvarez, et al. 2006).

- The ageing on lees influences the presence of biogenic amines not only as a source of amino acids which can be decarboxylated, but also as a microorganism reservoir (Perez-Serradilla and Luque de Castro, 2008).

2. Outline & Objectives of Thesis

2.1 Outline

Regarding this study ageing on lees of Grüne Veltliner wines was done during four vintages (2006 – 2009).

When it comes to ageing on lees the winemaker has to take many factors into consideration: lees quality, lees quantity, pH value, temperature and maybe the most important one is SO_2. During the first three years, ageing on lees was performed without SO_2 and malolactic fermentation started spontaneously. Due to this fact, the contact with lees was performed with and without SO_2 addition in 2009. The wine samples were maturated in the presence of lees for 9 months.

If no starter culture is used then LAB can originate from the vineyard, grapes or from the cellar equipment (Fleet, 1993). Therefore, it was interesting to find out which species was implicated.

During the four vintages, by producing Grüne Veltlinersur lie wines several factors were taken into account and different aspects were studied:

- The yeast during fermentation and sur lie ageing; fine lees and crude lees
- Exogenous enzymes
- Ageing time
- SO_2 addition
- Temperature

Several analysis were carried out at different moments of sur lie maturation:

- General composition
- Content of amino acids
- Content of higher alcohols
- Biogenic amines
- Identification of LAB involved in malolactic fermentation
- Sensorial analysis

Technology of Grüne Veltlinersur lie wines: Pressing (with or without destemming), racking, alcoholic fermentation, maturing on the total quantity of lees or racking and maturing in contact with fine lees, stabilization, filtration and botteling.

2.2 Objectives

This study is based on the hypothesis that “sur lie” ageing on lees can impart new characteristics and can improve the ageing potential of Grüner Veltliner wines. Therefore, the main objective of this study was to find out if the sur lie process is, in principle, suitable for the production of Grüner Veltliner wines and to analyse the impact of defined technological practices on the Grüner Veltliner wines matured on lees. In this context, the following objectives were defined:

- To examine the influence of ageing on lees on the chemical composition of Grüner Veltliner wines, as reflected by amino acids, higher alcohols and biogenic amines;

- To examine the influence of technological conditions (temperature, SO_2, lees quantity and quality) on the levels of free amino acids and on the sensory attributes of Grüner Veltliner wines;

- To monitor the sensory changes of Grüner Veltliner wines aged on lees compared with the usual method involving early racking;

- To point out possible effects of malolactic fermentation on wine quality;

3. Materials and methods

3.1 Wine samples and vinification methods

3.1.1 Vintage 2006

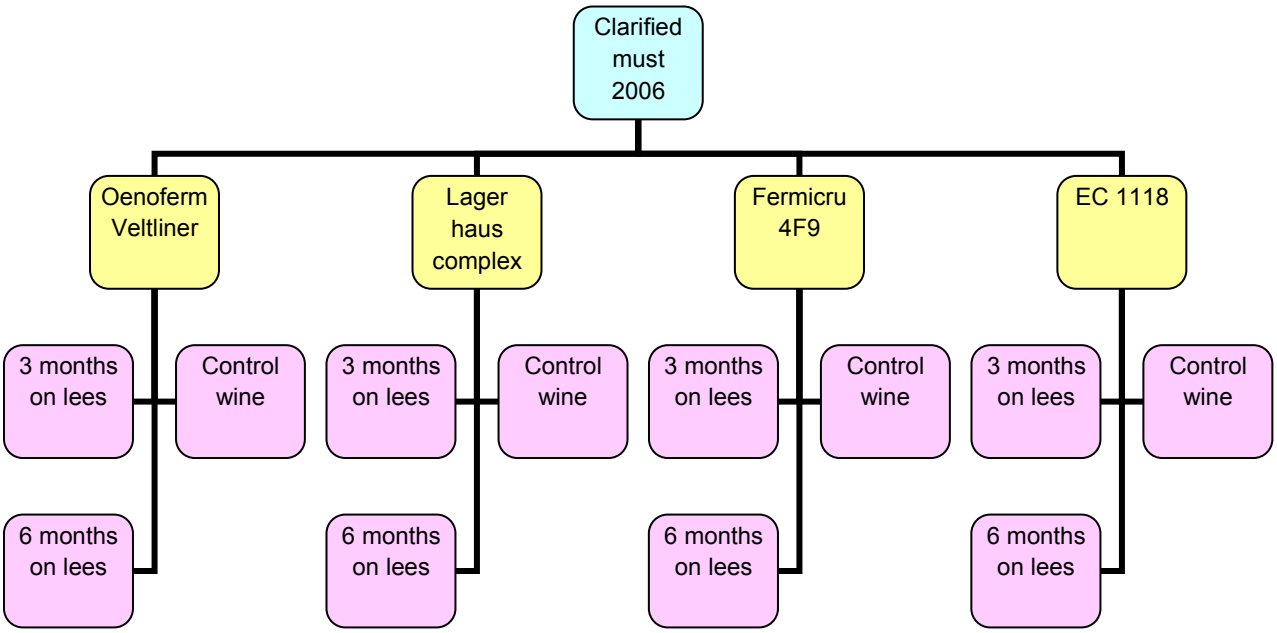

Figure 1: Wine samples and vinification methods, Vintage 2006

Factors taken into account: yeast strain, duration of lees contact

Figure 1 gives an overview of the performed trials. The clarified must of the Grüner Veltliner (2006 vintage) was inoculated with four different yeasts: *Saccharomyces cerevisiae* Oenoferm Veltliner (Erbslòh, Geisenheim, Germany) the most popular yeast for Grüner Veltliner,*Saccharomyces cerevisiae* Lagerhaus complex (Preziso, Austria) a new yeast on the market, Fermicru 4F9 (DSM, Ma delf, Netherlands) is a special yeast for autolysis, and *Saccharomyces bayanus* EC 1118 (Lallemand, Madrid, Spain) a highly vigorous yeast which is usually used in champagne production. The fermentations were conducted in 100 litre tanks. After the fermentation the wine was divided into 40 l glass carboys with a controlled amount of lees. The wines were stored on lees at 15°C for 3 and 6 months with periodic stirring. The control wine was racked, filtered, sulphited and also stored in 40 l glass carboys. After the desired yeast contact time wines were prepared for analysis: they were racked, sulphited, filtered and bottled. All wines were replicated two times.

3.1.2 Vintage 2007

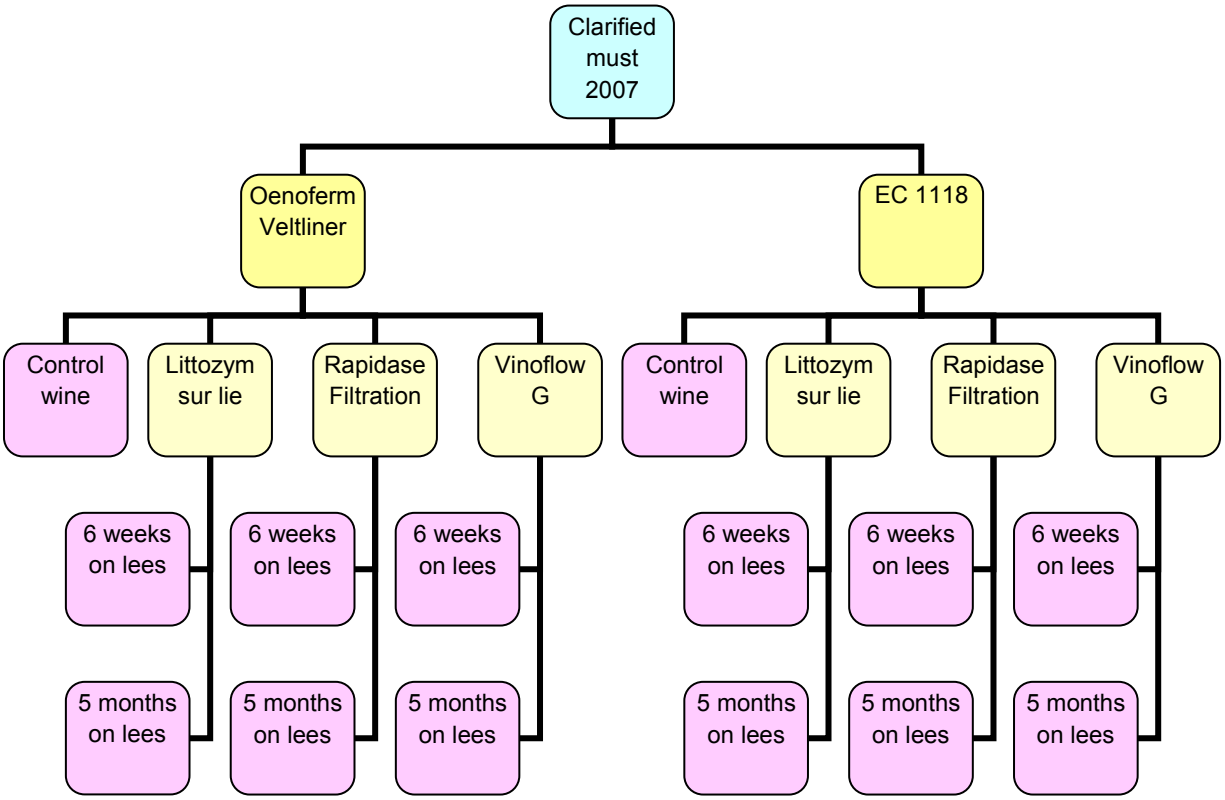

Figure 2: Wine samples and vinification methods, Vintage 2007

Factors taken into account: yeast strain, exogenic enzymes, duration of lees contact

Figure 2 gives an overview of the performed trials. The clarified must of the Grüner Veltliner (2007 vintage) was inoculated with two different yeasts: *Saccharomyces cerevisiae* Oenoferm Veltliner (Erbslòh, Geisenheim, Germany) and *Saccharomyces bayanus* EC 1118 (Lallemand, Madrid, Spain). The fermentations were conducted in 1000 l tanks. After the fermentation the wine was divided in 34 l carboys with controlled amount of lees. Three enzyme preparation were applied: Littozym sur lie (La LITTORALE, Béziers, France), Rapidase Filtration (DSM Food Specialties, Ma delf, The Netherlands) and Vinoflow G (Novozymes, Bagsvaerd, Denmark). The wines were stored on lees for 6 weeks and 5 months with periodic stirring. The control wine was racked, filtered, sulphited and also stored in 34 l glass carboys. After the desired yeast contact time wines were prepared for analysis: they were racked, sulphited, filtered and bottled. All wines were replicated two times.

3.1.3 Vintage 2008

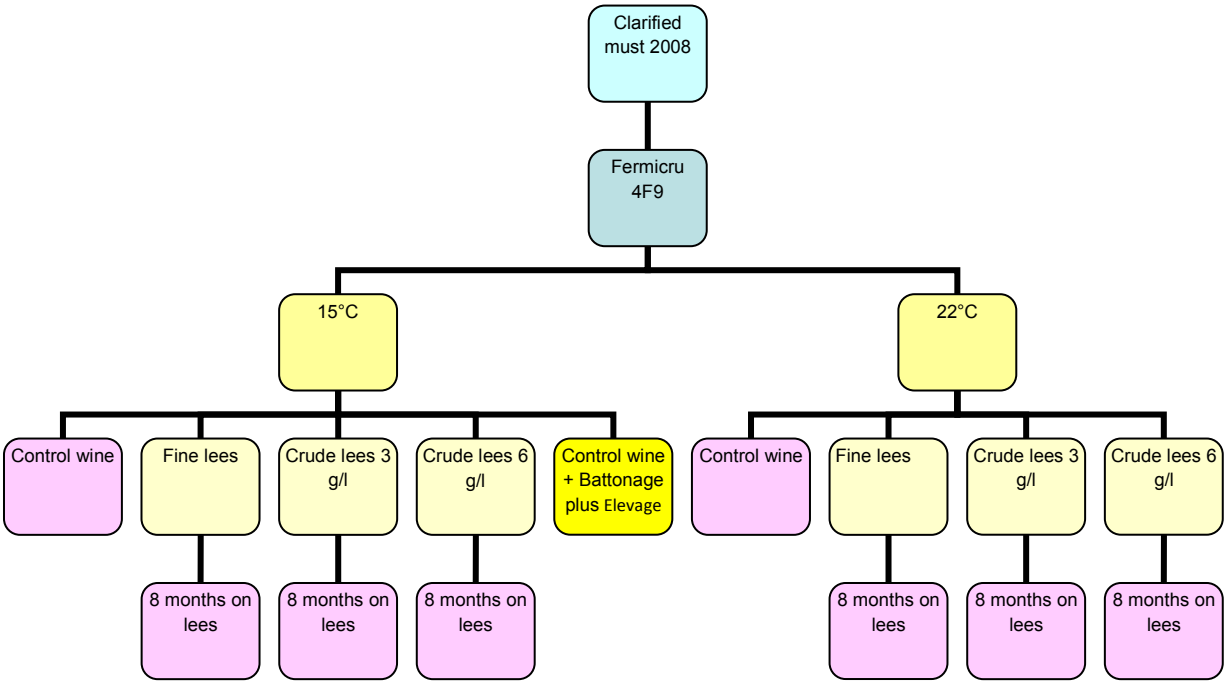

Figure 3: Wine samples and vinification methods, Vintage 2008

Factors taken into account: yeast lees quality and quanitity, temperature / malolactic fermentation

Figure 3 gives an overview of the performed trials. The clarified must of the Grüner Veltliner (2008 vintage) was inoculated with the yeast *Sacharomyces cerevisiae* Fermicru 4F9 (DSM, Ma delf, Netherlands). The fermentation was conducted into a 1000 l tank. After the fermentation the wine was divided in 34 l carboys with controlled amount of lees and 3 different treatments were setup: fine lees, crude lees 3g/l and crude lees 6g/l. The wines were stored on lees for 8 months with periodic stirring. The wines were stored during sur lie ageing at two different temperature levels: 15°C and 22°C. The control wine was racked, filtered, sulphited and also stored in 34 l glass carboys. At 15°C one more treatment was set up. Into a carboy with control wine a refining agent was added, Batonnage plus Elevage (AEB, Brescia, Italy). According to the supplier Batonnage plus Elevage is made out of yeast cells and the wines treated are more full-bodied and harmonious. This product is ment to replace long lasting sur lie ageing. After the desired yeast contact time wines were prepared for analysis: they were racked, sulphited, filtered and bottled. All wines were replicated two times.

3.1.4 Vintage 2009

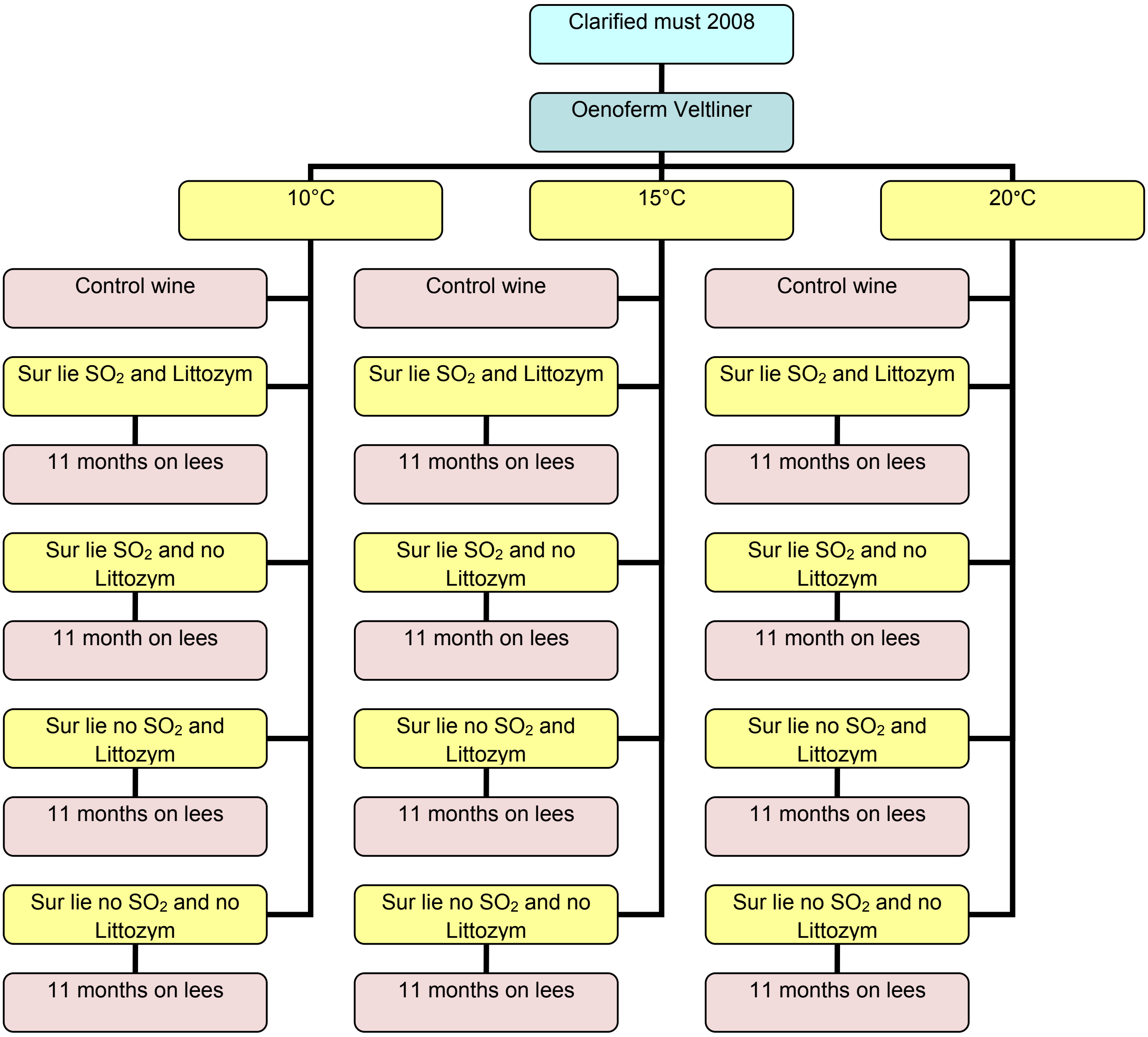

Figure 4: Wine samples and vinification methods, Vintage 2009

Factors taken into account: lees contact, temperature, S02 / malolactic fermentation, exogenic enzymes

Figure 4 gives an overview of the performed trials. The clarified must of the Grüner Veltliner (2009 vintage) was inoculated with the yeast: *Saccharomyces cerevisiae* Oenoferm Veltliner (Erbslòh, Geisenheim, Germany). The enzyme preparation Littozym sur lie (La LITTORALE, Béziers, France) was applied; dosage 8 g/hl. 4 g/hl of enzyme were added at the beginning of lees contact and 4 g/hl were added after 5 months of lees contact. The fermentation was conducted into a 1000 l tank. After the fermentation the wine was divided in 34 l carboys with controlled amount of lees. The wines were 11 months in contact with lees with periodic stirring. The wines were stored during sur lie ageing at three different temperature levels: 10°C, 15°C and 20°C. The control wine was racked, filtered, sulphited and also stored in 34 l glass carboys. After the desired yeast contact time wines were prepared for analysis: they were racked, sulphited, filtered and bottled. All wines were replicated two times.

3.2 Examination of wine samples

The basic wine composition was analysed by means of Fourier Transformed Infrared Spectroscopy (Foss WineScan FT 120, Foss Electric, Denmark). FTIR analyzes a sample by taking a spectral imprint of the light absorbed by the sample (interferogram). The interferogram is collected by the spectrometer and processed through the Fourier transform calculation. Data are programmed into the instrument mathematical equation using a calibration model identifying certain parts of the spectrum to reflect an analyte (Jacobson, 2006). The following parameters were analysed: density, alcohol, sugar, fructose, glucose, acidity, pH, volatile acidity, tartaric acid, malic acid, lactic acid.

3.3 Amino acids

The free amino acids were determined using a UPLC™ protocol established by Fiechter et al., 2011.

3.3.1 Extraction of free amino acids from wine samples

Extraction of the free amino acids from the wine samples was performed by clarification. Aliquots (10 ml) were mixed with 0.5 g polyvinylpolypyrolidone (binding agent and precipitate organic compounds) and stirred for 10 minutes at room temperature, prior to being centrifuged (16000 x g at 4°C for 15 min). Considering the fact that alkaline pH is needed for optimal derivatization, the acidic samples were subsequently neutralized with 0,05 M boric acid buffer (pH 9.0), and further diluted (1/10-1/25) to match the calibration range. The centrifuged supernatants were directly submitted to the derivatization procedure utilizing Waters AccQ.Fluor™ Reagent Kit.

3.3.2 Chemicals and standards

The chemical and standards employed were as follows: high purity amino acids standard (type H, 17 amino acids dissolved in 0.1 M HCl at 2.5 mM; L-cysteine at 1.25 mM), purchased from Pierce (Rockford, IL, USA). Additional amino acids (purity ≥99%) were obtained from a variety of suppliers; DL-alpha-n-amino butyric acid, L-tryptophan and L-asparagine from Sigma (St. Louis, MO, USA); L-citrulline L-ornithine hydrochloride and gamma-amino butyric acid from Fluka (Buchs, Switzerland); and L-glutamine from Pierce. Waters (Milford, MA, USA) supplied the AccQ.Tag ™ Eluent A concentrate as well as pre-column AQC derivatization reagent (AccQ.Fluor™ Reagent Kit). Additional bulk chemicals and solvents exhibited either analytical or HPLC grade. These were obtained from Roth (Karlsruhe, Germany) and Merck (Darmstadt, Germany). Ultrapure water from an Elga ultra-high quality (UHQ) system (High Wycombe, Buckinghamshire, UK) was used for the preparation of all solutions.

Fifty millimolar stock solutions of the respective solid amino acids were prepared in 0.1 M HCl, and subsequently combined to result a 2.5 mM intermediate composite solution. The mixed amino acid standards (5-160 µM for each of the 23 analytes; constant 40 µM for alpha-amino butyric acid (AABA) as internal standard) were prepared by dilution in ultrapure water, and subsequently merged with the commercial Pierce standard. Following derivatization (resulting in an additional 1/10 dilution), seven standard solutions in the range from 0.5 to 16 µM were analyzed with UPLC™ and further used for system calibration. Final calibration concentrations ranged from 2 to 64 pmol per injection (2, 4, 8, 16, 24, 32, 64 pmol/4 µL injection) for each amino acid and constant 16 pmol for AABA. Non-weighted linear calibration functions were calculated via Waters Empower 2 chromatography software.

3.3.3 6-aminoquinolyl-N-hydroxysuccinimidyl carbamate (AQC) derivatization of standards and samples

Pre-column Aqc (6-aminoquinolyl-N-hydroxysuccinimidyl carbamate) derivatization of amino acids was performed according to the Waters AccQ.Tag™ pre-column derivatization procedure (Waters., 1993). Briefly, for amino acid standards, 5 µL standard solution was mixed with 35 µL AccQ.Fluor™ borate buffer; while for wine samples, 5 µL neutralized sample, 5 µL internal standard (AABA at 40 µM) and 30 µL buffer were combined. Thus, 40

µL derivatization batches were obtained in both cases. To initiate derivatization, 10 µL derivatization reagent (~10 mM AccQ.Fluor™ reagent in acetonitrile) was admixed, mixtures were then immediately vertexed, left to rest for 1 min and finally heated at 55 °C for 10 min.

3.3.4 Equipment

The quantification of free amino acids was performed using ultra-performance liquid chromatography (Acqulty ™ UPLC ™) involving pre-column derivatization and reversed-phase separation according to the AccQ-Tag ™ method. A tunable ultraviolet detector set at 254 nm and software package “Empower 2 Chromatography“ was used; all instruments were from Waters (Milford, MA). Pre-column with 6-aminoquinolyl-N-Hyroxysucccinimidyl carbamate (AQC) derivatization of primary and secondary amino groups was performed according to Waters AccQ.Fluor ™ protocol.

3.3.5 Chromatographic conditions

Chromatographic setup consisted of a Waters Acquity™ Ultra Performance LC (UPLC™) system (Milford, MA, USA) equipped with an Acquity™ runable UV detector (TUV). Reversed-phase separations were performed on an Acquity UPLC™ BEH C_{18} column (1.7 µm, 2.1 x 100 mm) with pre-connected 0.20 µm column inline filter. Waters Empower 2 chromatography software package was used for data acquisition and management.

Using the AccQ.Tag™ package (Waters., 1993) as a basis, as originally designed for HPLC separations on Waters Nova-Pak™ columns, method transfer was applied to UPLC ™. The applied solvent system consisted of mobile phase A: Waters AccQ.Tag™ Eluent A concentrate diluted 1/11 with ultrapure water and adjusted to pH 4.92 with 10% (v/v) phosphoric acid; and mobile phase B: 60% (v/v) acetonitrile in ultrapure water. Ultraviolet detection was set to 254 nm.

Derivatized amino acid standards and wine samples were injected onto the column (4 µL injection volume) and eluted at a flow rate of 0.4 mL min^{-1} at 37°C according to the following gradient:

initial 0% B;

0.0-0.8 min/0-2% B;

0.8-10.0 min/2-6% B;

10.0-12.6 min/6-10% B;

12.6-22.0 min/10-33% B;

22.0-23.0 min/33-33% B;

23.0-24.0 min/33-100% B;

24.0-26.0/100-100% B;

26.0-28.0/100-0% B;

The column was subsequently re-equilibrated for 8 minutes back to its initial conditions, yielding net separations for all 24 amino acid derivatives within 23 minutes, with an overall

cycle time of 36 minutes, up until the next injection (including an intense column cleaning purge and re-equilibration with special regard to the high backpressure and column lifetime).

3.4 Higher alcohols, methanol and ethyl acetate

3.4.1 Equipment

Higher alcohols were analysed by means of gas liquid chromatography. The equipment used was a Gaschromatograph 5890 II, (Hewlett-Packard, Vienna, Austria) equipped with an FID-Detector, Automatic sampler HP, Injector and Controller 7673, data management system HP Chem Station, column: DB-WAX 60 m, 0,32 ID, 0,25 µm film , carrier gas: He 5.0, FID detector gases: hydrogen (H_2: Qual.min. 5.0) and compressed air.

3.4.2 Chemicals

Reagents: tetrahydrofuran p.A. (Merck, Darmstadt, Germany), ethanol CHROMASOLV min. 99,8% (Riedel de Haen 1170, ethyl acetate LiCrosolv, methanol CHROMASOLV, (RdH 34860), butanol-2 (d:0,81), p.A, propanol-1 p.A., i-butanol p.A., butanol-1 p.A., i-pentanol (RdH 32206), hexanol-1 p.A. (RdH 804393). All re-agents were of pure grade and of chromatographic purity.

3.4.3 Chromatographic conditions

Chromatographic conditions: column head pressure: 10 psi constant, injected volume 1 µL, split rate 1:30, injector-temperature 245° C, detector-temperature 250° C, initial temperature: 40°C for 10 min, programmed temperature:

Level	Rate(°C/min)	Final Temp.(°C)	Final time(min)
1	3,5	150	0,0
2	10,0	245	10,0

Table 1: Higher alcohols, methanol and ethyl acetat analysis, chromatographic conditions

3.4.4 Extraction of higher alcohols from wine samples

Sample preparation: Higher alcohols were separated by distillation of 100 ml wine, 80 ml of the distillate were collected and made up to 100 ml with distilled water. Tetrahydrofuran was added to the distillate as an internal standard before gas chromatographic analysis. The calibration was performed with the corresponding standard solutions in ethanol 10% (v/v) and treated in the same manner as the wine samples. The following compounds were determined in each sample: Ethyl acetate, methanol, 1-propanol, isobutanol, isopentanol.

3.5 Biogenic amines

3.5.1 Equipment

A Hewlett Packard 1090 Liquid Chromatograph was used, equipped with a column LiChrospher 100 RP-18 5 µm, 250 x 4 mm (Fa. Merck) and a fluorescence detection HP 1046 (Fa. Hewlett Packard) using 330 and 450 nm as the excitation and emission wavelengths, respectively.

3.5.2 Chemicals

Gradient elution of two solvents was used:	Solvent A 2.2681 g/L KH_2PO_4 + 5.933 g/L Na_2HPO_4; pH 7.2 with NaOH 50% Solvent B CH_3CN
Derivatization solution:	OPA: 25mg OPA 2.25mL CH_3OH 0.25µL 2-Mercaptoethanole 0.25mL BO3 buffer BO3 buffer: 3.0915g/100mL H_20 pH 9.5 with NaOH 50%
Internal standard:	210mg/L n-Hexylamin in 50/50 ethanol/H_20
Chromatographic conditions:	Factor Standard EW g/100mL H20 Histamine : x 0.605 0.08 Tyramine : x 0.795 0.07 Putrescine : x 0.56 0.09 Cadaverine : x 0.583 0.08 Phenylethylamine 0.05 Isophenylamine 0.05 IST Hexylamine 0.0210 50/50 ethanol/H_2O
Position in HPLC	0 H_20 1 OPA 2 Bo_3 buffer
Automatic derivatization in HPLC	1.) 3 µL Sample 2.) 0 µL H_2O

	3.) 4 µL Vial 1 OPA 4.) 4 µL Vial 2 BO3 5.) 0 µL H_2O 6.) 11 µL MIX 7.) Inject	
Gradient:	0 min 60% A 10 min 30% A 30 min 30% A 32 min 60% A Posttime 10 min	40% B 70% B 70% B 40% B

Table 2: Biogenic amines analysis, chemicals

3.5.3 Preparation of the wine samples

The samples were filtered to avoid the influence of solid residues (tartrates, precipitated proteins, microorganisms). 3 ml of the sample were mixed with 0.050mL internal standard, 1mL ethanol and 3g K2CO3 until complete solibilisation. To improve the separation of phases the solution was centrifuged for 10 min at 4000 rpm and the upper phase was collected with a pipette. The residue was treated again with 1 mL of ethanol K2CO3 mixture and centrifuged 10 min at 4000 rpm. Finally the upper phase was collected again with a pipette. Both ethanolic phases introduced in a vial were mixed well and were injected directly in the HPLC.

3.6 Microbiological methods

Most of the bacteria grown in wine can be isolated by traditional microbiological techniques (Pozo-Bayón et al., 2009).

3.6.1 Isolation of lactic acid bacteria

3.6.1.1 Principle

Isolation of LAB was done by plating wine samples directly or after dilution on favourable nutrient media.

3.6.1.2 Materials

Only wine samples, which underwent malolactic fermentation, were investigated.

Samples number	Samples identification
I	Sur lie without SO_2; + 8 g/hl Enzyme 10°C
II	Sur lie without SO_2; without Enzyme 10°C
III	Sur lie without SO_2; + 8 g/hl Enzyme 15°C
IV	Sur lie without SO_2; without Enzyme 15°C
V	Sur lie without SO_2; + 8 g/hl Enzyme 20°C
VI	Sur lie without SO_2; without Enzyme 20°C

Table 3: Isolation of LAB, wine samples

Media

One of the most popular growth media for anaerobic gram positive bacteria is MRS agar, named after its inventors: de Man, Rogosa and Sharpe. The addition of tomato or grape juice, malic acid and different sugars to MRS medium increases the growth of wine LAB (Wibowo et al. 1985).

Within this study the isolation of LAB was achieved using two different nourishing media: MRS agar + cysteine-HCl (0,5g/l) + cycloheximide (0,1g/l) and MLO, which is specific for the growth of *Oenococcus oeni* (Caspritz and Radler, 1983).

To isolate and purify wine lactic acid bacteria solid media with the addition of agar was used, while liquid media without agar were used for cultivation and to obtain biomass of the pure cultures.

Quantity	Substance
68.2 g	MRS agar (Merck)
0.5g	Cysteine-HCl
0.1g	Cycloheximide
1000 ml	H2O

Table 4: Media, Composition of MRS agar + cysteine-HCl + cycloheximide (g/l)

Cycloheximide (0,1g/l) was added to inhibit yeast growth, whereas Cysteine HCl is a reducing agent favouring the growth of LAB.

Quantity	Substance
52.5 g	MRS broth (Merck)
0.5g	Cysteine-HCl
0.1g	Cycloheximide
1000 ml	H2O

Table 5: Media, Composition of MRS broth + cysteine-HCl + cycloheximide (g/l)

Quantity	Substance
10g	Trypton
5g	Yeast extract
10g	Glucose
5g	Fructose
3.5g	Di-Ammoniumcitrate
0.5g	Cysteine-HCl
0.2g	MgSO4
0.5g	MnSO4
1ml	Tween*80
100ml	Tomato juice
0.1g	Cycloheximide
15g	Agar
900 ml	H2O

Table 6: Media, Composition of MLO agar (g/l)

Quantity	Substance
10g	Trypton
5g	Yeast extract
10g	Glucose
5g	Fructose
3.5g	Di-Ammoniumcitrate
0.5g	Cysteine-HCl
0.2g	MgSO4
0.5g	MnSO4
1ml	Tween*80
100ml	Tomato juice
0.1g	Cycloheximide
900ml	H2O

Table 7: Media, Composition of MLO broth (g/l)

Quantity	Substance
20 g	Pepton water (Oxoid)
1000 ml	H2O

Table 8: Media, Peptone water

Tools	Description	Manufacturer
Centrifuge	5804	Eppendorf
Vortexing tool	MS 3 basic	IKA
Scale	GPA 5202	Sartorius
Autoclave	18 Liter	Certoclav Steriliser
Precision Pippete	1000 µl	Eppendorf
Pipetting tipps	1000 µl	Eppendorf
Pipetting aid	Macro	Brand
Disposable pipette	2 ml	Sarstedt
Glas bottles	100 ml	Simax
Reaction tubes	1.5 ml	Eppendorf

Table 9: Media, equipment

3.6.1.3 Procedure

Spread plate

For the isolation of LAB by traditional microbiological techniques, wine samples were serially diluted. Peptone water was used to make dilutions (10^{-1} to 10^{-4}). Hence, 100 µl of sample were added to 900 µl peptone water and mixed. Diluted wine samples were plated on MRS-agar and BCM 133 medium. Subsequently, the plates were incubated at 30°C for 10 days under anaerobic conditions (85%N_2, 5%H, 10%CO_2).

Streak plate

Additionally, a streak plate method was applied on both media. Therefore, one loop of each sample was streaked on both media. Then the plates were incubated at 30°C for 10 days under anaerobic conditions (85%N_2, 5%H, 10%CO_2).

3.6.2 Cultivation of lactic acid bacteria

3.6.2.1 Principle

A total of 90 isolates were obtained from BCM 133 medium: 60 isolates from spread plates (10 isolates/sample) and 30 isolates from streak plates (5 isolates/sample). Each isolate was frozen in order to keep the cells viable and to store them for a longer time period. Glycerin and very low temperatures are necessary to apply this method. This particular storage method enables the possibility to resuscitate the bacteria on demand.

3.6.2.2 Materials

Tools	Description	Manufacturer
Centrifuge	5804	Eppendorf
Vortexing tool	MS 3 basic	IKA
Scale	GPA 5202	Sartorius
Autoclave	18 Liter	Certoclav Steriliser
Precision Pippete	1000 µl	Eppendorf
Pipetting tipps	1000 µl	Eppendorf
Pipetting aid	Macro	Brand
Disposable pipette	2 ml	Sarstedt
Glas bottles	100 ml	Simax
Reaction tubes	1.5 ml	Eppendorf

Table 10: Cultivation of LAB, equipment

Reagents	Description	Manufacturer
Glycerol, 87%, autoclaved	A0970	Applichem

Table 11: Cultivation of LAB, chemicals

3.6.2.3 Procedure

After evaluating and counting the colonies grown on the incubated spread and streak plates, colonies with different morphologies were streaked on agar plates to obtain pure isolates. Subsequently, plates were incubated at 30°C for 7 days using anaerobic conditions.

Respectively one colony of the pure culture was taken from the plates with a loop and suspended in 1.5 ml BCM 133-broth. Then the samples were incubated at 30°C for 4 days under anaerobic conditions (85%N_2, 5%H, 10%CO_2, 0%O_2). Finally, 200µl glycerol were added to each reaction tube containing 1.5 ml bacterial suspension. The tubes were mixed and cryopreserved at -80°C.

3.6.3 Molecular methods for the identification of lactic acid bacteria

Traditional methods used for identification are based on their phenotypic characteristic. Identification by phenotypic analysis is sometimes problematic. Clones are difficult to multiply in laboratory conditions and many sub-cultures are needed to obtain enough biomass for all of the tests. As the phenotypic characteristic is the result of a metabolic chain pathway, depending on the cell enzymatic activity, the use of inhibitors can modify a phenotype. Therefore, molecular methods are a better tool because the DNA composition is not influenced by culture conditions (Ribereau-Gayon et al. 2006).

Of the stored 90 isolates, 60 were tested in more detail. Therefore, they were resuscitated in 15 ml BCM 133. Additionally, the two starter cultures “Viniflora CH35” (*Oenococcus oeni)* and “Viniflora oenos” (*Oenococcus oeni)* were investigated. Hence, a loopful of each starter culture was transferred in 15 ml BCM 133. Then the isolates and the two starter cultures were incubated at 30°C for 5 days under anaerobic conditions (85%N_2, 5%H, 10%CO_2).

Cell harvest and cell wash

3.6.3.1 Principle

Cell harvest was done from incubated broth to obtain biomass. Subsequently, biomass was washed to gain a purified cell pellet for DNA-isolation.

3.6.3.2 Material

Reagents	Description	Manufacturer
Natriumchloride	27800.291	VWR

Table 12: Cell harvest and cell wash, material

Tools	Description	Manufacturer
Centrifuge	5804	Eppendorf
Vortexing tool	MS 3 basic	IKA
Busen burner	Nr. 05240	Schuett Phoenix
Autoclave	18 Liter	Certoclav Steriliser
Precision Pippete	1000 µl	Eppendorf
Pipetting tipps	1000 µl	Eppendorf
Scale	GPA 5202	Sartorius
Measuring cylinder	100 ml, 250 ml, 500 ml, 1000 ml	

Glas bottles	250 ml	Simax

Table 13: Cell harvest and cell wash, equipment

3.6.3.3 Procedure

For cell harvest, the incubated broth (15 ml) was centrifuged for 5 min at 5000 min^{-1}. The supernatant was discarded and cells were washed by adding 10 ml NaCl (0,9%), mixed and centrifuged again. Finally, the cell pellet was resuspended in 1 ml NaCl and the cell suspension was transferred to 2 ml reaction tubes and centrifuged for 6 min at 8000 min^{-1}. Afterwards, the supernatant was discarded.

3.6.4 Deoxyribonucleic acid (DNA) isolation

3.6.4.1 Principle

The DNA of all 60 isolates was extracted using the DNeasy Blood & Tissue kit following the manufacturer's instructions.

3.6.4.2 Material

Tools	Description	Manufacturer
Precision Pippete	10 µl, 100 µl, 1000 µl	Gilson
Pipetting tipps	10 µl, 100 µl, 1000 µl	Safe Seal Tips, Biozym
Centrifuge	Centrifuge 5417R	Eppendorf
Vortexing tool	MS2 Minishaker	IKA
Reaction tubes	1.5 ml	Eppendorf
Heating block	QBD4	Grant
Heating block/mixer	Thermomixer comfort	Eppendorf
Mixer	KS 130	IKA
DNA.Measuring tool	Nanodrop 200c	IKA
Disposable pipette	10ml, 25ml	Sarstedt
Plastic sample tubes with a screw cap	30 ml	Sterilin

Table 14: DNA Isolation, equipment

3.6.4.3 Procedure

The purified cell pellet (2.3.1) was dissolved in 180 µl cell lysate buffer, mixed, incubated at 37° C for 20 minutes and heated at 56°C for 30 min. Subsequently, 25 µl proteinase K and

200 µl AL-buffer (without ethanol) were added, mixed and incubated at 56°C for 30 min. Afterwards, 200 µl ethanol were added and mixed well to form a homogeneous solution.

A DNeasy Mini Spin column was placed in a 2 ml reaction tube. Then the sample was pipetted into the column and centrifuged at 8000 rpm for 1 min.

Afterwards, the flow-through was discarded and 500 µl AW1 buffer were pipetted into the spin column and centrifuged again at 8000 rpm for 1 min.

The previous step was repeated again but instead of 500 µl AW1 buffer 500 µl AW2 buffer were used and centrifuged at 14000 rpm for 1 min.

The DNeasy spin column was moved to a new 2ml Epi and 200 µl AE-buffer were pipetted directly on the DNeasy membrane, which was incubated at RT for 1min and centrifuged at 8000 rpm for 1 min to elute the DNA.

3.6.5 Polymerase chain reaction (PCR)

3.6.5.1 Principle

Polymerase chain reaction is a method that allows to amplify a fragment of DNA *in vitro.* Therefore, the DNA double helix is denaturated and after the annealing of the primers the enzyme DNA-polymerase produces a complementary DNA strand. For PCR several components are required:

- original DNA;
- two primers, one for the start of the synthesis and one to restrict the amplification stage;
- DNA-polymerase with the ability to react at higher temperatures;
- Nucleotides;
- buffer solutions to secure the chemical environment of the DNA-polymerase;

The PCR is carried out in a thermocycler. The number of cycles depends on the method and the sample (Newton und Graham, 1994). Basically, PCR is composed of five steps:

1. Initialization-denaturation step: this step is carried out to disrupt the hydrogen bounds yielding single-stranded DNA molecules from the double helix. This step lasts several minutes. In general temperatures between 94-95°C are applied (Newton und Graham, 1994).
2. Denaturation step: this step is equivalent to the initial denaturation step, but takes less time (Newton und Graham, 1994).
3. Annealing step: at this step the temperature will be kept constant for a short time (e.g. 30 s), allowing the annealing of the primers to the right place of the DNA. The annealing temperature strongly depends on the primers. If the temperature is too low, than the primer can anneal to a wrong place and unspecific reactions can be

produced. If the temperature is too high, the primer will not anneal because of the heat shock. Therefore no reaction will take place. In general temperatures between 55-65°C are applied (Newton und Graham, 1994).

4. Elongation step: single-stranded DNA molecules with the missing nucleotides will react with the DNA-polymerase. The temperature is between 68-72°C. This step takes about 30 s (Newton und Graham, 1994).

5. Final elongation: this step takes at least 5 minutes and is made to ensure that the remaining single-stranded DNA is fully extended (Newton und Graham, 1994).

In rep-PCR, the primers bind to repetitive sequences of the prokaryotic genome. The primer binding has to have a proper orientation and has to be within a distance that can be amplified by the enzyme polymerase. Subsequently, amplification products are obtained. Especially, the fingerprints made with the primer $(GTG)_5$ are used in bacterial taxonomy and are a reliable tool for identification of different bacterial groups (Svec et al. 2005).

Randomly amplified polymorphic DNA (RAPD) methods using arbitrary primers have been developed for studying genomic DNA. In this technique the primers are generally about 10 nucleotides long and are not directed at any known sequence of the bacterial genome. RAPD techniques are often used to examine lactic acid bacteria (Coeuret., et al. 2003).

Sequencing of the 16S rRNA gene is the most used technique to determine phylogenetic relationships among bacteria. The 16S rRNA gene is very preserved among bacterial species, but it has different zones that can be used for identification. These zones can be amplified by specific primers and the sequence can be inserted in a data base. The identification is made on the similarity with other sequences from the data base. The method is simple but it can not be used for the differentiation of subspecies (Pozo-Bayon et al. 2009; du Plessis et al. 2004)

3.6.5.2 Material

Reagent	Description	Manufacturer
10 x PCR-Buffer	F-511	Finnzymes
DNA-Polymerase	F-501-L	Finnzymes
dATP, 100mM	K035. 1	Carl Roth
dCTP, 100 mM	K036. 1	Carl Roth
dGTP, 100 mM	K037.1	Carl Roth
dTTP, 100 mM	K036.1	Carl Roth

Table 15: Polymerase chain reaction, reagents

Tools	Description	Manufacturer
Precision Pipette	2 µl, 10 µl, 20 µl, 100 µl, 1000 µl	Gilson

Pipette tipps	10 µl, 20 µl, 100 µl, 1000 µl	SafeSeal Tips
Laminar flow bench	OPTN/NO 58 11-13	Captair
Vortexing tool	MS2 Minishaker	IKA
Centrifuge	Centrifuge 5415 R	Eppendorf
PCR-tube	0.2 ml, 0.5 ml	Eppendorf
Thermocycler	Mastercycler	Eppendorf
Autoclave	18 l	Certoclav Steriliser

Table 16: Polymerase chain reaction, equipment

Quantity	solution/conc
100µl	dATP / 100mM
100µl	dGTP / 100mM
100µl	dTTP / 100mM
100µl	dCTP / 100mM
600µl	UHQ sterile water
1000µl	dNTP – mix / 10mM

Table 17: Polymerase chain reaction, preparation of solutions (dNTP-Mix, 10mM)

100 µl of ATP, CTP, GTP and TTP (100 mM) respectively were mixed with 600µl of sterile water and vortexed gently. The solution was stored in the freezer at -30°C until needed.

10 x PCR - Buffer	1000 µl
dNTP-Mix, 10mM	200 µl
Polymerase, 2U/ µl	200 µl
Deionised waster	7400 µl

Table 18: Polymerase chain reaction, MM-mastermix

All solutions were mixed in a tube and vortexed well. Afterwards the maxter mix was portioned and stored in the freezer until needed.

Primers

Primer	Conc.	Sequence	Literatur
(GTG)5	50 pmol/µl	GTG GTG GTG GTG GTG	Svec at al., 2005

Table 19: Primer for Rep-PCR

Primer	Conc.	Sequence	Literatur
Collado	25 pmol/µl	AGT CAG CCA C	Collado et al. (2006)
Torriani	25 pmol/µl	CCG CAG CCA A	Torriani et al. (1999)

Table 20: Primer for RAPD- PCR

Primer	Conc.	Sequence	Length (bp)	Specificity	Literatur
Cello-P0	7.5 pmol/µl	GAG AGT TTG ATC CTG GCT CAG	1470	16S rRNA	Di Cello et al. (1997)
Cello-P6	7.5 pmol/µl	CTA CGG CTA CCT TGT TAC GA			

Table 21: Primer for 16S rRNA – specific PCR and sequencing

PCR cycles

Cycles	*Time*	*Temperature*
1	*3 min*	*95°C*
40	*1 min*	*94°C*
	2 min	*37°C*
	5 min	*72°C*
1	*10 min*	*72°C*
1	∞	*4°C*

Table 22: Program for Rep-PCR (Primer: $(GTG)_5$)

Cycles	Time	Temperature
1	5 min	95°C

45	1 min	95°C
	1 min	36°C
	1 min	72°C
1	8 min	72°C
1	∞	4°C

Table 23: Program for RAPD-PCR (Primers: Collado, Torriani)

Cycles	Time	Temperature
1	2 min	95°C
5	30 sec	95°C
	30 sec	60°C
	3 min	68°C
5	30 sec	95°C
	30 sec	55°C
	3 min	68°C
25	30 sec	95°C
	30 sec	50°C
	3 min	68°C
1	10 min	68°C
1	∞	4°C

Table 24: Program for PCR for Sequencing (Primers: Cello P0 and Cello P6)

3.6.5.3 Procedure

30 min before starting the PCR, DNA, mastermix and the primers were removed from the freezer and stored in the fridge. Subsequently, the tubes (0.2 ml) for samples, negative and positive control were prepared. When all reagents were defrosted, the premix was calculated.

Per sample:

Mastermix MM 22µl

Primer 1 1µl

Primer 2 (Cello-PCR) / UHQ (rep/RAPD-PCR) 1µl

DNA 1µl

Ingredients needed for the Premix (MM, Primers) were vortexed and centrifuged. The premix was prepared, gently vortexed and pipetted into the PCR tubes (24 µl). If just one primer was used then instead of primer 2 UHQ was added. DNA samples were votexed and centrifuged. In each PCR tube containing 24 µl of premix, 1 µl of DNA was added. All the tubes were vortexed and given into the PCR-thermocycler. The suitable PCR-program was chosen. After the PCR run has been finished, the tubes with the PCR products were stored in the freezer at -30°C until needed.

3.6.6 Agarose gel electrophoresis

3.6.6.1 Principle

Agarose gel electrophoresis is a diagnostic method. The electrophoresis is used for separation of DNA fragments in many different areas and has the ability to electrically separate charged molecules in an electric field. The fragments are separated by size and charge. Regarding RAPD-PCR / rep-PCR electrophoresis uncovers the genomic DNA fingerprint from the amplification products (Lupski and Weinstock 1992).

Agarose is produced from algae and is a linear polysaccharide of D-galactose and L-galactose. Agarose is very soluble in warm water and produces a porous matrix after cooling down. The porosity, which is like a sieve for DNA fragments, depends on the used quantity of agarose in the medium. Small DNA fragments move faster in the electrical field than big fragments. Therefore, big fragments remain close to the cathode and the small fragments migrate to the anode. DNA is negatively charged, thus it always migrates to the anode.

3.6.6.2 Material

Buffers & solutions

Tris	242 g	484 g
Glacial acetic acid	57,5 ml	115 ml
0,5M EDTA; pH 8.0	100 ml	200 ml
	in **1l** deion. H_2O	in **2l** deion. H_2O

Table 25: Preparation of 50xTAE-Buffer, composition

Tris was weighed into a beaker and carried quantitatively over into a 2 l volumetric flask. The funnel and beaker were rinsed with deionized water. EDTA and glacial acetic acid were added with a column and a pipette. The volumetric flask was filled up to app. 1.5 l with deionized water and let stirred on a magnetic stirrer until everything has dissolved. Then the flask was filled up to the mark with deionized water. The buffer was transferred into two 1 l flasks and autoclaved at 121°C for 15 min.

Preparation of 0,75xTAE-Buffer:

15 ml 50xTAE-Buffer were filled. The buffer was transferred into a 500 ml measuring cylinder, which was subsequently filled up to 500 ml with deionized water. The buffer was transferred into a 1 l flask and filled up to 1l with deionized water.

0,25% Bromphenol blue	0,025 g
0,25% Xylene Cyanol	0,025 g
15% Ficoll Type 400	1 g
Sterile UHQ-water	9 ml

Table 26: Loading dye, composition

All dyes were weighted into a Sterilin tube and dissolved in 9 ml sterile UHQ- water. The loading dye is stored at room temperature.

3.6.6.3 Procedure

Approximately 10 min before gel preparation the water bath was turned on.

1,4 g of aragose were weighted into a sterile flask and filled up with 70 ml 0,75xTAE- Buffer. The agarose was dissolved in a microwave oven, and then cooled in water bath (50°C).The gel tray was prepared and filled with the solution. After putting the comb into the gel, the gel was left for polymerization for 30 min.

The electrophoresis apparatus was filled with approximately 1.9 l 0.75xTAE buffer. The temperature was adjusted to 20°C and the pump was switched on. After the gel was polymerized, the combs were removed and the gel was put into the chamber. The pump was turned off to grant a better lowering of the samples into the slots. 5 µl of the sample were mixed with 1.5 µl gel loading buffer on parafilm and filled into the slots. After sample application, the electrodes were connected to the power supply and the electrophoresis was switched on (80 V, 2000 mA). The pump was switched on after 5 min in which the samples had diffused into the gel. After 1 h 50 min the electrophoresis was switched off and the gel was stained, destained and documented.

3.6.7 Staining and documentation

3.6.7.1 Principle

The coloring has to be made with a fluorescent agent. Therefore ethidium bromide was used. Ethidium bromide is a red, fluorescent coloring agent. It is applied in molecular biology for staining the fragments of nucleic acids. The capacity of ethidium bromide to bind to DNA fragments depends on the concentration and the length of DNA. The fluorescence of ethidium bromide is more intense after binding to DNA. When exposed to ultraviolet, the gel area which contains DNA and thus ethidium bromide fluorescence with more intensity than the area without (Lodisch, 2001).

After staining it is possible to make the DNA bands visible using a transilluminator and to photograph the gels with a digital camera.

3.6.7.2 Material

Tools	Description	Manufacturer
Pipette	100 µl	Gilson
Pippete tipps	100 µl	VWR
Staining container	-	Interbox
Measuring cylinder	1000 ml	ISO-LAB Germany
Transilluminator	Chemilmager	Alpha Innotech

Table 27: Staining and documentation, equipment

Reagent	Description	Manufacturer
Ethidiumbromide, 1%, 10 mg/ml	2218.2	Carl Roth
Deiobised water	-	-
0.75xBuffer	Self mixed	-

Table 28: Staining and documentation, reagents

3.6.7.3 Procedure

After preparing the staining solution by adding 35 µl ethidium bormide (1%) to 350 ml sterile water, the gel was placed into this solution and left there for 30 minutes. Subsequently the gel was destained in 0.75% TAE buffer for 15 minutes. Then the gel was placed into the transillumantor and photographed.

3.7 Sensory analysis

3.7.1 Sensory attributes of Grüner Veltliner wines

The typical attributes of the variety Grüner Veltliner are peppery and spicy flavours.

3.7.2 Materials and methods

3.7.2.1 Wine samples

The ageing on lees of Grüner Veltliner wine was done during four vintages: 2006, 2007, 2008 and 2009. Each year some possible influence factors were taken into account:

Vintage 2006
Two yeast strains *Saccharomyces cerevisiae* Fermicru 4F9 (DSM, Delft, Netherlands) and *Saccharomyces bayanus* Lalvin EC 1118 (Lallemand, Madrid, Spain), contact time 3 and 6 month; compared to the control wine with no lees contact;

Vintage 2007
Two yeast strains *Saccharomyces bayanus* Lalvin EC 1118 (Lallemand, Madrid, Spain) and *Saccharomyces cerevisiae* Oenofem Veltliner (Erbslòh, Geisenheim, Germany), contact time 6 weeks and 5 months; compared to the control wine with no lees contact;

Vintage 2008
One yeast strain *Saccharomyces cerevisiae* Fermicru 4F9, quality (fine and crude) and amount of lees (3 and 6g/l), temperature; compared to the control wine with no lees contact;

Vintage 2009
One yeast strain *Saccharomyces cerevisiae* Oenofem Veltliner, 3 temperature levels (10, 15, 20°C), enzymes and sulphur dioxide addition; 9 month lees contact; compared to the control wine with no lees contact;

3.7.2.2 Tasting intervals

Vintage 2006
The tasting sessions were made after 5 years of bottle ageing. Sensory analysis of Grüner Veltliner wines was made by two different panels, one consisting of 13 tasters with experience and one consisting of 44 viticulture students.

Vintage 2007
The tasting sessions were made 3 months after bottling. The sensory analysis of Grüner Veltliner wines was performed by a panel of 10 expert wine tasters.

Vintage 2008
The tasting sessions were made 3 months after bottling. The sensory analysis of Grüner Veltliner wines was performed by a panel of 10 expert wine tasters.

Vintage 2009

The tasting sessions were made 3 months after bottling. The sensory analysis of Grüner Veltliner wines was performed by a panel of 14 wine tasters.

3.7.2.3 Descriptive sensory analysis

The panelists noted the intensity of each descriptor on a scale from 0 for low or no intensity to 10 for high intensity. The mean values for each descriptor were used to obtain a profile of the experimental wines.

3.7.2.4 Principal components analysis

Principal component analysis (PCA) is particular useful in seeing the correlation between attributes. If attributes are highly correlated it means that the attributes are similarly perceived by the tasters.The PCA biplot represents both variables and observations of a matrix of multivariate data on the same plot. The PCA biplot provides a useful tool of data analysis and allows visual appraisal of the structure of large data matrices (Gabriel, 1971).

3.8 Statistical methods

Statistical analyses were performed using SPSS (15.0.1) programme. Univariate analysis of variance (ANOVA) was applied to detect significant differences provoked by different treatments (yeast strain, duration of lees contact, enzyme addition, lees quantity and quality, temperature, malolactic fermentation and SO_2 addition). Significant level was $p < 0.05$.

4. Results

4.1 Influence of different fermentation conditions (yeast strain, quantity and quality of lees, SO_2, malolactic fermentation) on the general composition of Grüner Veltliner wines.

4.1.1 Vintage 2006

The alcoholic fermentation was completed with all the selected yeast strains. The sugar level of wines after the alcoholic fermentation was below 1.5 g/l. The ethanol level was high (14 vol%). Only slight differences were noted regarding the alcohol level. The decrease in the acidity level and the increase of pH and volatile acidity are the consequence of malolactic fermentation. The MLF started spoltaneuosly and after 6 months on lees the malic acid was almost depleted. The general composition of wines was virtually identical.

Yeast	Description	Time months	Density [20°C/ 20°C]	Alcohol [Vol%]	Sugar [g/L]	Fructose [g/L]	Glucose [g/L]	Acidity [g/L]	pH	Volatile acidity [g/L]	Tartaric acid [g/L]	Malic acid [g/L]	Lactic acid [g/L]
O.Veltliner	Control wine	0	0.9898	14.3	0.9	1.6	0.8	5.3	3.5	0.5	1.9	2.1	n.n.
O.Veltliner	sur lie	3	0.9902	14.2	1.3	2.2	0.9	5.3	3.5	0.5	1.6	2.2	0.1
O.Veltliner	sur lie	6	0.9892	14.3	0.8	1.5	1.1	4.2	3.7	0.6	1.8	0.4	1.5
Weiss Komplex	Control wine	0	0.9899	13.9	0.8	1.4	1.0	5.0	3.5	0.3	1.9	1.9	0.1
Weiss Komplex	sur lie	3	0.9899	14.1	0.9	1.7	1.0	5.1	3.5	0.4	1.5	2.1	0.1
Weiss Komplex	sur lie	6	0.9893	14.4	1.1	1.4	1.3	4.1	3.7	0.5	1.9	0.3	1.6
Fermicru 4F9	Control wine	0	0.9899	14.3	0.8	1.7	0.8	5.2	3.5	0.4	1.8	2.0	0.1
Fermicru 4F9	sur lie	3	0.9903	14.1	0.9	1.7	0.9	5.4	3.5	0.4	1.8	2.2	0.1
Fermicru 4F9	sur lie	6	0.9895	14.3	1.2	1.7	1.2	4.1	3.7	0.5	2.0	0.3	1.5
EC 1118	Control wine	0	0.9898	14.1	0.8	1.6	0.8	5.1	3.5	0.4	1.6	1.9	0.1
EC 1118	sur lie	3	0.9899	14.2	0.9	1.8	0.9	5.2	3.5	0.4	1.5	2.0	0.1
EC 1118	sur lie	6	0.9894	14.4	1.0	1.5	1.2	4.2	3.7	0.5	1.9	0.3	1.5

Table 29: General composition of Grüner Veltliner wine fermented with the yeasts Oenoferm Veltliner, Weiss Komplex, Fermicru 4F9 and EC 1118 during their ageing on lees

4.1.2 Vintage 2007

The alcohol level was similar in the wines fermented with the two yeasts. The alcohol level was 12.7 vol%, lower than in the wines produced in 2006. Both yeasts completed the alcoholic fermentation. In the wines stored on lees for 5 months malolactic fermentation started spontaneously and was completed. The acidity decreased and pH and volatile acidity increased, 1 g/l of lactic acid was produced.

Yeast	Description	Time	Density [20°C/20°C]	Alcohol [Vol%]	Sugar [g/L]	Fructose [g/L]	Glucose [g/L]	Acidity [g/L]	pH	Volatile acidity [g/L]	Tartaric acid [g/L]	Malic acid [g/L]	Lactic acid [g/L]
O.Veltliner	control wine	0	0.9912	12.7	1.3	2.1	1.2	5.3	3.4	0.3	2.8	1.5	n.n.
O.Veltliner	Sur lie	6 weeks	0.9912	12.7	1.4	2.0	1.4	5.4	3.4	0.3	2.8	1.5	n.n.
O.Veltliner	Sur lie	5 months	0.9910	12.5	1.6	2.1	1.6	4.4	3.5	0.4	2.6	0.3	1.0
EC 1118	control wine	0	0.9907	12.8	1.0	1.7	1.3	5.4	3.4	0.3	3.0	1.4	n.n.
EC 1118	Sur lie	6 weeks	0.9907	12.8	1.2	1.8	1.3	5.1	3.4	0.3	2.7	1.2	0.2
EC 1118	Sur lie	5 months	0.9905	12.7	1.3	1.8	1.5	4.5	3.5	0.3	2.7	0.2	1.0

Table 30: General composition of Grüner Veltliner wine fermented with the yeasts Oenoferm Veltliner and EC 1118 during ageing on lees

4.1.3 Vintage 2008

The wine was obtained with a single yeast strain, therefore the alcohol level was the same (12,4 vol%). The fermentation was completed. The wines stored on lees at 15°C presented slightly differences in acidity content. In all the wines stored on lees at 22°C malolactic fermentation started spontaneously. After 8 months on lees malolactic fermentation was not completed. The residual amount of 0.6 g/l malic acid was still present. The pH and volatile acidity increased in the samples were malolactic fermentation occurred. The levels of tartaric acid were higher in the samples stored on lees at 22°C.

Yeast	Description	Time	Density [20°C/20°C]	Alcohol [Vol%]	Sugar [g/L]	Fructose [g/L]	Glucose [g/L]	Acidity [g/L]	pH	Volatile acidity [g/L]	Tartaric acid [g/L]	Malic acid [g/L]	Lactic acid [g/L]
Fermicru 4F9	15 Control wine	8 months	0.9919	12.4	1.2	1	0.8	5.3	3.6	0.3	1.7	2.5	0.1
Fermicru 4F9	15 i ne lees	8 months	0.9926	12.7	1.7	1.3	1.1	5.8	3.7	0.4	1.9	2.8	0.4
Fermicru 4F9	15 Crude lees 3g L	8 months	0.9921	12.4	1.3	1.1	1.1	5.2	3.6	0.4	1.8	2.3	0.4
Fermicru 4F9	15 Crude lees g L	8 months	0.9923	12.3	1.4	1	1.1	5.1	3.7	0.5	1.8	2	0.7
Fermicru 4F9	15 ° Batannage plus Elevage	8 months	0.9921	12.3	1.2	1	1	5.2	3.6	0.4	1.6	2.5	0.1
Fermicru 4F9	22 Control wine	8 months	0.9923	12.4	1.2	1	0.8	5.5	3.6	0.3	2.1	2.6	0.2
Fermicru 4F9	22 i ne lees	8 months	0.9921	12.7	2	1.1	1.5	4.3	3.8	0.6	2.3	0.6	2
Fermicru 4F9	22 Crude lees 3g L	8 months	0.9917	12.4	1.4	0.9	1.1	4	3.7	0.5	2.3	0.6	1.7
Fermicru 4F9	22 Crude lees g L	8 months	0.9919	12.4	1.5	1	1.3	4	3.8	0.5	2.4	0.6	1.7

Table 31: General composition of Grüner Veltliner wine fermented with the yeast Fermicru 4F9, stored on lees at 15° and 22°C with different lees quantity and quality

4.1.4 Vintage 2009

The yeast Oenoferm Veltliner completed the alcoholic fermentation. The ethanol level was 13.3 vol%. The wines were stored on lees at three different temperature levels, with and without SO_2 addition. In the samples without SO_2, malolactic fermentation started spontaneously at all three temperature levels. As a consequence of malolactic fermentation, acidity decreased and pH and volatile acidity increased. After 11 months on lees malolactic fermentation was completed. Tartaric acid content displayed a slight increase in the samples were malolactic fermentation was finished.

Description	Time	Density [20°C/20°C]	Alcohol [Vol%]	Sugar [g/L]	Fructose [g/L]	Glucose [g/L]	Acidity [g/L]	pH	Volatile acidity [g/L]	Tartaric acid [g/L]	Malic acid [g/L]	Lactic acid [g/L]
Control Wine 20° C	0	0.9902	13.2	1.0	0.6	0.7	4.9	3.5	0.4	1.8	1.7	n.n.
sur lie, SO_2 and enzyme 20°C	11 months	0.9907	13.1	1.7	0.8	1.1	5.1	3.6	0.5	1.7	1.7	0.1
sur lie. SO_2, no enyzme 20°C	11 months	0.9904	13.3	1.0	0.7	0.6	4.9	3.6	0.4	1.8	1.4	0.3
sur lie, no SO_2, enzyme 20°C	11 months	0.9899	13.3	1.7	0.9	1.5	3.9	3.7	0.6	1.9	0.2	1.4
sur lie, no SO_2, no enzyme 20°C	11 months	0.9897	13.3	1.0	0.7	0.9	4.3	3.7	0.6	2.1	0.1	1.8
Control Wine 15° C	0	0.9904	13.1	1.3	0.7	0.6	5.2	3.5	0.4	2.0	1.7	n.n.
sur lie, SO_2 and enzyme 15°C	11 months	0.9909	13.2	1.7	0.8	1.0	5.2	3.6	0.4	1.9	1.9	0.1
sur lie. SO_2, no enyzme 15°C	11 months	0.9906	13.2	1.2	0.7	0.6	5.2	3.6	0.4	1.9	1.9	n.n.
sur lie, no SO_2, enzyme 15°C	11 months	0.9902	13.2	1.7	0.9	1.4	4.0	3.7	0.6	2.3	0.2	1.4
sur lie, no SO_2, no enzyme 15°C	11 months	0.9899	13.3	1.5	0.7	1.3	3.9	3.7	0.6	2.2	0.3	1.4
Control Wine 10° C	0	0.9901	13.2	1.0	0.6	0.6	5.0	3.5	0.4	1.8	1.7	n.n.
sur lie, SO_2 and enzyme 10°C	11 months	0.9907	13.1	1.6	0.8	1.0	5.1	3.6	0.4	1.8	1.8	n.n.
sur lie. SO_2, no enyzme 10°C	11 months	0.9904	13.2	1.0	0.7	0.8	5.1	3.6	0.4	1.8	1.9	n.n.
sur lie, no SO_2, enzyme 10°C	11 months	0.9900	13.2	1.9	0.9	1.4	3.9	3.7	0.6	2.1	0.3	1.4
sur lie, no SO_2, no enzyme 10°C	11 months	0.9897	13.3	1.4	0.8	1.2	3.9	3.7	0.5	2.1	0.2	1.3

Table 32: General composition of Grüner Veltliner wines fermented with the yeast Oenoferm Veltliner, stored on lees at 20°C, 15°C and 10°C, with and without sulphur dioxide, with and without the addition of enzymes

4.2 Free amino acids in Grüner Veltliner sur lie wines

4.2.1 Vintage 2006

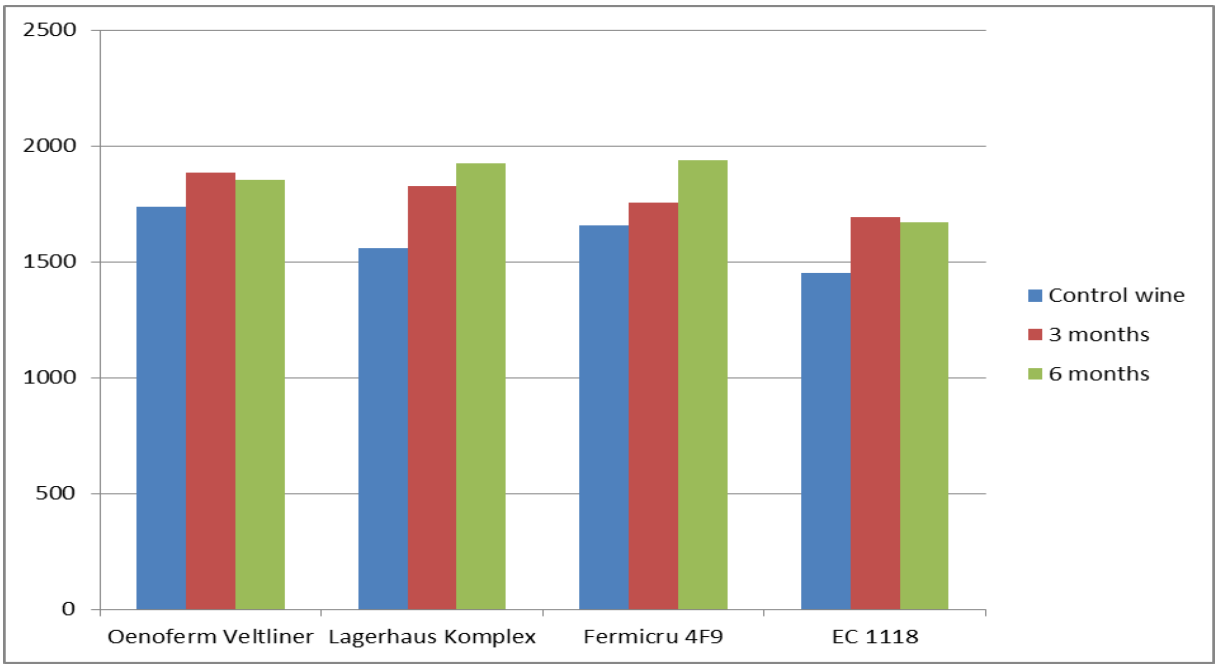

Figure 5: Total amino acids concentrations (mg/l) in Grüner Veltliner wines produced with the yeasts Oenoferm Veltliner, Lagerhaus Komplex, Fermicru 4F9 and EC 1118 at different stages of ageing on lees

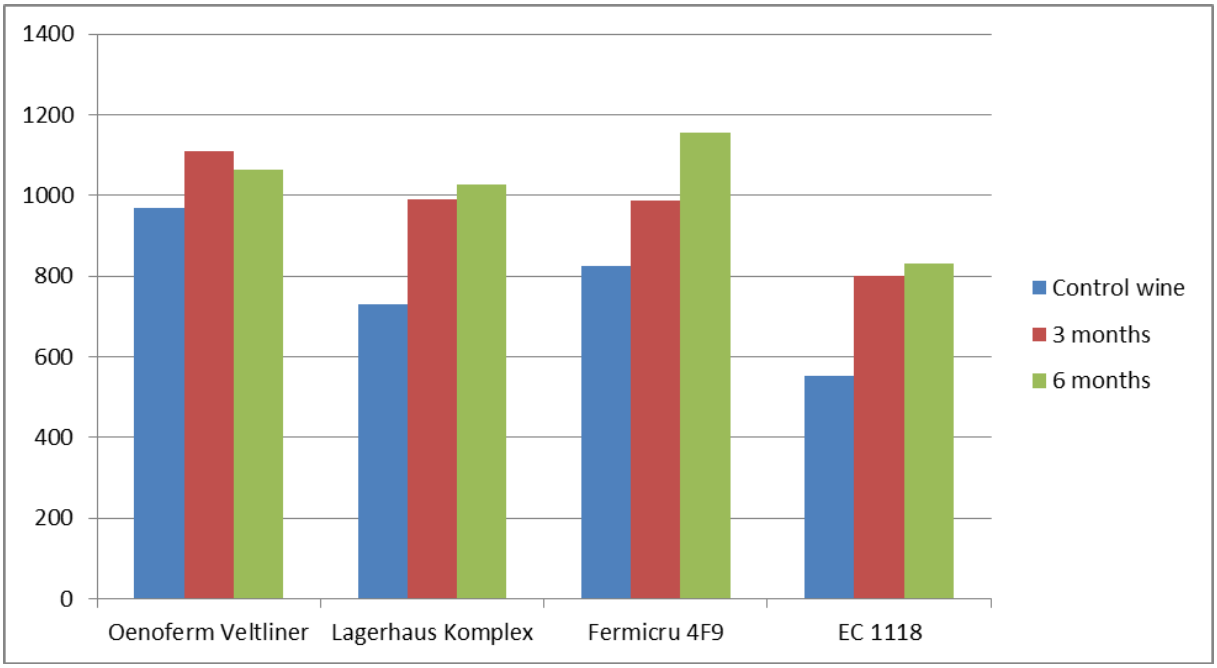

Figure 6: Total amino acids concentrations without Proline (mg/l) in Grüner Veltliner wines produced with the yeasts Oenoferm Veltliner, Lagerhaus Komplex, Fermicru 4F9 and EC 1118 at different stages of ageing on lees

The amino acid content of wines increased during the maturation on lees (Figure 5 and 6). Normally it was expected an increase of the amino acid content of wines aged on lees as

compared with the control wine as a result of autolysis of yeast cells. In general, the analytical data showed an increasing trend, but some exceptions are noticed. After fermentation yeast Oenoferm Veltliner presented the highest amount of free amino acids 968 mg/l followed by yeasts Fermicru 4F9 and Lagerhaus Komplex 825 mg/l respectively 730 mg/l. The lowest amount of free amino acids presented yeast EC 1118 551 mg/l. After 3 months contact with lees the biggest increase in amino acids presented the yeast EC 1118 (45% leading to a total content of 800 mg/l) followed by yeast Lagerhaus Komplex (35% leading to a total content of 989mg/l). For the yeasts Fermicru 4F9 and Oenoferm Veltliner the increase was lower (19% and 14% leading to a total content of 985 mg/l and 1110 mg/l). After 6 months ageing on lees the yeast Fermicru 4F9 was the only yeast with similar increase rate like in the first 3 months (17% leading to a total content of 1156 mg/ for the sum of free amino acids). The yeasts Lagerhaus Komplex and EC 1118 presented after 6 months of contact with lees a slightly similar increase. The sum of free amino acids showed after 6 months ageing on lees a slightly decrease for the yeast Oenoferm Veltliner.

	Content			ANOVA	ANOVA
Amino acid	Control wine	3 months	6 months	Yeast	Time
Aspartic acid	40.24	47.40	51.02	ns	$p < 0.05$
Serine	12.95	14.64	11.32	$p < 0.05$	$p < 0.05$
Asparagine	14.67	17.63	18.40	ns	$p < 0.05$
Glutamic acid	68.95	77.89	74.96	$p < 0.05$	$p < 0.05$
Glycine	20.05	21.19	24.15	$p < 0.05$	$p < 0.05$
Histidine	11.23	13.08	15.30	ns	$p < 0.05$
Glutamine	4.77	5.09	4.40	$p < 0.05$	ns
Arginine	451.14	497.99	428.44	$p < 0.05$	$p < 0.05$
Citrulline	7.69	7.91	8.35	$p < 0.05$	ns
Threonine	9.90	12.31	12.78	ns	$p < 0.05$
Alanine	67.87	75.69	63.05	ns	ns
Proline	769.72	774.72	788.47	$p < 0.05$	ns
ɣ Aminobutyric acid	124.31	130.28	127.53	$p < 0.05$	ns
Cysteine	2.01	2.55	2.91	$p < 0.05$	$p < 0.05$
Tyrosine	18.51	23.86	27.27	ns	$p < 0.05$
Valine	12.10	15.69	18.52	ns	$p < 0.05$
Methionine	8.08	9.93	12.11	ns	$p < 0.05$
Ornithine	8.74	9.09	10.93	$p < 0.05$	$p < 0.05$
Lysine	35.71	53.89	62.57	ns	$p < 0.05$
Isoleucine	6.93	10.89	13.63	ns	$p < 0.05$
Leucine	24.18	36.81	44.88	ns	$p < 0.05$
Phenylalanine	16.90	24.65	29.91	ns	$p < 0.05$
Tryptophan	1.30	1.89	2.37	ns	$p < 0.05$

Table 33: Amino acids concentration (mg/l) in Grüner Veltliner wines (2006) fermented with yeast Oenoferm Veltliner at different stages of maturation on lees

The changes in amino acids composition in wines produced with the Oenoferm Veltliner yeast were monitored during ageing on lees (Table 33). It is clear from the observation that the concentration of almost all amino acids increased during maturation process. Amino acids aspartic acid, asparagine, tyrosine, valine, lysine, isoleucine, leucine, phenylalanine and tryptophan doubled or almost doubled their concentration during maturation process.

Glycine, histidine, citruline, threonine, cysteine, methionine and ornithine slightly increased during ageing on lees. The amount of serine, glutamic acid, glutamine, arginine, alanine and ɣ aminobutyric acid slightly increased during the first period of ageing on lees and subsequently the amount decreased. The concentration of amino acids arginine and alanine presented during the second ageing period a heavy decrease, even lower than the initial concentration, this could be due to the fact that in all samples maturated 6 months on lees malolactic fermentation started spontaneously.

LAB require amino acids to grow. The effect of lactic acid bacteria strain on free amino acid content is little known. Besides autolysis, some other changes affect the amino acid content of wines. The uptake of amino acids by lactic acid bacteria for their growth – the needs are related to the species and strain. The decarboxylation of amino acids by lactic acid bacteria resulting biogenic amines. Adsorption on the lees and other reactions can also not be excluded.

	Content			ANOVA	ANOVA
Amino acid	Control wine	3 months	6 months	Yeast	Time
Aspartic acid	24.78	38.48	52.13	ns	$p < 0.05$
Serine	8.13	10.78	10.03	$p < 0.05$	$p < 0.05$
Asparagine	9.19	18.14	24.15	ns	$p < 0.05$
Glutamic acid	42.79	64.79	74.20	$p < 0.05$	$p < 0.05$
Glycine	17.91	23.02	26.34	$p < 0.05$	$p < 0.05$
Histidine	11.12	17.05	21.21	ns	$p < 0.05$
Glutamine	3.09	4.38	4.54	$p < 0.05$	ns
Arginine	280.33	372.81	271.70	$p < 0.05$	$p < 0.05$
Citrulline	7.98	9.49	9.91	$p < 0.05$	ns
Threonine	6.80	11.67	14.85	ns	$p < 0.05$
Alanine	76.43	87.47	92.43	ns	ns
Proline	827.41	838.19	895.66	$p < 0.05$	ns
ɣ Aminobutyric acid	121.43	122.22	122.79	$p < 0.05$	ns
Cysteine	2.20	2.42	2.93	$p < 0.05$	$p < 0.05$
Tyrosine	14.41	24.82	31.69	ns	$p < 0.05$
Valine	8.79	16.67	22.40	ns	$p < 0.05$
Methionine	7.10	9.60	14.29	ns	$p < 0.05$
Ornithine	22.23	20.73	34.74	$p < 0.05$	$p < 0.05$
Lysine	27.51	55.35	79.75	ns	$p < 0.05$
Isoleucine	5.28	10.48	16.26	ns	$p < 0.05$
Leucine	19.09	41.16	61.43	ns	$p < 0.05$
Phenylalanine	12.21	25.59	36.55	ns	$p < 0.05$
Tryptophan	1.23	2.60	3.54	ns	$p < 0.05$

Table 34: Amino acids concentration (mg/l) in Grüner Veltliner wines (2006) fermented with yeast Lagerhaus Komplex at different stages of maturation on lees

Table 34 shows the content of amino acids in wines produced with yeast Lagerhaus Komplex during maturation on lees. The concentration of amino acids aspartic acid, asparagine, glutamic acid, glycine, histidine, threonine, alanine, tyrosine, valine, methionine, ornithine, lysine, isoleucine, leucine, phenylalanine and tryptophan heavily increased during ageing on lees. The amount of glutamine, citrulline and cysteine slightly increased during contact with

lees. In the wines produced with yeast Lagerhaus Komplex only the concentration of amino acids serine and arginine increased during the first 3 months of ageing, then during the second period presented a heavy decrease. ɣ aminobutyric acid was the only one amino acid in the wines produced with yeast Lagerhaus Komplex which presented almost no variation during contact with lees.

Amino acid	Content			ANOVA	ANOVA
	Control wine	3 months	6 months	Yeast	Time
Aspartic acid	31.41	45.22	63.26	ns	$p < 0.05$
Serine	13.55	15.72	12.69	$p < 0.05$	$p < 0.05$
Asparagine	13.65	22.62	28.53	ns	$p < 0.05$
Glutamic acid	55.43	69.83	94.30	$p < 0.05$	$p < 0.05$
Glycine	20.50	23.74	26.83	$p < 0.05$	$p < 0.05$
Histidine	10.80	15.51	17.65	ns	$p < 0.05$
Glutamine	4.92	6.23	6.02	$p < 0.05$	ns
Arginine	359.11	392.97	418.65	$p < 0.05$	$p < 0.05$
Citrulline	8.82	9.53	8.87	$p < 0.05$	ns
Threonine	7.46	11.82	15.79	ns	$p < 0.05$
Alanine	55.88	62.38	80.47	ns	ns
Proline	830.41	771.20	779.30	$p < 0.05$	ns
ɣ Aminobutyric acid	127.38	123.09	125.82	$p < 0.05$	ns
Cysteine	2.00	2.33	2.85	$p < 0.05$	$p < 0.05$
Tyrosine	16.70	23.01	29.25	ns	$p < 0.05$
Valine	10.13	16.60	21.61	ns	$p < 0.05$
Methionine	4.89	8.75	11.42	ns	$p < 0.05$
Ornithine	11.11	10.53	26.72	$p < 0.05$	$p < 0.05$
Lysine	29.71	51.60	68.46	ns	$p < 0.05$
Isoleucine	5.05	10.04	13.60	ns	$p < 0.05$
Leucine	21.46	38.02	50.05	ns	$p < 0.05$
Phenylalanine	14.00	24.06	31.19	ns	$p < 0.05$
Tryptophan	1.47	2.25	2.76	ns	$p < 0.05$

Table 35: Amino acids concentration (mg/l) in Grüner Veltliner wines (2006) fermented with yeast Fermicru 4F9 at different stages of maturation on lees

The evolution of free amino acids in the wines produced with yeast Fermicru 4F9, a special yeast for ageing on lees (Table 35) showed an increasing trend during maturation process. Amino acids aspartic acid, asparagine, glutamic acid, histidine, threonine, alanine, tyrosine, valine, methionine, lysine, isoleucine, leucine, phenylalanine, tryptophan doubled or almost doubled their concentration during ageing process. It can be observed that for yeast Fermicru 4F9 only four amino acids (glycine, glutamine, arginine and cysteine) presented small changes during maturation process, their concentration increased slightly. The concentration of amino acids serine and citrulline increased during the first 3 months of ageing then during the second period presented a slight decrease. The concentration of serine after 6 months of ageing on lees was lower than in the control wine. The concentration of citrulline was the same in control wine and in wine with 6 months lees contact. Ornithine was the only one amino acid which presented a light decrease during the first 3 months, then

during the second period of ageing a heavy increase 140%. ɣ aminobutyric was the only one amino acid in the wines produced with yeast Fermicru 4F9 which presented small changes during maturation process. During the first period of ageing the concentration decreased and during the second ageing period the concentration increased slightly. The amount of ɣ aminobutyric was lower after 6 months of ageing than in the control wine.

Amino acid	Content			ANOVA	ANOVA
	Control wine	3 months	6 months	Yeast	Time
Aspartic acid	25.54	37.32	51.41	ns	$p < 0.05$
Serine	13.44	17.13	16.32	$p < 0.05$	$p < 0.05$
Asparagine	11.32	17.14	21.69	ns	$p < 0.05$
Glutamic acid	38.77	54.90	67.69	$p < 0.05$	$p < 0.05$
Glycine	17.17	20.40	22.91	$p < 0.05$	$p < 0.05$
Histidine	10.38	15.30	19.51	ns	$p < 0.05$
Glutamine	4.28	3.78	4.55	$p < 0.05$	ns
Arginine	128.27	215.84	138.13	$p < 0.05$	$p < 0.05$
Citrulline	6.06	6.58	7.79	$p < 0.05$	ns
Threonine	6.60	10.29	13.56	ns	$p < 0.05$
Alanine	59.64	73.05	78.68	ns	ns
Proline	900.33	893.46	840.04	$p < 0.05$	ns
ɣ Aminobutyric acid	113.29	122.30	115.69	$p < 0.05$	ns
Cysteine	2.28	2.74	3.02	$p < 0.05$	$p < 0.05$
Tyrosine	16.00	24.16	30.24	ns	$p < 0.05$
Valine	9.36	17.15	21.83	ns	$p < 0.05$
Methionine	5.11	10.18	13.56	ns	$p < 0.05$
Ornithine	12.34	13.28	17.86	$p < 0.05$	$p < 0.05$
Lysine	28.56	55.13	74.69	ns	$p < 0.05$
Isoleucine	5.28	11.18	15.20	ns	$p < 0.05$
Leucine	22.10	43.46	58.84	ns	$p < 0.05$
Phenylalanine	14.17	26.37	35.36	ns	$p < 0.05$
Tryptophan	1.60	2.70	3.40	ns	$p < 0.05$

Table 36: Amino acids concentration (mg/l) in Grüner Veltliner wines (2006) fermented with yeast EC 1118 at different stages of maturation on lees

Table 36 shows the evolution of free amino acids during ageing on lees in wines produced with yeast EC 1118. After 6 months of ageing on lees, amino acids aspartic acid, asparagine, glutamic acid, histidine, threonine, alanine, tyrosine, valine, methionine, ornithine, lysine, isoleucine, leucine, phenylalanine and tryptophan doubled or almost doubled their concentration. It can be noticed that for yeast EC 1118 amino acids glycine, citrulline and cysteine presented small changes during the maturation process, their concentration increased slightly. The concentration of serine, arginine and ɣ aminobutyric acid increased slightly during the first period of ageing, but during the second period their concentration decreased.

Univariate analysis of variance (ANOVA) was carried out to examine significant differences between amino acids concentration due to two factors: time and yeast strain. The factor

yeast strain influenced significantly ($p < 0.05$) the following amino acids serine, glutamic acid, glycine, glutamine, arginine, citrulline, proline, ɣ Aminobutyric acid, cysteine and ornithine. The factor time displayed significant differences in amino acid concentration with some exceptions: glutamine, citrulline, alanine, ɣ aminobutyric acid, cysteine.

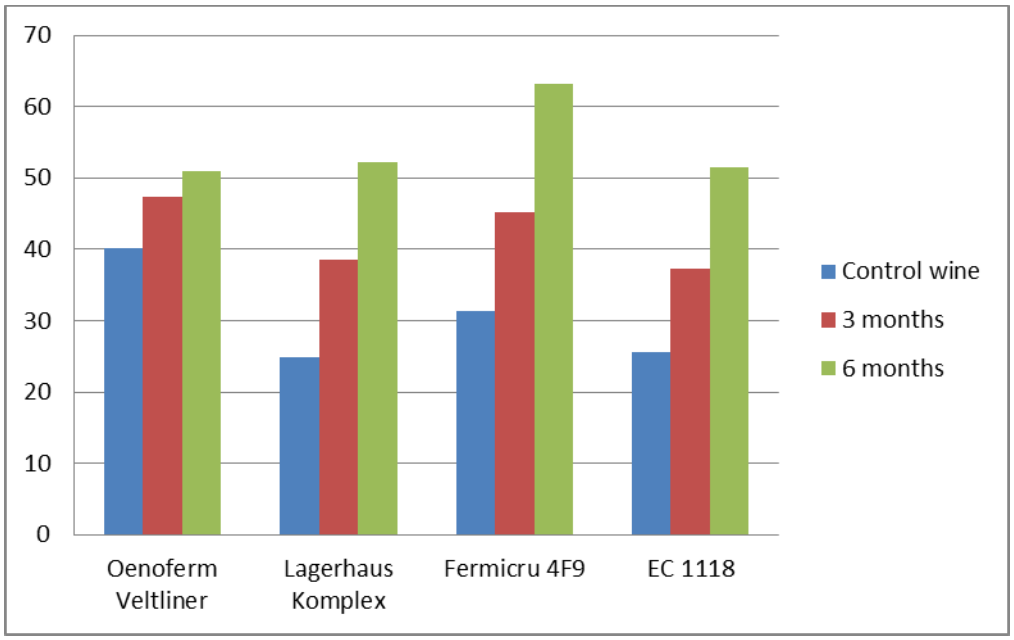

Figure 7: Aspartic acid concentrations (mg/l) in Grüner Veltliner wines produced with the yeasts Oenoferm Veltliner, Lagerhaus Komplex, Fermicru 4F9 and EC 1118 at different stages of ageing on lees

The yeast Oenoferm Veltliner absorbed the lowest amount of aspartic acid during the fermentation followed by yeast Fermicru 4F9 (Figure 7). The yeasts Lagerhaus Komplex and EC 1118 absorbed almost the same quantity of aspartic acid. The yeast Oenoferm Veltliner presented the lowest rate of changes with the milieu.

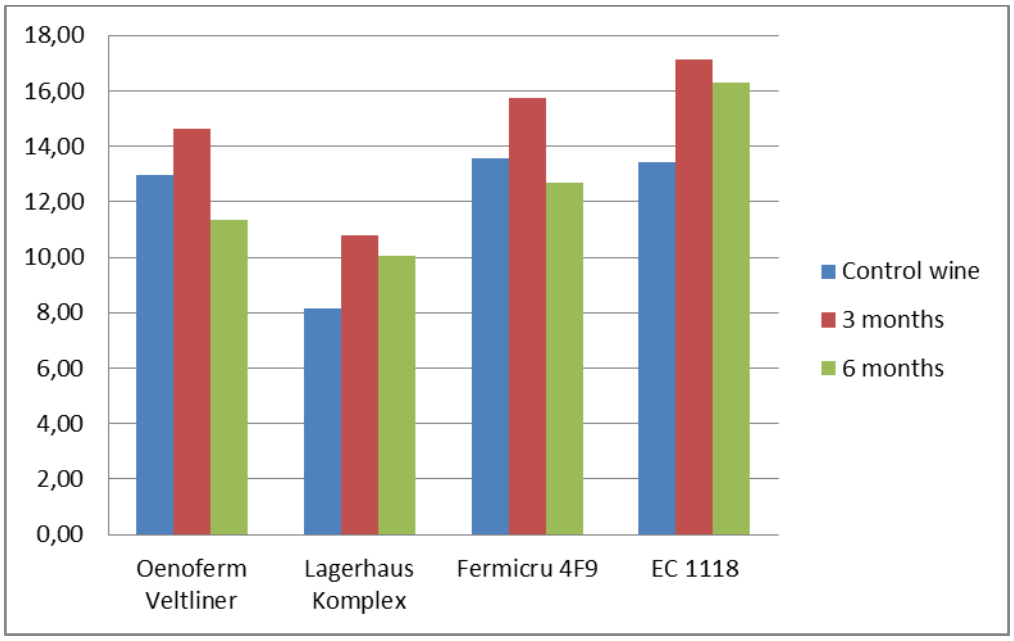

Figure 8: Serine concentrations (mg/l) in Grüner Veltliner wines produced with the yeasts Oenoferm Veltliner, Lagerhaus Komplex, Fermicru 4F9 and EC 1118 at different stages of ageing on lees

The yeast Lagerhaus Komplex took up the lowest amount of serine during the fermentation. The other three yeasts used almost the same quantity of serine during the fermentation. After 3 months of storage on lees the amount of serine increased in all the samples. After 6 months of storage on lees in the samples produced with Oenoferm Veltiner and Fermicru 4F9 the serine content presented a heavy decrease. For the yeasts Lagerhaus Komplex and EC 1118 just a slight decrease was observed. In all samples the malolactic fermentation started spontaneously, therefore different bacteria with different population fermented malolactic the wines. This can explain the decrease difference of serine.

4.2.2 Vintage 2007

Figures 9 and 10 show the total concentration of free amino acids at different stages of ageing in the wine produced with the yeast Oenoferm Veltliner, in comparison with the wine produced with the yeast EC 1118. Proline levels were not included in the total amount of amino acids represented in the Figure 10, but were considered in the results listed in Table 37 and Table 38. At the end of fermentation, the wine produced with Oenoferm Veltliner had higher amounts of free amino acids (167 mg/l) compared to the wine produced with the yeast EC 1118 (97 mg/l). This indicates either a lower rate of nitrogen uptake by the yeast Oenoferm Veltliner or advanced cell death and autolysis phenomenon. Alternatively, exsorption from still living cells could also be a reason. The concentration of total free amino acids increased generally for both yeasts during maturation, whereas the development was faster during the first six weeks of ageing on lees and subsequently declined. The augmentation of amino acids was lower for Oenoferm Veltliner (increase by 34%, leading to a total content of 225 mg/l) and higher for the yeast EC 1118 (increase by 101%, leading to a total content of 195 mg/l). After five months ageing on lees, the increase of free amino acids was similar for the two yeasts applied, 37% for Oenoferm Veltliner leading to a total content of 307 mg/l and 36% for EC 1118 leading to a total concentration of 266 mg/l.

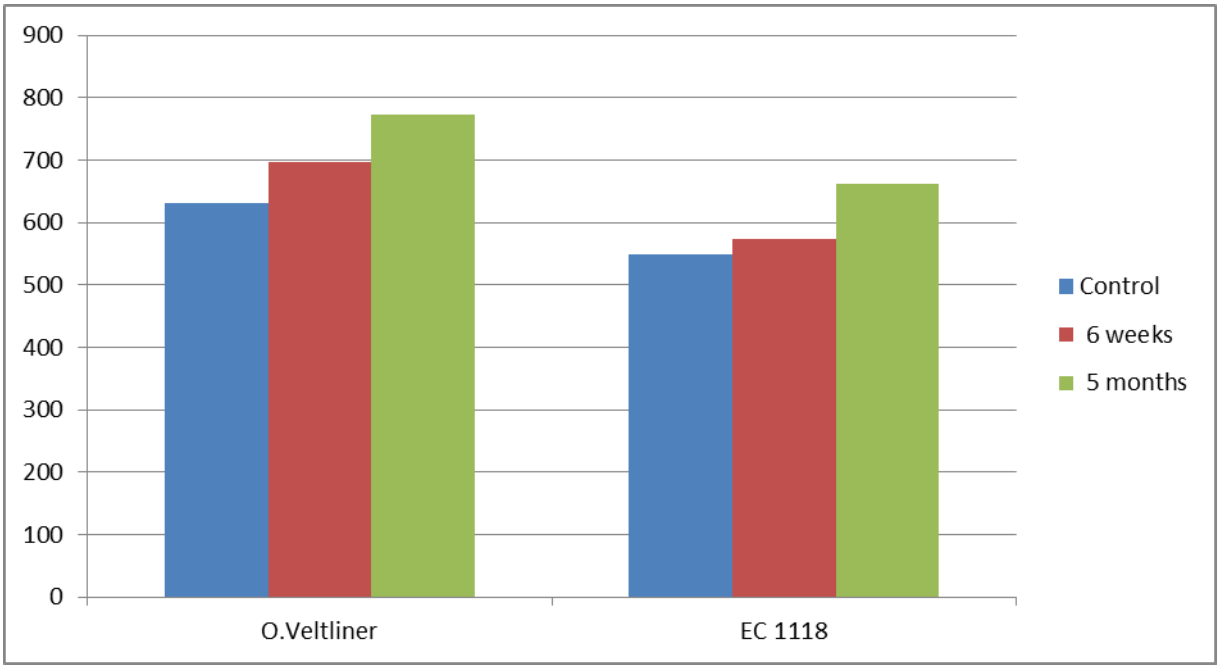

Figure 9: Total amino acid concentrations with proline (mg/l) in wines produced with yeast Oenoferm Veltliner and yeast EC 1118 at different stages of ageing on lees with no enzyme addition

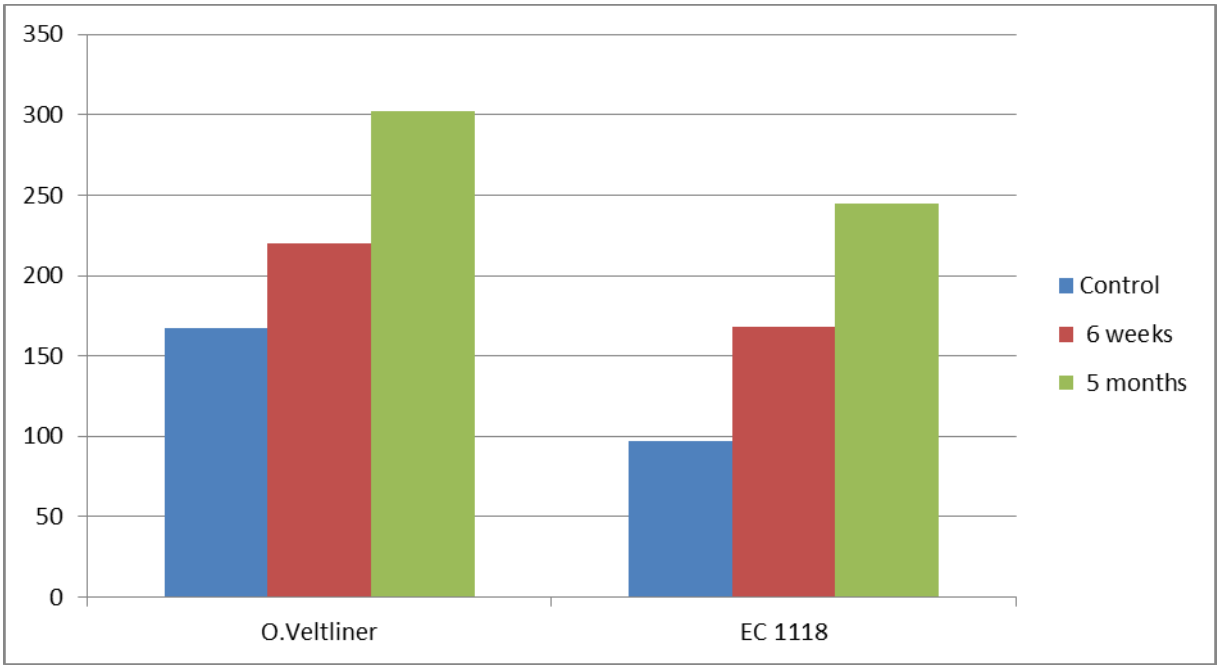

Figure 10: Total amino acid concentrations without proline (mg/l) in wines produced with yeast Oenoferm Veltliner and yeast EC 1118 at different stages of ageing on lees with no enzyme addition

Content [mg/l]										ANOVA		
Amino acids	***Control***	***No enzyme 6w***	***No ezyme 5m***	***Littozym 6w***	***Littozym 5m***	***Rapidase 6w***	***Rapidase 5m***	***Vinoflow 6w***	***Vinoflow 5m***	**Time**	**Yeast**	**Enzyme**
Aspartic acid	11,70	15,57	21,65	15,67	21,45	15,73	21,61	15,38	21,37	$p < 0,05$	$p < 0,05$	ns
Serine	6,01	7,21	9,04	6,64	9,87	7,38	9,89	7,48	10,02	$p < 0,05$	$p < 0,05$	$p < 0,05$
Asparagine	5,66	7,27	10,61	6,96	10,69	7,12	10,45	7,00	10,02	$p < 0,05$	$p < 0,05$	ns
Glutamic acid	27,76	31,50	38,61	32,44	39,35	32,22	39,58	31,37	39,31	$p < 0,05$	$p < 0,05$	ns
Glycine	6,00	7,14	8,97	6,94	8,85	7,10	8,94	6,95	8,83	$p < 0,05$	$p < 0,05$	ns
Histidine	7,40	9,19	12,05	9,63	12,35	9,71	12,30	9,57	12,39	$p < 0,05$	$p < 0,05$	$p < 0,05$
Glutamine	1,95	3,01	3,44	2,68	3,67	3,06	3,58	2,94	3,79	$p < 0,05$	$p < 0,05$	ns
Arginine	14,54	22,40	31,64	23,02	32,13	23,52	32,13	23,66	32,91	$p < 0,05$	$p < 0,05$	ns
Threonine	3,58	4,59	6,55	4,39	6,69	4,60	6,78	4,70	6,81	$p < 0,05$	$p < 0,05$	ns
Alanine	14,98	17,24	21,61	17,81	21,51	17,57	22,01	17,08	22,08	$p < 0,05$	$p < 0,05$	ns
Proline	462,70	475,96	470,48	458,98	480,64	468,18	477,21	455,44	488,14	ns	$p < 0,05$	ns
ɣ Aminobutyric acid	5,65	6,48	6,99	6,37	6,66	5,97	6,43	5,79	6,64	$p < 0,05$	$p < 0,05$	ns
Cysteine	1,14	1,57	1,76	1,52	1,86	1,52	1,82	1,48	2,07	$p < 0,05$	$p < 0,05$	ns
Tyrosine	9,33	11,44	16,45	11,71	16,37	11,94	16,42	11,74	16,84	$p < 0,05$	$p < 0,05$	ns
Valine	4,47	6,53	9,90	6,60	9,92	6,93	10,13	6,84	10,28	$p < 0,05$	$p < 0,05$	$p < 0,05$
Methionine	3,27	4,73	6,96	4,69	7,20	4,84	7,24	4,85	7,06	$p < 0,05$	$p < 0,05$	ns
Ornithine	1,01	0,88	1,96	0,87	2,01	0,89	2,09	0,87	2,08	$p < 0,05$	ns	ns
Lysine	17,05	26,18	37,87	26,62	39,40	27,04	39,31	27,25	39,88	$p < 0,05$	$p < 0,05$	$p < 0,05$
Isoleucine	2,74	4,16	6,66	4,38	6,61	4,61	7,01	4,61	6,55	$p < 0,05$	$p < 0,05$	ns
Leucine	13,67	20,29	29,79	20,37	30,01	21,54	29,99	21,41	29,39	$p < 0,05$	$p < 0,05$	ns
Phenylalanine	8,56	12,00	18,21	12,34	18,03	12,44	18,29	12,70	18,81	$p < 0,05$	$p < 0,05$	$p < 0,05$
Tryptophan	0,99	1,49	1,88	1,54	1,87	1,56	1,81	1,66	1,96	$p < 0,05$	$p < 0,05$	$p < 0,05$

Table 37: Amino acids concentration (mg/l) in Grüner Veltliner wines (2007) fermented with yeast Oenoferm Veltliner at different stages of maturation on lees with different enzymes

Content [mg/l]										ANOVA		
Amino acids	***Control***	***No enzyme 6w***	***No ezyme 5m***	***Littozym 6w***	***Littozym 5m***	***Rapidase 6w***	***Rapidase 5m***	***Vinoflow 6w***	***Vinoflow 5m***	**Time**	**Yeast**	**Enzyme**
Aspartic acid	6,27	11,52	17,81	12,79	18,27	12,33	17,98	13,64	19,59	$p < 0,05$	$p < 0,05$	ns
Serine	4,11	5,65	7,57	6,11	8,33	6,50	8,48	7,33	9,52	$p < 0,05$	$p < 0,05$	$p < 0,05$
Asparagine	3,25	5,39	9,40	5,88	8,84	6,11	9,16	6,96	9,88	$p < 0,05$	$p < 0,05$	ns
Glutamic acid	20,83	25,26	33,52	28,92	36,27	28,62	35,87	29,51	37,53	$p < 0,05$	$p < 0,05$	ns
Glycine	3,72	5,23	7,93	5,81	7,47	6,04	7,66	6,46	8,46	$p < 0,05$	$p < 0,05$	ns
Histidine	5,22	7,61	10,72	8,59	11,28	8,93	11,14	8,62	11,11	$p < 0,05$	$p < 0,05$	$p < 0,05$
Glutamine	1,39	2,26	2,99	2,35	3,23	2,20	3,12	2,54	3,24	$p < 0,05$	$p < 0,05$	ns
Arginine	6,94	15,69	18,57	18,33	20,44	18,51	20,87	20,11	25,68	$p < 0,05$	$p < 0,05$	ns
Threonine	2,08	3,60	5,88	4,16	5,87	4,03	6,06	4,23	6,48	$p < 0,05$	$p < 0,05$	ns
Alanine	9,73	14,16	18,64	15,24	19,41	16,06	19,73	16,86	21,28	$p < 0,05$	$p < 0,05$	ns
Proline	450,99	405,12	415,33	426,19	442,81	447,81	447,31	465,42	467,71	ns	$p < 0,05$	ns
ɣ Aminobutyric acid	4,16	6,46	7,13	7,04	7,49	7,29	7,52	7,57	8,16	$p < 0,05$	$p < 0,05$	ns
Cysteine	0,79	1,29	1,61	1,37	1,56	1,33	1,57	1,21	1,52	$p < 0,05$	$p < 0,05$	ns
Tyrosine	5,61	9,03	13,71	9,72	14,11	10,75	14,21	10,75	14,63	$p < 0,05$	$p < 0,05$	ns
Valine	2,06	4,88	7,88	5,46	8,17	5,46	8,23	5,69	8,80	$p < 0,05$	$p < 0,05$	$p < 0,05$
Methionine	1,55	3,67	5,60	3,81	5,99	3,95	5,93	4,13	6,15	$p < 0,05$	$p < 0,05$	ns
Ornithine	0,42	0,46	2,48	0,67	2,67	0,61	2,51	0,74	2,36	$p < 0,05$	ns	ns
Lysine	7,43	18,68	30,49	21,76	33,15	21,86	33,25	23,52	35,36	$p < 0,05$	$p < 0,05$	$p < 0,05$
Isoleucine	1,18	2,95	5,31	3,38	5,45	3,41	5,47	3,64	6,21	$p < 0,05$	$p < 0,05$	ns
Leucine	5,63	14,03	22,51	15,88	23,99	16,01	24,02	18,09	26,31	$p < 0,05$	$p < 0,05$	ns
Phenylalanine	4,64	8,97	14,41	9,97	15,18	10,26	15,30	10,98	16,09	$p < 0,05$	$p < 0,05$	$p < 0,05$
Tryptophan	0,66	1,25	1,60	1,41	1,60	1,50	1,72	1,59	1,79	$p < 0,05$	$p < 0,05$	$p < 0,05$

Table 38: Amino acids concentration (mg/l) in Grüner Veltliner wines (2007) fermented with yeast EC 1118 at different stages of maturation on lees with different enzymes

The levels of the individual free amino acids in wines produced with yeast Oenoferm Veltliner and EC 1118 after six weeks and after five months of storage on lees respectively are listed in Tables 37 and 38. Only proline, which is the most abundant amino acid in wine, showed almost no variation, as it cannot be utilized as a source of nitrogen assimilation (Flanzy, 1998). It is evident from our observations that for Oenoferm Veltliner the amino acids arginine, valine, methionine, lysine, isoleucine, leucine and phenylalanine increased by more than 100% during contact with yeast. Aspartic acid, asparagine, glutamine, threonine, tyrosine, ornithine and tryptophan increased by more than 70%. Serine, glutamic acid, glycine, histidine, alanine and cysteine increased by more than 40% during ageing process. The lowest increase was presented by gamma-aminobutyric acid only 23%.

The highest increase for the yeast EC 1118 was presented by amino acid ornithine 450%. Lysine, isoleucine and leucine increased by more than 300%. Valine, methionine and phenylalanine increased by more than 200% during lees contact. Aspartic acid, asparagine, arginine and threonine increased by more than 150%. Glycine, histidine, glutamine, cysteine, tyrosine and tryptophan increased by more than 100%. The amino acids serine, glutamic acid, alanine and gamma-aminobutyric presented the lowest increase during ageing process only by more than 50%.

During the ageing on lees the increase of amino acid concentrations was different for the two yeasts used. Gamma-amino butyric acid was the only amino acid that did not significantly differ between the two yeasts applied ($P<0.05$).

Univariate analysis of variance (ANOVA) was carried out to examine significant differences between amino acids concentration due to three factors: time, yeast strain and enzyme. The factor yeast strain influenced significantly ($p < 0.05$) all examined amino acids with one exception: amino acid ornithine. As expected, proline was the only amino acid which was not influenced by the factor time. The factor enzyme displayed significant differences for the following amino acids: serine, histidine, valine, lysine, phenylalanine and tryptophan.

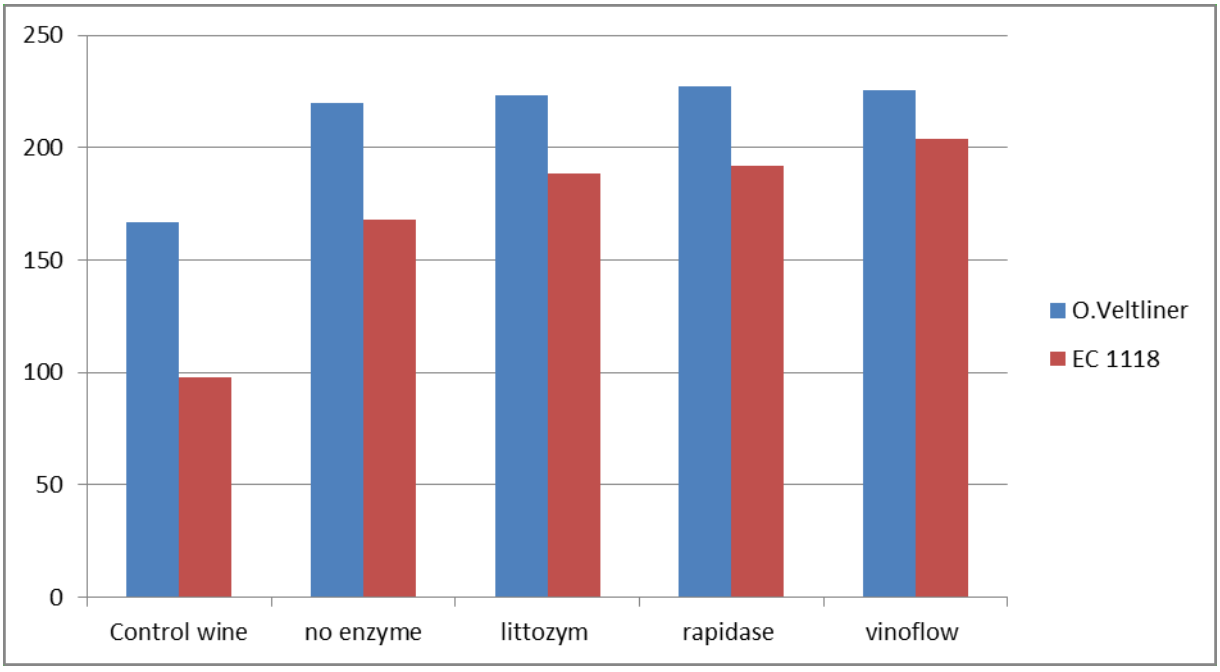

Figure 11: Total concentration of amino acids (mg/l) in wines produced with yeast Oenoferm Veltliner and yeast EC 1118 and different enzymes after 6 weeks of contact with lees

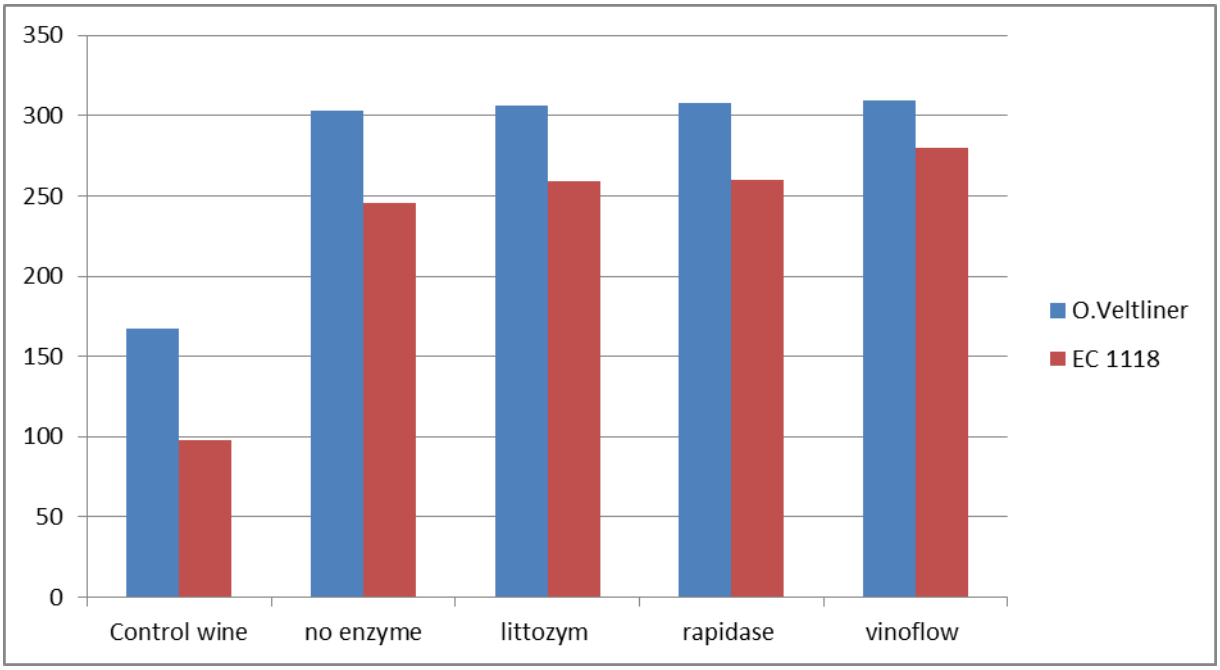

Figure 12: Total concentration of amino acids (mg/l) in wines produced with yeast Oenoferm Veltliner and yeast EC 1118 and different enzymes after 5 months of contact with lees

The total amount of amino acids is increased already after 6 weeks of contact with the lees. The results show that the enzymes did not greatly affect the content of amino acids during the ageing on lees. For the yeast Oenoferm Veltliner there are no significant differences in total amino acids between the samples with enzyme treatment and the sample without enzyme. For the yeast EC 1118 (Figures 11 and 12) the samples with different enzymes preparation presented slightly more free amino acids than the sample with no enzyme. The data suggests that the yeasts have different capacities to valorise amino acids and also different rates and capacities to release amino acids during autolysis. In such cases the addition of an enzyme with beta-glucanase activity like Vinoflow has positive effect on the release of amino acids during the contact with lees.

4.2.3 Vintage 2008

It is evident for Figures 13 and 14 that the samples with fine lees presented after 8 months of sur lie ageing the highest amount of total amino acids at both temperature levels. At 15°C, the sample with fine lees increased by 34%, leading to a total content of 1084 mg/l. At 22°C, the increase was even greater at 51%, leading to a total content of 1188 mg/l. The sample with crude lees 3 g/l showed after the ageing period (816 mg/l) almost the same amount of total free amino acids like the control wine (803 mg/l) at 15°C. At 22°C, the sample with crude lees presented slightly more free amino acids than the control wine. As expected, the augmentation of amino acids was higher with double amount of lees 6 g/l at both temperature levels, but lower than the sample with fine lees.

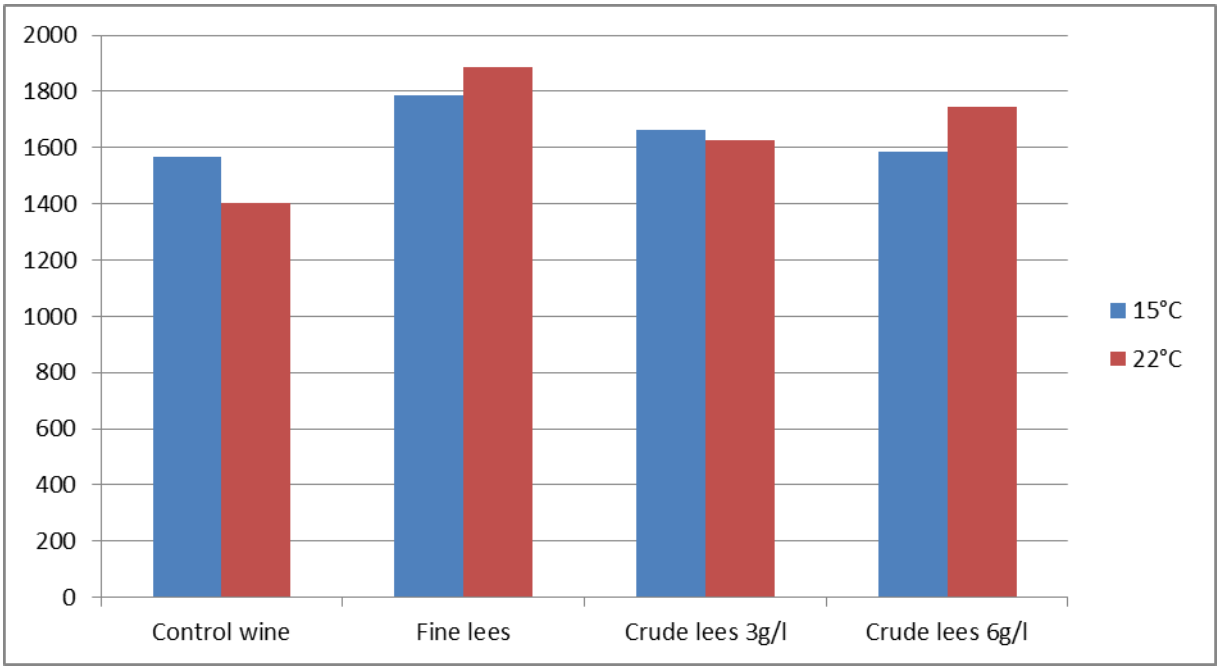

Figure 13: Total concentration of free amino acids with proline in sur lie wines (8 months lees contact), fermented with yeast Fermicru 4F9 stored at 15°C and 22°C with different lees quantity and quality

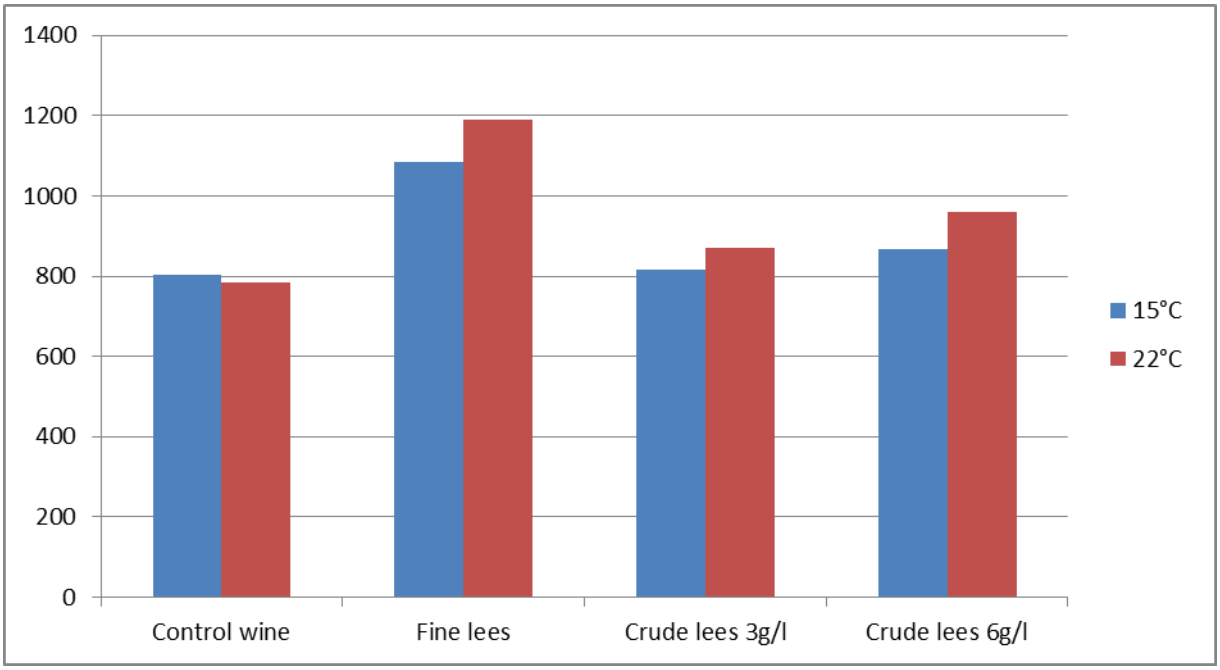

Figure 14: Total concentration of free amino acids without proline in sur lie wines (8 months lees contact), fermented with yeast Fermicru 4F9 stored at 15°C and 22°C with different lees quantity and quality

		Content [mg/l]				ANOVA	ANOVA
Amino acid	**Control wine**	**Control wine + Batonnage Plus Elevace**	**Fine lees**	**Crude lees 3g/l**	**Crude lees 6g/l**	**Lees quantity/quality**	**Temperatur/MLF**
Aspartic acid	36.79	52.74	60.40	45.98	52.55	$p < 0.05$	$p < 0.05$
Serine	18.44	20.52	28.91	16.04	21.40	$p < 0.05$	$p < 0.05$
Asparagine	8.66	7.61	27.57	13.88	14.50	$p < 0.05$	$p < 0.05$
Glutamic acid	42.75	57.74	64.93	51.42	55.75	$p < 0.05$	$p < 0.05$
Glycine	17.96	21.29	26.19	22.73	23.22	$p < 0.05$	$p < 0.05$
Glutamine	1.37	1.833	1.98	0.83	1.30	$p < 0.05$	ns
Arginine	144.63	106.43	160.36	142.24	129.90	$p < 0.05$	$p < 0.05$
Threonine	18.37	12.63	28.19	26.99	27.13	$p < 0.05$	ns
Alanine	40.79	45.34	55.00	41.95	46.99	$p < 0.05$	$p < 0.05$
Proline	721.54	731.191	650.68	782.29	670.82	ns	ns
Hydroxyproline	41.20	56.483	52.93	65.61	46.51	$p < 0.05$	$p < 0.05$
Tyrosine	275.75	279.34	288.81	270.67	274.31	$p < 0.05$	$p < 0.05$
Valine	9.87	8.91	18.55	8.79	11.18	$p < 0.05$	ns
Methionine	16.68	16.54	17.83	13.04	13.97	ns	ns
Ornithine	16.99	36.96	17.39	14.99	18.32	ns	$p < 0.05$
Lysine	90.79	75.49	148.44	77.00	87.40	$p < 0.05$	ns
Isoleucine	7.79	9.85	19.10	8.97	12.12	$p < 0.05$	$p < 0.05$
Leucine	25.83	30.91	59.80	29.73	38.02	$p < 0.05$	$p < 0.05$
Phenylalanine	18.74	20.56	38.40	19.60	24.64	$p < 0.05$	$p < 0.05$
Tryptophan	11.06	13.55	22.72	11.95	15.86	$p < 0.05$	$p < 0.05$

Table 39: Free amino acids in sur lie wines (8 months lees contact), fermented with yeast Fermicru 4F9, stored at 15°C with different lees quantity and quality

		Content [mg/l]			ANOVA	ANOVA
Amino acid	**Control wine**	**Fine lees**	**Crude lees 3g/l**	**Crude lees 6g/l**	**Lees quantity/quality**	**Temperature/ MLF**
Aspartic acid	46.40	76.51	53.07	60.29	$p < 0.05$	$p < 0.05$
Serine	16.76	42.40	25.97	31.62	$p < 0.05$	$p < 0.05$
Asparagine	7.01	16.61	10.96	12.72	$p < 0.05$	$p < 0.05$
Glutamic acid	44.18	76.23	56.85	63.19	$p < 0.05$	$p < 0.05$
Glycine	18.86	31.81	23.36	25.66	$p < 0.05$	$p < 0.05$
Glutamine	1.01	1.82	1.40	1.70	$p < 0.05$	ns
Arginine	135.43	148.65	120.78	129.65	$p < 0.05$	$p < 0.05$
Threonine	12.53	26.80	16.54	19.75	$p < 0.05$	ns
Alanine	43.38	70.16	52.51	58.52	$p < 0.05$	$p < 0.05$
Proline	592.38	663.42	722.13	752.21	ns	ns
Hydroxyproline	25.42	33.59	32.66	32.77	$p < 0.05$	$p < 0.05$
Tyrosine	278.00	297.55	283.72	288.86	$p < 0.05$	$p < 0.05$
Valine	7.86	20.43	10.53	13.15	$p < 0.05$	ns
Methionine	14.79	15.69	15.19	15.78	ns	ns
Ornithine	20.83	38.16	34.15	35.85	ns	$p < 0.05$
Lysine	72.28	149.50	77.00	91.51	$p < 0.05$	ns
Isoleucine	7.45	22.79	10.69	14.25	$p < 0.05$	$p < 0.05$
Leucine	25.93	76.02	38.02	47.37	$p < 0.05$	$p < 0.05$
Phenylalanine	20.58	49.16	26.47	32.01	$p < 0.05$	$p < 0.05$
Tryptophan	10.92	28.19	14.57	18.57	$p < 0.05$	$p < 0.05$

Table 40: Free amino acids in sur lie wines (8 months lees contact), fermented with yeast Fermicru 4F9, stored at 22°C with different lees quantity and quality

The evolution of free amino acids in sur lie wines stored at 15°C is presented in Table 39. Asparagine showed the greatest evolution, after 8 month lees contact the content was 220% higher in the wine with fine lees than in the control wine. The samples with 3 g/l and 6 g/l crude lees presented much lower levels of asparagine, but still more than the control wine. Amino acids isoleucine, leucine, phenylalanine and tryptophan increased by more than 100% in the wine with fine lees. Aspartic acid, glutamic acid, glycine, threonine, alanine, serine, glutamine, valine and lysine increased between 34% and 80% in the wine with fine lees after the ageing period. Methione and ornithine presented the lowest increase 7% respectively 2%. The levels of amino acids aspartic acid, asparagine, glutamic acid, glycine, threonine, isoleucine and leucine were higher in the wine with 3 g/l crude lees than in the control wine after 8 months sur lie ageing. The levels of amino acids arginine, alanine, phenylalanine and tryptophan were in the wine with 3 g/l crude lees, after 8 months equal or almost equal with the amounts from the control wine. Serine, glutamine, valine, methione, ornithine and lysine presented lower amounts than in the control wine. In the wine with 6 g/l crude lees the amino acids aspartic acid, serine, asparagine, glutamic acid, glycine, threonine, alanine, valine, ornithine, isoleucine, leucine, phenylalanine and tryptophan presented higher levels than in the control wine. Only the concentration of glutamine was like in the control wine. Arginine, methionine and lysine showed lower levels after 8 months of lees contact than the levels from the control wine. The wine treated with Batonnage plus Elevage showed minor differences regarding the amino acids concentration compared to the control wine. The following amino acids presented slightly higher concentration in the wine treated with Batonnage plus Elevage: aspartic acid, glutamic acid, glutamine, alanine, hydroxyproline. Arginine, threonine and lysine showed lower concentration in the wine treated with Batonnage plus Elevage than in the control wine. The amino acid composition of this wine was not comparable to the sur lie wines.

The changes in the free amino acids concentration during ageing on lees at 22°C are showed in Table 40. The wine with fine lees stored at 22°C presented itself similar to the wine with fine lees from 15°C the highest levels of free amino acids. The amino acids isoleucine and leucine presented the highest increase after the ageing period. Isoleucine increased by more than 200% and lecine increased by more than 190%. The concentration of serine, valine and tryptophan increased by more than 150% in the wine with fine lees after 8 months sur lie ageing. Asparagine, threonine, lysine and phenylalanine increased by more than 100%. Amino acids aspartic acid, glutamic acid, glycine, glutamine, alanine and ornithine presented increases between 61% and 90%. The concentration of methionine showed the lowest increase during ageing process 6%. The increase of arginine was in the wine with fine lees at both temperature levels 10%. The concentrations of aspartic acid, serine, asparagine, glutamic acid, glycine, glutamine, threonine, alanine, valine, methionine, ornithine, lysine, isoleucine, leucine, phenylalanine and tryptophan were slightly higher in the wine with 3 g/l crude less than in the control wine. Arginine concentration was lower in the wine with 3 g/l crude lees than in the control wine. In the wine with 6 g/l crude lees at 22°C only arginine presented lower concentration than the control wine.

Univariate analysis of variance (ANOVA) was carried out to examine significant differences between amino acids concentration due to two factors: quantity/quality and temperature/MLF (malolactic fermentation).

The factor quantity/quality displayed significant differences ($p < 0.05$) in amino acid concentration with the following exceptions: proline, methionine and ornithine.

Amino acids glutamine, threonine, proline, valine, methionine and lysine were not significantly influenced by the factor temperature/MLF.

4.2.4 Vintage 2009

The total concentration of free amino acids was almost equal in control wines stored at 20°C and 15°C (627 mg/l respectively 600 mg/l).The total concentration of free amino acids was 26% higher in control wines stored at 10°C, 758 mg/l. A possible explanation for this fact can be the Strecker degradation. The Strecker degradation is temperature dependent reaction therefore at 10°C takes place slower that at 20°C. All the wine samples stored in contact with lees presented more free amino acids than the control wines. The sur lie samples with SO_2 and enzyme addition stored at 20°C and 10°C presented slightly more free amino acids (891 mg/l respectively 865 mg/l) than the samples stored at 15°C (797 mg/l). The sur lie samples with SO_2 addition but no enzyme stored at 20°C showed higher concentration of free amino acids (898 mg/l) than the samples stored at 15°C and 10°C (833 mg/l respectively 817 mg/l). The sur lie wines stored without SO_2 but with enzyme addition presented the same level of total free amino acids at all three temperature levels 880 mg/l. The sur lie samples without SO_2 and without enzyme addition stored at 20°C showed the highest concentration of free amino acids (974 mg/l). The sur lie samples without SO_2 and without enzyme addition stored at 15°C and 10°C presented almost the same quantity of free amino acids (887 mg/l respectively 870 mg/l).

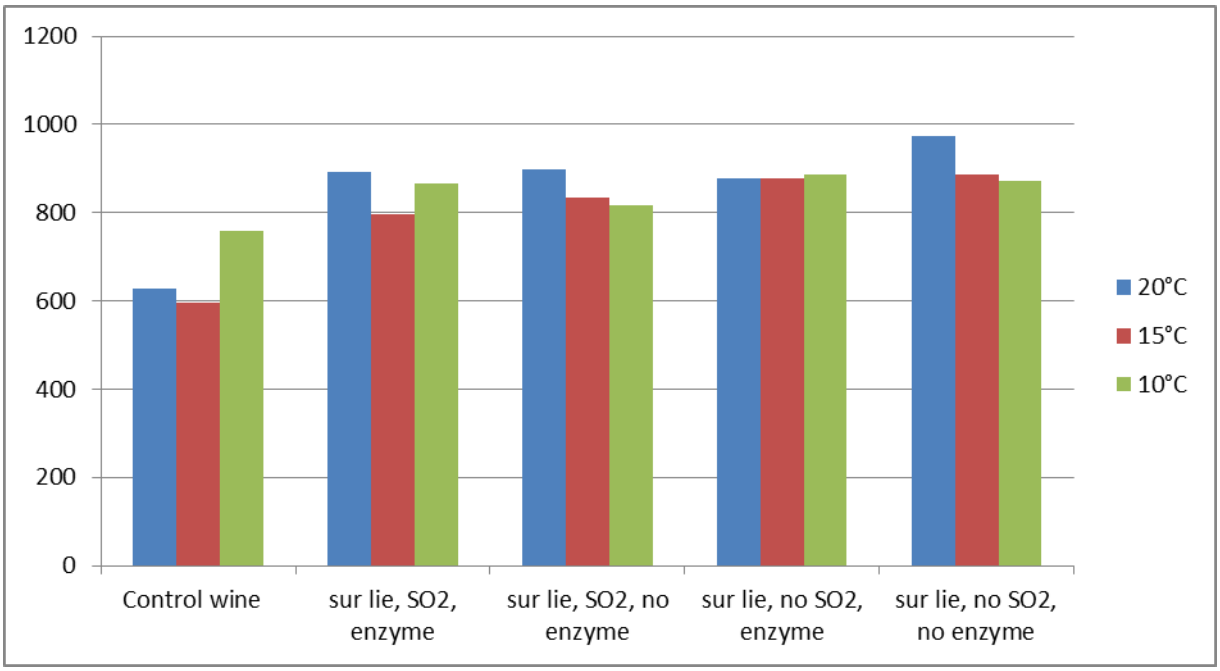

Figure 15: Total concentration of amino acids (mg/l) in wines produced with yeast Oenoferm Veltliner at different temperature levels; with and without sulphur dioxide, with and without enzyme

Content (mg/l)						ANOVA	ANOVA	ANOVA	ANOVA
Amino acid	**Control wine**	**Sur lie, SO_2 and enzyme**	**Sur lie, SO_2, no enzyme**	**Sur lie, no SO_2, enzyme**	**Sur lie, no SO_2, no enzyme**	**Lees contact**	**Temperature**	**SO_2/MLF**	**Enzyme**
Aspartic acid	11.98	25.21	26.71	28.38	37.50	$p < 0.05$	ns	$p < 0.05$	ns
Serine	10.22	19.50	20.61	22.74	30.12	$p < 0.05$	$p < 0.05$	$p < 0.05$	$p < 0.05$
Asparagine	9.54	17.06	15.97	16.75	19.13	$p < 0.05$	$p < 0.05$	ns	$p < 0.05$
Glutamic acid	36.03	52.65	53.78	59.44	71.26	$p < 0.05$	ns	$p < 0.05$	ns
Glycine	13.99	19.52	20.91	22.11	28.34	$p < 0.05$	$p < 0.05$	$p < 0.05$	$p < 0.05$
Glutamine	1.20	1.66	1.90	1.86	1.73	$p < 0.05$	$p < 0.05$	$p < 0.05$	ns
Arginine	64.61	94.82	89.19	40.45	60.37	$p < 0.05$	ns	$p < 0.05$	ns
Threonine	6.29	11.54	12.19	12.66	17.45	$p < 0.05$	ns	$p < 0.05$	ns
Alanine	88.84	104.98	106.22	108.45	116.76	$p < 0.05$	ns	$p < 0.05$	ns
Proline	456.65	620.82	716.13	812.70	665.40	ns	$p < 0.05$	ns	ns
Hydroxyproline	17.49	34.89	50.00	68.21	68.83	ns	ns	ns	ns
Tyrosine	272.35	293.98	291.36	297.17	299.69	$p < 0.05$	$p < 0.05$	$p < 0.05$	ns
Valine	4.95	12.05	12.11	11.81	13.66	$p < 0.05$	$p < 0.05$	$p < 0.05$	ns
Methionine	14.64	17.37	18.45	19.82	19.23	$p < 0.05$	ns	$p < 0.05$	ns
Ornithine	12.96	16.72	24.01	12.83	5.68	ns	$p < 0.05$	$p < 0.05$	ns
Lysine	55.37	111.84	107.23	120.26	132.32	$p < 0.05$	$p < 0.05$	$p < 0.05$	ns
Isoleucine	3.15	11.95	12.81	13.82	18.94	$p < 0.05$	ns	$p < 0.05$	ns
Leucine	8.88	38.59	40.54	42.39	48.50	$p < 0.05$	ns	$p < 0.05$	ns
Phenylalanine	7.48	26.71	28.34	28.67	32.36	$p < 0.05$	ns	$p < 0.05$	ns
Tryptophan	4.81	14.88	15.82	17.06	21.98	$p < 0.05$	ns	$p < 0.05$	ns

Table 41: Free amino acids in sur lie wines (11 months fine lees contact), fermented with yeast Oenoferm Veltliner, stored at 20°C with and without sulphur dioxide, with and without enzyme

Content (mg/l)						ANOVA	ANOVA	ANOVA	ANOVA
Amino acid	**Control wine**	**Sur lie, SO_2 and enzyme**	**Sur lie, SO_2, no enzyme**	**Sur lie, no SO_2, enzyme**	**Sur lie, no SO_2, no enzyme**	**Lees contact**	**Temperature**	**SO_2/MLF**	**Enzyme**
Aspartic acid	12.90	28.25	26.54	34.15	38.06	$p < 0.05$	ns	$p < 0.05$	ns
Serine	10.73	16.36	17.56	22.75	23.85	$p < 0.05$	$p < 0.05$	$p < 0.05$	$p < 0.05$
Asparagine	9.70	14.01	14.35	16.52	12.82	$p < 0.05$	$p < 0.05$	ns	$p < 0.05$
Glutamic acid	36.99	53.27	53.96	67.54	70.79	$p < 0.05$	ns	$p < 0.05$	ns
Glycine	14.01	18.01	18.22	24.23	24.81	$p < 0.05$	$p < 0.05$	$p < 0.05$	$p < 0.05$
Glutamine	0.95	1.34	1.77	1.69	2.17	$p < 0.05$	$p < 0.05$	$p < 0.05$	ns
Arginine	61.04	89.99	92.68	27.76	14.89	$p < 0.05$	ns	$p < 0.05$	ns
Threonine	6.32	9.95	10.33	14.19	15.88	$p < 0.05$	ns	$p < 0.05$	ns
Alanine	84.08	95.75	102.84	111.67	119.04	$p < 0.05$	ns	$p < 0.05$	ns
Proline	619.03	545.55	600.36	450.72	725.41	ns	$p < 0.05$	ns	ns
Hydroxyproline	55.74	65.50	79.03	63.81	92.67	ns	ns	ns	ns
Tyrosine	256.08	277.15	287.23	298.79	311.24	$p < 0.05$	$p < 0.05$	$p < 0.05$	ns
Valine	4.10	8.46	9.54	11.01	10.53	$p < 0.05$	$p < 0.05$	$p < 0.05$	ns
Methionine	15.32	18.03	18.21	18.85	17.12	$p < 0.05$	ns	$p < 0.05$	ns
Ornithine	10.67	16.78	16.15	27.74	11.66	ns	$p < 0.05$	$p < 0.05$	ns
Lysine	45.09	74.33	80.09	100.05	111.17	$p < 0.05$	$p < 0.05$	$p < 0.05$	ns
Isoleucine	3.65	9.49	10.94	12.60	13.71	$p < 0.05$	ns	$p < 0.05$	ns
Leucine	11.18	32.06	35.41	42.32	43.96	$p < 0.05$	ns	$p < 0.05$	ns
Phenylalanine	8.82	21.95	24.15	28.57	28.29	$p < 0.05$	ns	$p < 0.05$	ns
Tryptophan	5.45	12.52	13.73	16.37	17.22	$p < 0.05$	ns	$p < 0.05$	ns

Table 42: Free amino acids in sur lie wines (11 months fine lees contact), fermented with yeast Oenoferm Veltliner, stored at 15°C with and without sulphur dioxide, with and without enzyme

Content (mg/l)						ANOVA	ANOVA	ANOVA	ANOVA
Amino acid	**Control wine**	**Sur lie, SO_2 and enzyme**	**Sur lie, SO_2, no enzyme**	**Sur lie, no SO_2, enzyme**	**Sur lie, no SO_2, no enzyme**	**Lees contact**	**Temperature**	**SO_2/MLF**	**Enzyme**
Aspartic acid	21.72	26.36	21.72	24.18	26.06	$p < 0.05$	ns	$p < 0.05$	ns
Serine	13.71	16.79	16.61	22.04	22.02	$p < 0.05$	$p < 0.05$	$p < 0.05$	$p < 0.05$
Asparagine	11.56	16.17	14.23	17.56	14.49	$p < 0.05$	$p < 0.05$	ns	$p < 0.05$
Glutamic acid	50.67	57.61	50.14	59.52	60.64	$p < 0.05$	ns	$p < 0.05$	ns
Glycine	13.21	14.26	17.69	22.16	22.02	$p < 0.05$	$p < 0.05$	$p < 0.05$	$p < 0.05$
Glutamine	1.60	2.38	2.49	3.44	3.57	$p < 0.05$	$p < 0.05$	$p < 0.05$	ns
Arginine	84.44	105.44	91.17	12.75	7.79	$p < 0.05$	ns	$p < 0.05$	ns
Threonine	10.34	12.79	9.41	12.55	12.30	$p < 0.05$	ns	$p < 0.05$	ns
Alanine	106.32	112.46	102.39	109.89	110.99	$p < 0.05$	ns	$p < 0.05$	ns
Proline	1000.76	1184.52	1264.90	1343.35	1176.80	ns	$p < 0.05$	ns	ns
Hydroxyproline	102.47	145.53	153.57	177.52	145.91	ns	ns	ns	ns
Tyrosine	294.72	300.00	295.15	308.67	310.00	$p < 0.05$	$p < 0.05$	$p < 0.05$	ns
Valine	5.61	9.16	9.09	10.20	9.58	$p < 0.05$	$p < 0.05$	$p < 0.05$	ns
Methionine	16.28	16.83	17.42	19.87	18.67	$p < 0.05$	ns	$p < 0.05$	ns
Ornithine	12.18	14.03	15.06	82.51	81.10	ns	$p < 0.05$	$p < 0.05$	ns
Lysine	58.06	76.35	78.57	90.00	82.50	$p < 0.05$	$p < 0.05$	$p < 0.05$	ns
Isoleucine	7.84	10.63	9.98	12.17	12.11	$p < 0.05$	ns	$p < 0.05$	ns
Leucine	23.55	36.65	32.31	38.97	37.75	$p < 0.05$	ns	$p < 0.05$	ns
Phenylalanine	15.62	24.27	21.86	25.04	24.33	$p < 0.05$	ns	$p < 0.05$	ns
Tryptophan	10.69	13.66	12.54	14.93	15.04	$p < 0.05$	ns	$p < 0.05$	ns

Table 43: Free amino acids in sur lie wines (11 months fine lees contact), fermented with yeast Oenoferm Veltliner, stored at 10°C with and without sulphur dioxide, with and without enzyme

The evolution of individual amino acids at 20°C is presented in Table 41. All the sur lie samples stored at 20°C presented more free amino acids than the control wine. The highest evolution was observed in sample without SO_2 and no enzyme addition. Isoleucine and leucine presented the highest increase 500% and 440% respectively. Phenylalanine and tryptophan increased by more than 300% after 9 months of lees contact in the sample without SO_2 and no enzyme addition. Aspartic acid and serine increased by 200%. Threonine and valine presented an increase by more than 170% and asparagine, glutamic acid, glycine and lysine doubled their concentration increased by 100%. Alanine showed the lowest increase after the sur lie process only 31%. The concentration of methionine was almost equal in all sur lie wine samples, but slightly higher in the samples without SO_2 with and without enzyme addition, increased by 30%. The amino acid glutamine showed after the ageing period a slight higher concentration in the samples with lees contact than in the control wines, the highest concentration was presented by the sample with SO_2 and no enzyme addition. The sample with SO_2 and enzyme addition presented the highest concentration of arginine after the ageing period, increased by more than 40%. Ornithine showed the highest concentration in the sample with SO_2 and no enzyme addition.

Table 42 shows the evolution of free amino acids in the wines stored at 15°C. All the samples aged on lees showed more free amino acids than the control wine. As at 20°C, the highest evolution was observed in the sample without SO_2 and no enzyme addition. The increase of each amino acid was obvious lower than the increase in wines stored at 20°C. Isoleucine and leucine showed the highest increase, by more than 250%. Phenylalanine, tryptophan and aspartic acid increased by more than 200% in the sample without SO_2 and no enzyme addition. The concentration of threonine, valine and lysine increased by more than 150% Asparagine and glutamine showed an increase by more than 120% after ageing period. Glutamic acid and glycine increased by more than 90% %, respectively 70%. Asparagine presented the highest concentration in the sample without SO_2 and enzyme addition, an increase by 70%. Arginine showed the highest concentration in the sample with SO_2 and no enzyme addition, an increase by 50%. Ornithine presented the highest concentration in the sample without SO_2 and enzyme addition, the amount of ornithine was in this sample much higher at 15°C than at 20°C.

Table 43 shows the evolution of free amino acids in the wines stored at 10°C. As at 20°C and 15°C all the samples aged on lees were richer in free amino acids than the control wine. The evolution of amino acids in the sur lie wine samples at 10°C was almost the same for all aged samples. Aspartic acid, threonine and alanine presented the highest increase in the sample with SO_2 and enzyme addition. Aspartic acid and threonine increased by more than 20% during lees contact. Glutamic acid, glutamine and tryptophan showed the highest amount in the sample without SO_2 and without enzyme addition. Glutamine increased by more than 120%. Serine, asparagine, glycine, valine, methionine, lysine, isoleucine, leucine, and phenylalanine showed the highest evolution in the sample without SO_2 and enzyme addition. Valine presented the greatest increase by more than 80%. Ornithine showed the highest concentration in the sample without SO_2 and enzyme addition. The evolution of ornithine at 10°C was the highest evolution, increased by more than 570%.

Univariate analysis of variance (ANOVA) was carried out to examine significant differences between amino acids concentration due to four factors: lees contact, temperature, SO_2/MLF and enzyme.

The factor lees contact displayed significant differences ($p < 0.05$) on amino acid concentration with some exceptions: proline, hydroxyproline and ornithine.

The following amino acids were significantly influenced by the factor temperature: serine, asparagine, gycine, glutamine, proline, valine, ornithine and lysine.

The factor SO_2/MLF displayed significant differences ($p < 0.05$) on amino acid concentration with some exceptions: asparagine, proline, hydroxyproline.

Only serine, asparagine and glycine were significantly influenced by the factor enzyme.

4.3 Higher alcohols in Grüner Veltliner sur lie wines

4.3.1 Vintage 2006

	O.Veltliner			Weiss Komplex			Fermicru 4F9			EC 1118			ANOVA Yeast	ANOVA Time
Time (months)	0	3	6	0	3	6	0	3	6	0	3	6		
Ethyl acetate	46.5	63.2	47.2	39.1	34.1	50	42.3	43.1	47.8	42.8	55.9	24.4	ns	ns
Methanol	26.6	36.4	36.3	27.2	32.1	28	27.4	27.2	29	25.8	29.5	21.1	ns	ns
1-propanol	21.6	27	22.4	34.2	36.5	37	29.5	29	31.3	38.6	43.4	34.1	p <0.05	ns
Iso-butanol	24.8	31.5	25.6	36.3	39.3	40	29.8	28.4	60.1	36.6	43.6	33.4	ns	ns
isopentanol	106	133	103	86.3	101	91	103	102	101	88.3	106.8	78.3	p <0.05	p <0.05

Table 44: Concentration of higher alcohols, ethyl acetate and methanol in Grüner Veltliner wines fermented with four yeasts at different stages of maturation

The concentration of ethyl acetate increased after the first 3 months of ageing on lees and than subsequently decreased for the yeast Oenoferm Veltliner and EC 1118. In the wines fermented with Weiss Komplex, the concentration of ethyl acetate decreased slightly after 3 months of ageing on lees but after 6 months of lees contact the concentration increased. The samples produced with Fermicru 4F9 presented almost no changes in ethyl acetate concentration during ageing on lees.

For the yeast Oenoferm Veltliner, methanol concentration increased after 3 months of lees contact, then presented no changes until the ageing process was done. In the wines fermented with the yeasts Weiss Komplex and EC 1118, the methanol concentration increased after 3 months of ageing than decreased slightly. The samples produced with Fermicru 4F9 showed no changes in methanol concentration during the maturation process.
The concentration of 1-propanol increased after the first 3 months, followed by a decrease during the following period for the yeasts Oenofem Veltliner and EC 1118. The wines fermented with the yeast Weiss Komplex presented a slight increase in 1-propanol content during the maturation process. The samples produced with Fermicru 4F9 showed almost no changes during the lees contact.

The iso-butanol content increased after the first period of lees contact than the concentration presented a decrease for the yeast Oenoferm Veltliner and EC 1118. The iso-butanol concentration in the wines was produced with the yeast Fermicru 4F9 after 3 months of ageing, equal with the control wine, but after 6 months presented a sharp increase. Iso-butanol concentration showed almost no changes during the ageing period in wines produced with yeast Weiss Komplex.

The concentration of isopentanol increased during the first ageing period, than was followed by a decrease in the wines fermented with the yeasts Oenoferm Veltliner, Weiss Komplex and EC 1118. The wines produced with Fermicru 4F9 showed almost no changes during the maturation process.

Univariate analysis of variance (ANOVA) was carried out to examine significant differences between higher alcohols, ethyl acetate and methanol concentration due to two factors: time and yeast strain. The factor yeast strain influenced significantly ($p < 0.05$) the content of 1-

propanol and isopentanol. Isopentanol was the only one higher alcohol influenced significantly by the factor time.

4.3.2 Vintage 2007

The two yeasts applied Oenofem Veltliner and EC 1118 produced during the fermentation different amounts of higher alcohols. At the end of alcoholic fermentation the yeast EC 1118 had higher amounts of hexanol, 1-propanol, isobutanol and ethyl acetate. The concentrations of 1-hexanol, 1-propanol, isobutanol, methanol and ethyl acetate were very similar during lees contact in wines produced with the yeasts Oenoferm Veltliner and EC 1118. The yeast Oenoferm Veltliner presented at the end of fermentation higher concentration of isopentanole. The concentration of isopentanol increased slightly in all the samples fermented with Oenofem Veltliner after 6 weeks of ageing on lees. After the second period of ageing on lees (5 months), the concentration of isopentanol decreased. This could also be observed in the wines fermented with the yeast EC 1118, but the differences between concentrations were lower.

1-Butanol, 2-butanol, benzaldehyde and ethyl lactate levels were not detectable in the wines produced with the two yeasts.

Univariate analysis of variance (ANOVA) was carried out to examine significant differences between higher alcohols, ethyl acetate and methanol concentration due to three factors: time, yeast strain and enzyme.

The factor yeast strain influenced significantly ($p < 0.05$) the concentration of the following higher alcohols: 1-hexanol, 1-propanol, isobutanol and isopentanol.

The factor time displayed significant differences on the content of isopentanol.

										ANOVA		
Higher alcohols	***Control***	***No enzyme 6w***	***No enzyme 5m***	***Littozym 6w***	***Littozym 5m***	***Rapidase 6w***	***Rapidase 5m***	***Vinoflow 6w***	***Vinoflow 5m***	**Time**	**Yeast**	**Enzyme**
1-Butanol	n.n	n.n	n.n	n.n	n.n	n.n	n.n	n.n	n.n			
1-Hexanol	1.7	1.8	1.8	1.7	1.7	1.8	1.7	1.7	1.6	ns	p <0.05	p <0.05
1-Propanol	37.2	36.6	36.2	37.5	35.7	37.4	35.2	36.6	35.2	ns	p <0.05	ns
2-Butanol	n.n	n.n	n.n	n.n	n.n	n.n	n.n	n.n	n.n			
Benzaldehyd	n.n	n.n	n.n	n.n	n.n	n.n	n.n	n.n	n.n			
Ethylacetat	38.3	40.3	40.9	41.7	40.4	41.3	39.8	40.9	39.1	ns	ns	ns
Ethyllactat	n.n	n.n	n.n	n.n	n.n	n.n	n.n	n.n	n.n			
Isobutanol	28.3	27.3	27.1	27.9	26.9	27.9	26.5	27.3	26.3	ns	p <0.05	ns
Isopentanol	153.2	158.9	156.6	163.4	155.1	163.4	151.5	160.1	152.3	p<0.05	p <0.05	ns
Methanol	41.1	42.2	41.5	43.0	40.9	43.2	40.3	41.9	40.5	ns	ns	ns

Table 45: Concentration of higher alcohols, ethyl acetate, ethyl lactate and methanol (mg/l) in Grüner Veltliner wines fermented with the yeast Oenofem Veltliner at different stages of maturation

										AVOVA		
Higher alcohols	***Control***	***No enzyme 6w***	***No enzyme 5m***	***Littozym 6w***	***Littozym 5m***	***Rapidase 6w***	***Rapidase 5m***	***Vinoflow 6w***	***Vinoflow 5m***	**Time**	**Yeast**	**Enzyme**
1-Butanol	n.n	n.n	n.n	n.n	n.n	n.n	n.n	n.n	n.n			
1-Hexanol	1.9	1.8	1.9	1.9	1.8	1.9	1.9	1.8	1.8	ns	p<0.05	p <0.05
1-Propanol	40.2	38.6	39.6	41.6	40.1	41.7	39.6	40.6	39.2	ns	p<0.05	ns
2-Butanol	n.n	n.n	n.n	n.n	n.n	n.n	n.n	n.n	n.n			
Benzaldehyd	n.n	n.n	n.n	n.n	n.n	n.n	n.n	n.n	n.n			
Ethylacetat	40.3	40.0	40.4	42.8	40.7	42.6	41.4	40.9	40.5	ns	ns	ns
Ethyllactat	n.n	n.n	n.n	n.n	n.n	n.n	n.n	n.n	n.n			
Isobutanol	32.9	31.4	32.4	33.9	32.6	33.9	32.4	33.0	32.0	ns	p<0.05	ns
Isopentanol	141.2	134.4	138.4	144.3	139.0	144.6	137.9	141.1	136.4	p<0.05	p<0.05	ns
Methanol	41.8	40.3	40.9	42.9	41.6	43.1	41.0	42.0	40.7	ns	ns	ns

Table 46: Concentration of higher alcohols, ethyl acetate, ethyl lactate and methanol (mg/l) in Grüner Veltliner wines fermented with the yeast EC 1118 at different stages of maturation

4.3.3 Vintage 2008

Higher alcohols	Control wine	Fine lees	Crude lees 3g/l	Crude lees 6g/l	Lees quantity/quality	Temp./MLF
1-Butanol	ns	ns	ns	ns		
1-Hexanol	1	1	1	0	ns	ns
1-Propanol	62	60	62	61	ns	ns
2-Butanol	ns	ns	ns	ns		
Benzaldehyd	ns	ns	ns	ns		
Ethylacetat	40	39	44	63	ns	ns
Ethyllactat	18	19	32	47	ns	$p < 0.05$
Isobutanol	28	26	26	26	ns	ns
Isopentanol	129	129	129	129	ns	ns
Methanol	51	50	52	50	ns	ns

Table 47: Concentration of higher alcohols, ethyl acetate, ethyl lactate and methanol in Grüner Veltliner sur lie wines (8 months lees contact), fermented with the yeast Fermicru 4F9 at different stages of maturation, stored at 15°C with different lees quantity and quality

Higher alcohols	Control wine	Fine lees	Crude lees 3g/l	Crude lees 6g/l	Lees quantity/quality	Temp./MLF
1-Butanol	ns	ns	ns	ns		
1-Hexanol	0	0	0	0	ns	ns
1-Propanol	64	67	62	61	ns	ns
2-Butanol	ns	ns	ns	ns		
Benzaldehyd	ns	ns	ns	ns		
Ethylacetat	34	50	46	45	ns	ns
Ethyllactat	22	130	120	123	ns	$p < 0.05$
Isobutanol	29	28	27	26	ns	ns
Isopentanol	131	136	132	130	ns	ns
Methanol	52	54	51	51	ns	ns

Table 48: Concentration of higher alcohols, ethyl acetate, ethyl lactate and methanol in Grüner Veltliner sur lie wines (8 months lees contact), fermented with the yeast Fermicru 4F9 at different stages of maturation, stored at 22°C with different lees quantity and quality

The concentration of 1-hexanol, isobutanol, isopentanol and methanol were applied very similar in all the treatments. The concentration of ethyl acetate was higher in the sur lie wine with crude lees 6g/l stored at 15°C than in the other wines. In the wines stored at 22°C the concentration of ethyl acetate was higher in the sur lie wine with fine lees than in the control wine after 8 months of lees contact. At 15°C the concentration of ethyl lactate was higher in the sur lie wine stored on lees than in the control wine. This could be the evidence of starting malolactic activity in the wines. At 22°C, all the sur lie wines underwent malolactic fermentation, the concentration of ethyl lactate in the sur lie wines was 6 times higher than

the control wine. The sur lie wine with fine lees stored at 22°C showed the highest concentration of ethyl lactate.

Univariate analysis of variance (ANOVA) was carried out to examine significant differences between higher alcohols, ethyl acetate, ethyl lactate and methanol concentration due to two factors: quantity/quality and temperature/MLF (malolactic fermentation).

Only ethyl lactate concentration was significantly influenced by the factor temperature/MLF.

4.3.4 Vintage 2009

Content (mg/l)						ANOVA	ANOVA	ANOVA	ANOVA
Amino acid	**Control wine**	**Sur lie, SO_2 and enzyme**	**Sur lie, SO_2, no enzyme**	**Sur lie, no SO_2, enzyme**	**Sur lie, no SO_2, no enzyme**	**Lees contact**	**Temp.**	**SO_2/ MLF**	**Enzyme**
1-Butanol	1.7	1.8	0.9	1.7	0.9	ns	ns	ns	ns
1-Hexanol	0.5	0.6	0.5	0.9	0.0	ns	ns	ns	ns
1-Propanol	54.7	57.1	56.8	55.8	55.9	ns	$p < 0.05$	$p < 0.05$	ns
2-Butanol	ns	ns	ns	ns	ns				
Benzaldehyde	ns	ns	ns	ns	ns				
Ethyl acetate	46.1	47.5	48.7	52.3	52.5	ns	ns	$p < 0.05$	ns
Ethyl lactate	15.9	19.7	36.1	88.5	99.1	ns	$p < 0.05$	$p < 0.05$	ns
Isobutanol	65.8	67.6	67.8	67.6	68.1	ns	$p < 0.05$	$p < 0.05$	ns
Isopentanole	155.1	159.9	158.2	158.1	158.4	ns	ns	ns	ns
Methanol	39.2	40.5	39.8	40.4	40.5	ns	ns	ns	ns

Table 49: Concentration of higher alcohols, ethyl acetate, ethyl lactate and methanol in sur lie wines (11 months fine lees contact), fermented with yeast Oenoferm Veltliner, stored at 20°C with and without sulphur dioxide, with and without enzyme

Content (mg/l)						ANOVA	ANOVA	ANOVA	ANOVA
Amino acid	**Control wine**	**Sur lie, SO_2 and enzyme**	**Sur lie, SO_2, no enzyme**	**Sur lie, no SO_2, enzyme**	**Sur lie, no SO_2, no enzyme**	**Lees contact**	**Temp.**	**SO_2/ MLF**	**Enzyme**
1-Butanol	1.8	1.8	0.9	0.0	1.8	ns	ns	ns	ns
1-Hexanol	0.0	0.0	0.0	0.0	0.6	ns	ns	ns	ns
1-Propanol	56.6	56.0	54.6	54.8	55.8	ns	$p < 0.05$	$p < 0.05$	ns
2-Butanol	ns	ns	ns	ns	ns				
Benzaldehyde	ns	ns	ns	ns	ns				
Ethyl acetate	48.5	47.4	47.3	52.2	53.7	ns	ns	$p < 0.05$	ns
Ethyl lactate	15.5	22.3	13.7	62.5	66.0	ns	$p < 0.05$	$p < 0.05$	ns
Isobutanol	65.1	67.4	65.6	65.8	67.3	ns	$p < 0.05$	$p < 0.05$	ns
Isopentanole	165.0	160.7	155.9	157.7	160.8	ns	ns	ns	ns
Methanol	43.4	41.2	39.5	39.8	39.4	ns	ns	ns	ns

Table 50: Concentration of higher alcohols, ethyl acetate, ethyl lactate and methanol in sur lie wines (11 months fine lees contact), fermented with yeast Oenoferm Veltliner, stored at 15°C with and without sulphur dioxide, with and without enzyme

Content (mg/l)						ANOVA	ANOVA	ANOVA	ANOVA
Amino acid	**Control wine**	**Sur lie, SO_2 and enzyme**	**Sur lie, SO_2, no enzyme**	**Sur lie, no SO_2, enzyme**	**Sur lie, no SO_2, no enzyme**	**Lees contact**	**Temp.**	**SO_2/ MLF**	**Enzyme**
1-Butanol	1.9	1.8	1.8	2.1	1.9	ns	ns	ns	ns
1-Hexanol	0.0	0.0	1.1	0.0	0.0	ns	ns	ns	ns
1-Propanol	57.8	55.6	54.9	60.2	60.2	ns	$p < 0.05$	$p < 0.05$	ns
2-Butanol	ns	ns	ns	ns	ns				
Benzaldehyde	ns	ns	ns	ns	ns				
Ethyl acetate	50.2	47.9	49.1	50.6	51.5	ns	ns	$p < 0.05$	ns
Ethyl lactate	38.6	12.4	11.9	23.5	34.3	ns	$p < 0.05$	$p < 0.05$	ns
Isobutanol	70.2	67.9	67.3	73.5	72.9	ns	$p < 0.05$	$p < 0.05$	ns
Isopentanole	153.7	155.3	157.2	159.3	156.1	ns	ns	ns	ns
Methanol	39.6	40.0	40.0	41.2	40.8	ns	ns	ns	ns

Table 51: Concentration of higher alcohols, ethyl acetate, ethyl lactate and methanol in sur lie wines (11 months fine lees contact), fermented with yeast Oenoferm Veltliner, stored at 10°C with and without sulphur dioxide, with and without enzyme

1-butanol, 1-hexanol, isopentanol and methanol concentrations were very similar in the treatments applied.

1-propanol evolution was different throughout the ageing temperatures applied. At 20°C, the sur lie wines with SO_2 had, after the ageing period, the highest 1-propanol concentration. At 15°C, the wines presented almost the same concentration and at 10°C the highest concentration, after the ageing period was in the sur lie wines without the addition of SO_2.

The amount of ethyl acetate was recorded slightly higher in the sur lie wines without SO_2 at all three temperature levels.

The ester ethyl lactate had different evolution at all three temperature levels. At 20°C, the control wine showed the lowest concentration of ethyl acetate. The sur lie wines without SO_2 had 6 times more ethyl lactate than the control wine. The sur lie wine without SO_2 and no enzyme addition showed the highest concentration of ethyl lactate after the ageing period. At 15°C the lowest concentration was found in the sur lie sample with SO_2 and no enzyme addition. The concentration was slightly lower than in the control wine. The sur lie wines without SO_2 showed as well the highest concentration of ethyl lactate but only 4 times higher than the control wine. At 10°C the lowest concentration was recorded in the sur lie wine with SO_2 and no enyzme addition after the ageing period. As at 20°C and 15°C, the sur lie wines without SO_2 had, after the ageing process, the highest amount of ethyl lactate, but only 2 times higher than the lowest concentration found in wines stored at 10°C.

The concentration of isobutanol was slightly higher in the control wine stored at 10°C compared to the control wines stored at 15 and 10°C. The sur lie wines stored at 15°C and 20°C had after the ageing period higher amounts of isobutanol than the control wines. At 10°C the sur lie wines with SO_2 had after the ageing period lower concentration of isobutanol than the control wine.

The concentration of isobutanol was slightly higher in the control wine stored at 10°C than in the control wines stored at 15 and 10°C. The sur lie wines stored at 15°C and 20°C had after the ageing period higher amounts of isobutanol than the control wines. At 10°C the sur lie wines with SO_2 had, after the ageing period, lower concentration of isobutanol than the control wine.

Univariate analysis of variance (ANOVA) was carried out to examine significant differences between higher alcohols, ethyl acetate, ethyl lactate and methanol concentration based on four factors: lees contact, temperature, SO_2 /MLF and enzyme.

The factors lees contact and enzyme displayed no significant differences on the higher alcohols, ethyl acetate, ethyl lactate and methanol content. 1-propanol, ethyl lactate and isobutanol were significantly influenced by the factors temperature and SO_2/MLF. The content on ethyl acetate was significanty influenced by the factor SO_2/MLF.

4.4 Biogenic amines in Grüner Veltliner sur lie wines

4.4.1 Vintage 2008

Biogenic amines	Control wine	Fine lees	Crude lees 3g/l	Crude lees 6g/l	Lees quantity/quality	Temp./MLF
2-Phenylethylamine	0.25	0.29	0.28	0.26	ns	ns
Cadaverine	nd	nd	nd	nd		
Histamine	nd	nd	nd	nd		
Isopentylamine	1.31	1.34	1.32	1.27	ns	ns
Putrescine	0.53	0.51	0.53	0.56	ns	ns
Tyramine	nd	nd	nd	nd		
Total	2.09	2.13	2.13	2.09		

Table 52: Concentration of biogenic amines in Grüner Veltliner sur lie wines (8 months lees contact), fermented with the yeast Fermicru 4F9 at different stages of maturation, stored at 15°C with different lees quantity and quality

Biogenic amines	Control wine	Fine lees	Crude lees 3g/l	Crude lees 6g/l	Lees quantity/quality	Temp./MLF
2-Phenylethylamine	0.27	0.28	0.27	0.26	ns	ns
Cadaverine	nd	nd	nd	nd		
Histamine	nd	nd	nd	nd		
Isopentylamine	1.30	1.32	1.28	1.27	ns	ns
Putrescine	0.65	0.46	0.38	0.43	ns	ns
Tyramine	nd	0.41	nd	nd		
Total	2.22	2.47	1.93	1.95		

Table 53: Concentration of biogenic amines in Grüner Veltliner sur lie wines (8 months lees contact), fermented with the yeast Fermicru 4F9 at different stages of maturation, stored at 22°C with different lees quantity and quality

The content of biogenic amines was very similar among the Grüner Veltliner wines aged on lees. Cadaverine, histamine and tyramine were not detectable in the wines produced in 2008. At 22°C, the concentration of putrescine was slightly higher in the control wine than in the wines matured on lees after maturation. The level of total biogenic amines was slightly lower in the wines with crude lees stored at 22°C.

Univariate analysis of variance (ANOVA) was carried out to examine significant differences between biogenic amines due to two factors: quantity/quality and temperature/MLF. No significant difference was recorded.

4.4.2 Vintage 2009

Content (mg/l)						ANOVA	ANOVA	ANOVA	ANOVA
Amino acid	**Control wine**	**Sur lie, SO_2 and enzyme**	**Sur lie, SO_2, no enzyme**	**Sur lie, no SO_2, enzyme**	**Sur lie, no SO_2, no enzyme**	**Lees contact**	**Temp.**	**SO_2/ MLF**	**Enzyme**
2-Phenylethylamine	0.06	0.06	0.05	0.08	0.07	ns	ns	ns	ns
Cadaverine	nd	nd	nd	0.23	0.28				
Histamine	1.20	0.76	2.33	0.00	0.00	ns	ns	ns	ns
Isopentylamine	0.33	0.33	0.32	0.34	0.33	ns	ns	ns	ns
Putrescine	0.86	0.98	1.10	28.38	17.95	ns	ns	$p < 0.05$	ns
Tyramine	0.05	nd	nd	nd	nd				
Total	2.51	2.13	3.80	29.03	18.62				

Table 54: Concentration of biogenic amines in sur lie wines (11 months fine lees contact), fermented with yeast Oenoferm Veltliner, stored at 20°C with and without sulphur dioxide, with and without enzyme

Content (mg/l)						ANOVA	ANOVA	ANOVA	ANOVA
Amino acid	**Control wine**	**Sur lie, SO_2 and enzyme**	**Sur lie, SO_2, no enzyme**	**Sur lie, no SO_2, enzyme**	**Sur lie, no SO_2, no enzyme**	**Lees contact**	**Temp.**	**SO_2/ MLF**	**Enzyme**
2-Phenylethylamine	0.06	0.05	0.05	0.07	0.09	ns	ns	ns	ns
Cadaverine	nd	nd	nd	nd	0.17				
Histamine	0.90	1.02	1.22	2.57	0.00	ns	ns	ns	ns
Isopentylamine	0.29	0.32	0.32	0.34	0.33	ns	ns	ns	ns
Putrescine	1.04	3.25	1.05	31.10	49.70	ns	ns	$p < 0.05$	ns
Tyramine	nd	nd	nd	nd	nd				
Total	2.28	4.64	2.64	34.08	50.28				

Table 55: Concentration of biogenic amines in sur lie wines (11 months fine lees contact), fermented with yeast Oenoferm Veltliner, stored at 15°C, with and without sulphur dioxide, with and without enzyme

Content (mg/l)						ANOVA	ANOVA	ANOVA	ANOVA
Amino acid	**Control wine**	**Sur lie, SO_2 and enzyme**	**Sur lie, SO_2, no enzyme**	**Sur lie, no SO_2, enzyme**	**Sur lie, no SO_2, no enzyme**	**Lees contact**	**Temp.**	**SO_2/ MLF**	**Enzyme**
2-Phenylethylamine	0.06	0.09	0.06	0.05	0.06	ns	ns	ns	ns
Cadaverine	nd	nd	nd	nd	nd				
Histamine	0.96	1.17	1.05	4.98	5.23	ns	ns	ns	ns
Isopentylamine	0.33	0.42	0.32	0.32	0.34	ns	ns	ns	ns
Putrescine	1.15	1.11	1.10	0.89	0.94	ns	ns	$p < 0.05$	ns
Tyramine	nd	nd	nd	nd	nd				
Total	2.50	2.79	2.53	6.24	6.56				

Table 56: Concentration of biogenic amines in sur lie wines (11 months fine lees contact), fermented with yeast Oenoferm Veltliner, stored at 10°C with and without sulphur dioxide, with and without enzyme

Concentration of 2-phenylethylamine and isopentylamine presented almost no changes among the Grüner Veltliner wines aged on lees. Cadaverine and tyramine were not detectable in the wines produced in 2009. Histamine was present in the control wine and in

the sur lie samples with SO_2 and enzyme addition stored at 20°C, but in the sur lie wines without SO_2 histamine was not detectable. At 15°C, the histamine concentration detected in the sur lie wine without SO_2 and no enzyme addition was slightly higher than in the other samples. In the sur lie wines without SO_2 stored at 10°C the concentration of histamine was 5 times higher than in the control wine and the sur lie samples with SO_2 addition. Putrescine concentration was very high in the sur lie wines without SO_2 stored at 20 and 15°C. The highest value of putrescine was found in the sur lie wine without SO_2 and no enzyme addition stored at 15°C. At 10°C the level of putrescine was in the sur lie wines without SO_2 slightly lower than in the control wine and the sur lie samples with SO_2 addition.

The level of total biogenic amines was higher after malolactic fermentation at all three temperature levels.

Univariate analysis of variance (ANOVA) was carried out to examine significant differences between biogenic amines based on four factors: lees contact, temperature, SO_2/MLF and enzyme. Only putrescine was significantly influenced by the factor SO_2/MLF.

4.5 Identification of lactic acid bacteria isolated from Grüner Veltliner sur lie wines

4.5.1 Wine composition

Table 3 (General composition 2009) shows the general composition of wines. After alcoholic fermentation the pH value was high (pH 3.6). At the end of malolactic fermentation the value increased to pH 3.8. The volatile acidity after the alcoholic fermentation was more than the average value of white wines (0.1-0.3 g/l) and as expected, malolactic fermentation produced a further increase. Malolactic fermentation was completed for all sur lie samples without SO_2, after nine months of ageing on lees. Citric acid presented no changes during ageing on lees. After the malolactic fermentation, the acidity decreased from 5 g/l to 4 g/l.

4.5.2 Viable counts

The analysis of colony counts was carried out with wine samples, where malolactic fermentation had occurred. Table 57 presents the colonies counted at each dilution of both growth media, while Figures 16 and 17 illustrate bacterial count results.

Sample - dilution	Medium	Population	Medium	Population
I -1	MRS = Medium 1	> 300 pinpoints (n.a.)	BCM 133 = Medium 2	101
I -2		> 300 pinpoints (n.a.)		no growth
I -3		no growth		no growth
I -4		no growth		no growth
II -1		> 300 pinpoints (n.a.)		96
II -2		43 pinpoints		1
II -3		no growth		no growth
II -4		no growth		no growth
III -1		338 white, 104 pinpoints		373
III -2		22 pinpoints		46
III -3		4 pinpoints		6 (Contamination!)
III -4		no growth		1
IV -1	MRS = Medium 1	132 white, 108 pinpoints ($^1/_4$)	BCM 133 = Medium 2	128 auf ¼ (n.a.)
IV -2		76 white, 42 pinpoints		81
IV -3		4 white		8
IV -4		no growth		1
V -1		108 white, 35 pinpoints		108
V -2		12 white, 3 pinpoints		15
V-3		2 white		no growth
V -4		no growth		no growth

VI -1		42 white K.		65
VI -2		5 white K.		5
VI -3		no growth		3
VI -4		no growth		k. W.

Table 57: Colony count

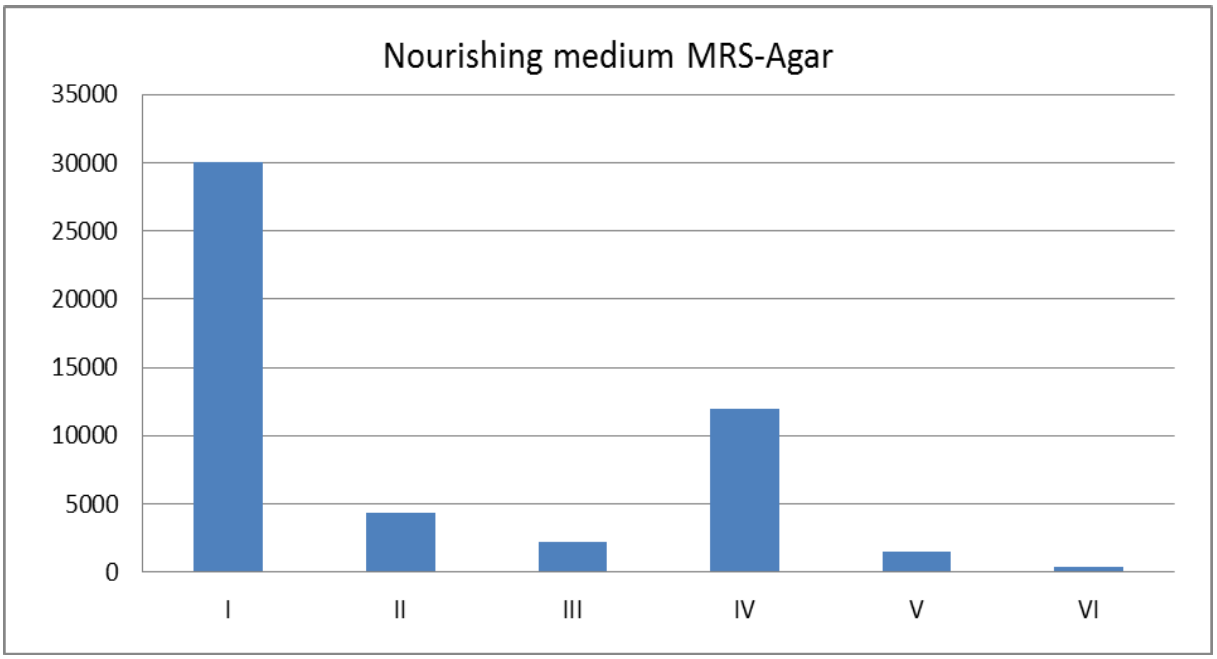

Figure 16: LAB population of Grüner Veltliner samples on MRS-agar after nine months on lees (UFC/ml)

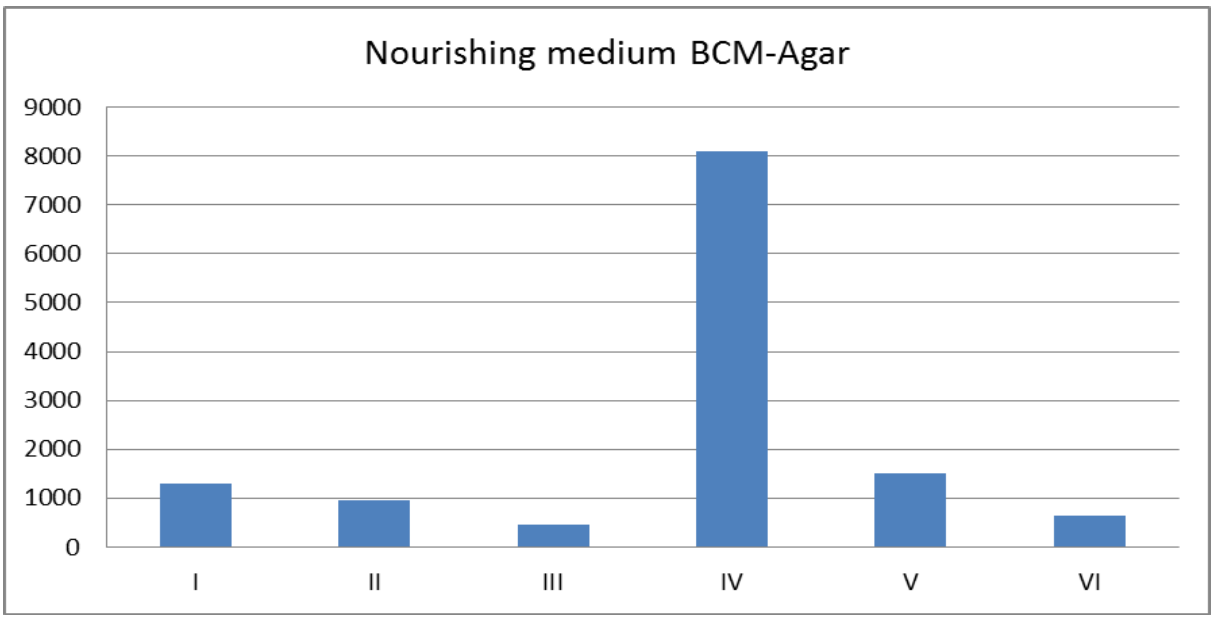

Figure 17: LAB population of Grüner Veltliner samples on BCM-agar after nine months on lees (UFC/ml)

4.5.3 Identification

It was interesting to detect the species, which was implicated in spontaneous malolactic fermentation. Therefore, it was necessary to discriminate and identify the lactic acid bacteria which grew and completed malolactic fermentation. Thus, typing of the isolates using rep-PCR (Figure 18, 19) and RAPD-PCR (Figure 20-23) was primarily performed. Based on the received patterns, representatives of obtained groups were identified, and Chello-PCR of all isolates was applied (Figure 24) and PCR-products of six isolates were sent to sequencing (Table 58).

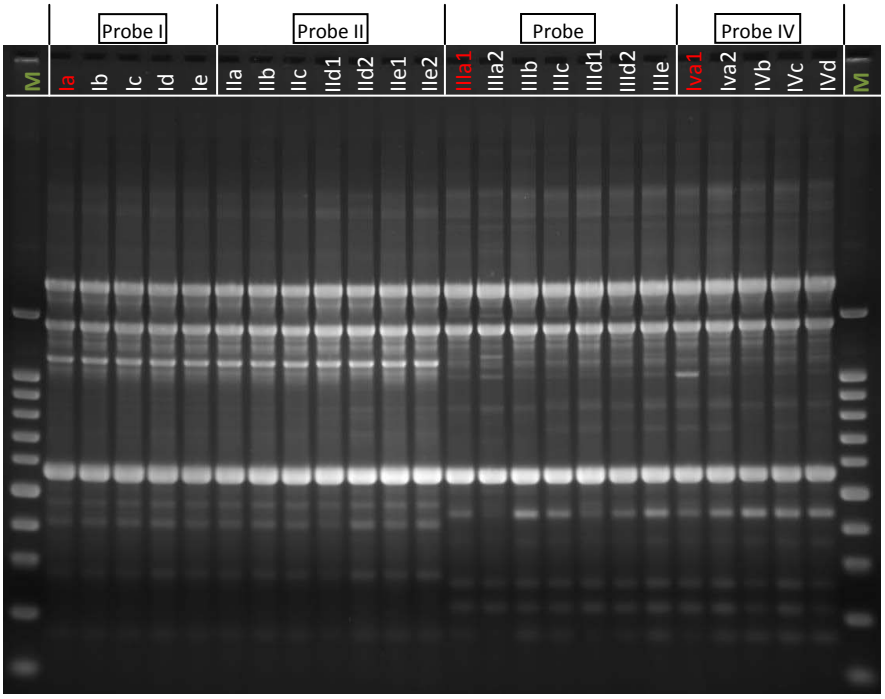

Figure 18: Products of rep-PCR show the fragments patterns of the bacterial strains isolated from samples 1 – 4

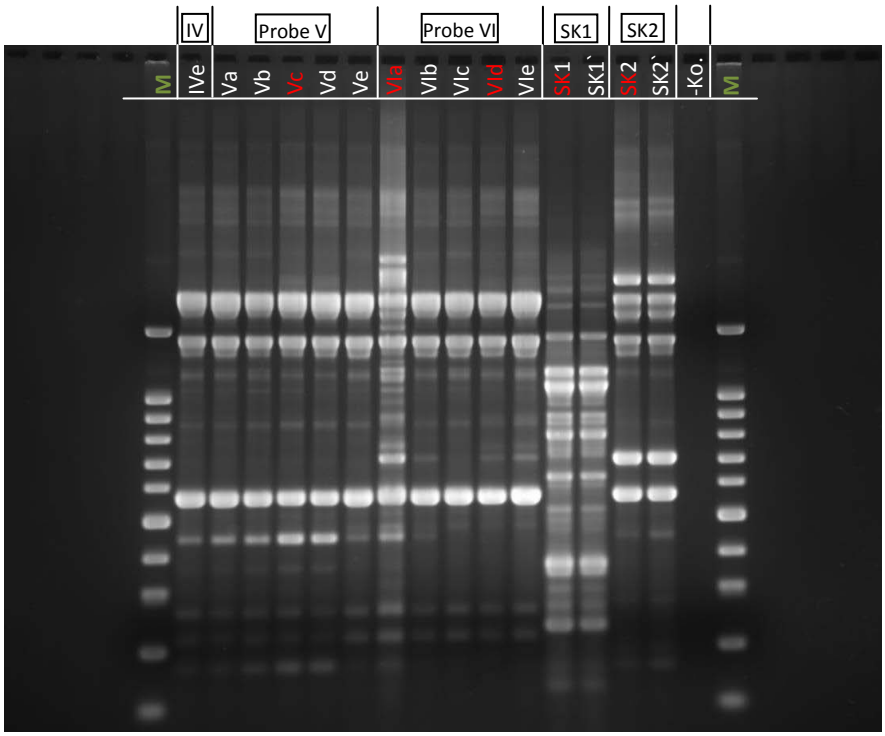

Figure 19: Products of rep-PCR show the fragments patterns of the bacterial strains isolated from samples 4 – 6 and the starter cultures SK1 and SK2

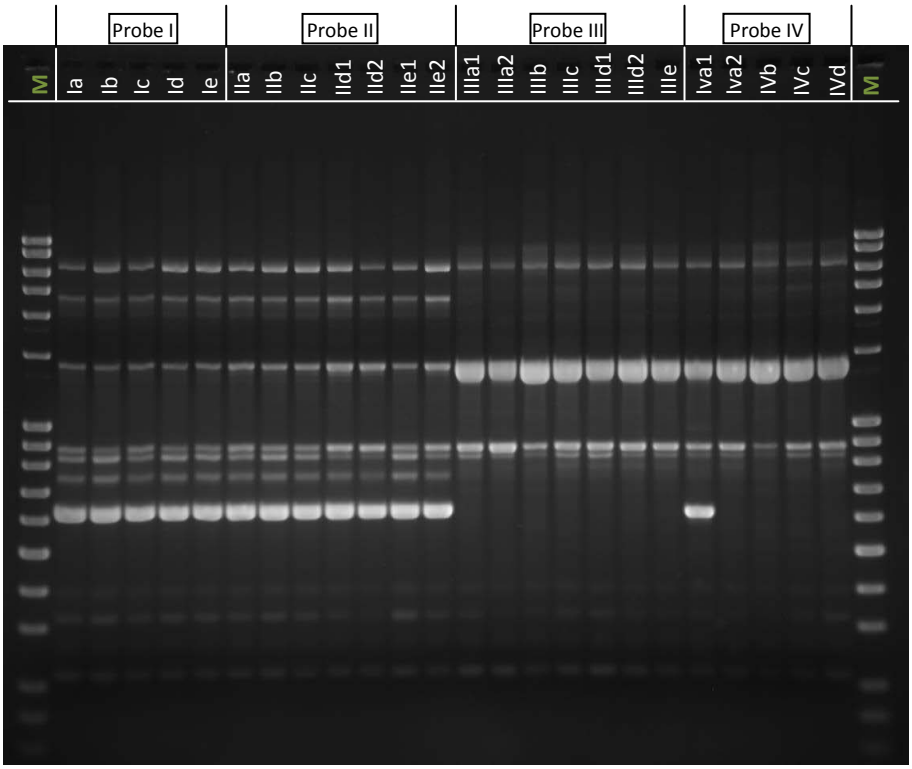

Figure 20: Products of RAPD-PCR gel Collado show the fragments patterns of the bacterial strains isolated from samples 1 – 4

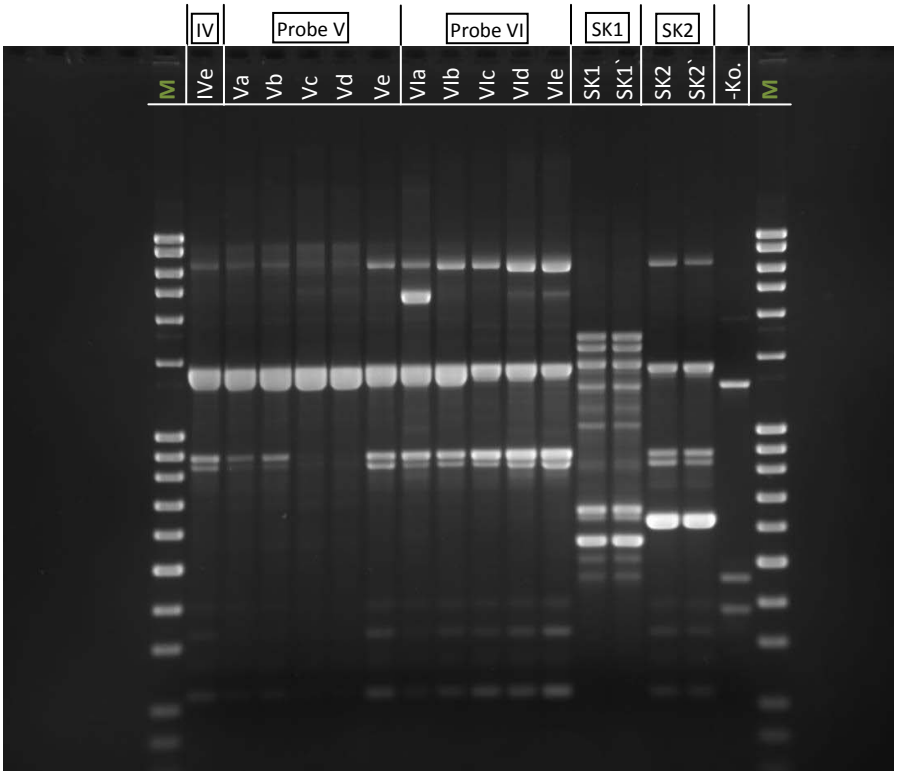

Figure 21: Products of RAPD-PCR gel Collado show the fragments patterns of the bacterial strains isolated from samples 4 – 6 and the starter cultures SK1 and SK2.

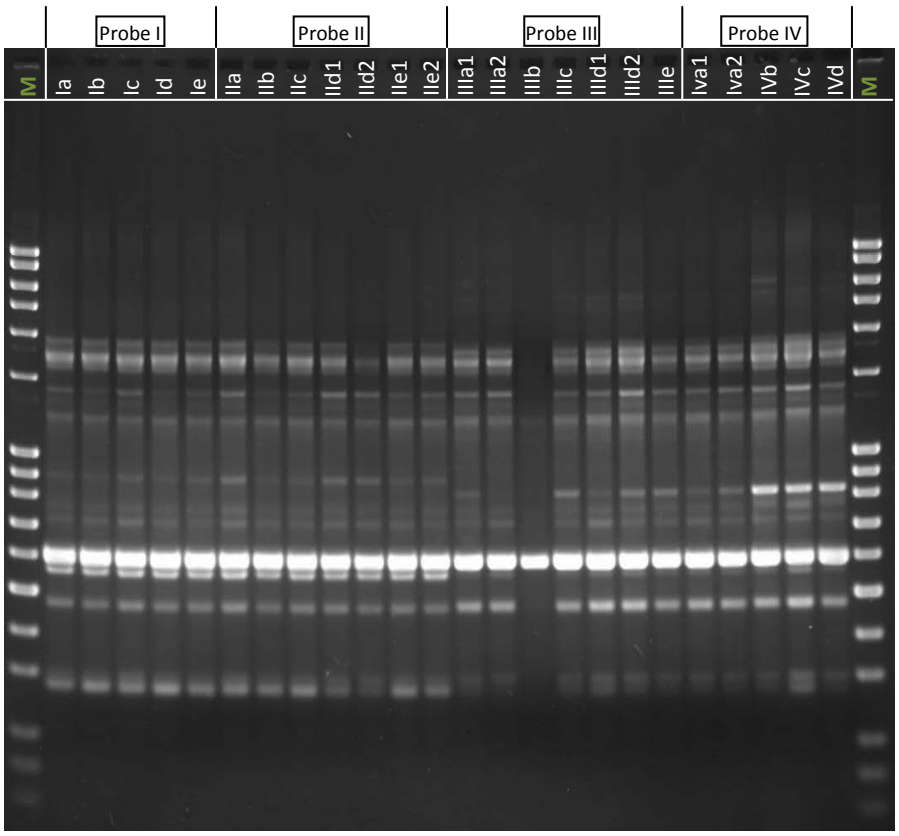

Figure 22: Products of RAPD-PCR gel Torriani show the fragments patterns of the bacterial strains isolated from samples 1 – 4

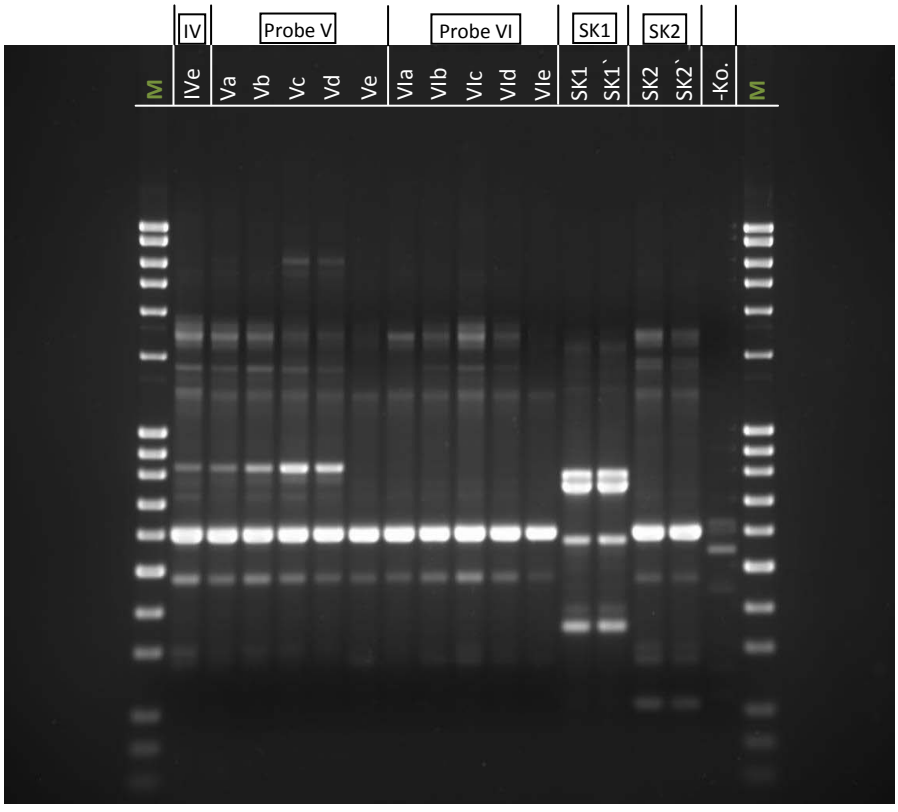

Figure 23: Products of RAPD-PCR gel Torriani show the fragments patterns of the bacterial strains isolated from samples 4 – 6 and the starter cultures SK1 and SK2.

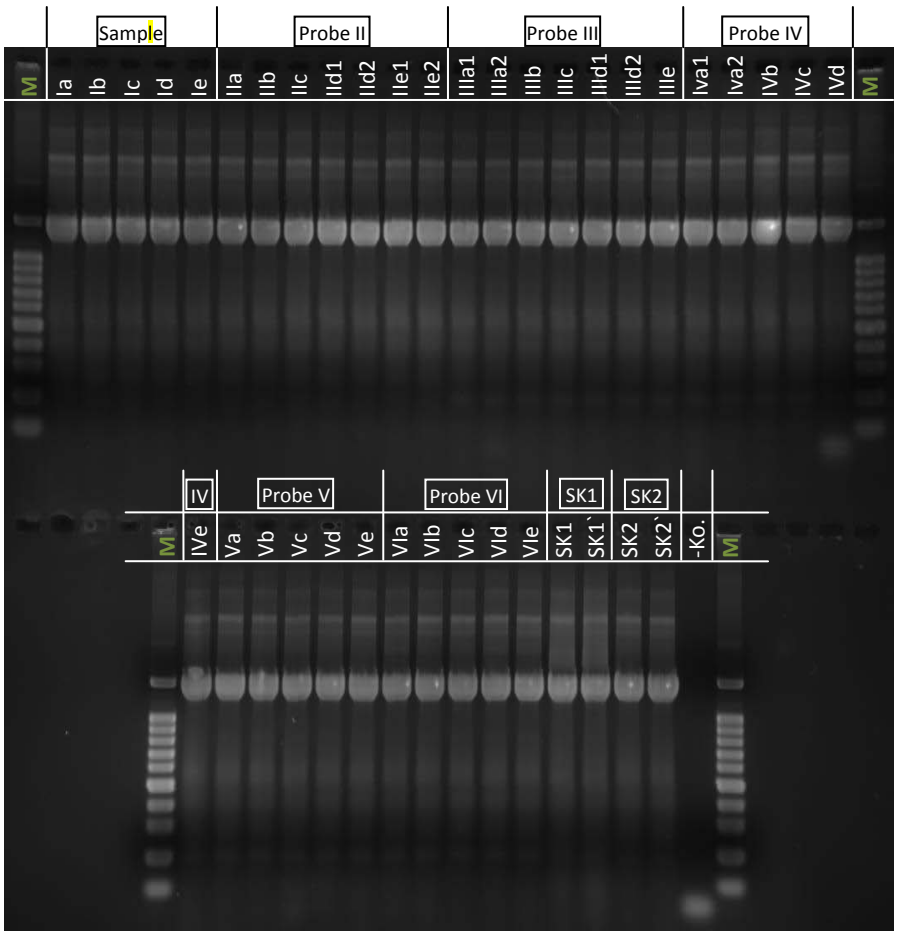

Figure 24: PCR products of Cello PCR show the fragments patterns of the bacterial strains isolated from samples 1 - 6 and the starter cultures SK1 and SK2.

Ia: ASH002PG24 - CelloP0	*Oenococcus oeni* strain 100%
IIIa1: ASH002PG25 - CelloP0	*Oenococcus oeni* strain 99%
IVa1: ASH002PG26 - CelloP0	*Oenococcus oeni* strain 99%
Vc: ASH002PG27 - CelloP0	*Oenococcus oeni* strain 99%
VIa: ASH002PG28 - CelloP0	*Oenococcus oeni* strain 99%
VId: ASH002PG29 - CelloP0	*Oenococcus oeni* strain 99%
SK1: ASH002PG30 - CelloP0	*Lactobacillus plantarum* strain 100%
SK2: ASH002PG31 - CelloP0	*Oenococcus oeni* strain 100%

Table 58: Results of blast

At the end of the alcoholic fermentation, when the yeast population decreases, the lactic acid bacteria (LAB) starts to proliferate. LAB use the remaining sugars, pentoses and hexoses to grow. The main transformation produced by LAB in wine is the conversion of L-malic acid to L-lactic acid and CO_2, called malolactic fermentation (MLF). MLF is an important process to deacidify of young wines, thereby increasing the body and mouthfeel of them and changing wine properties, mainly wine acidity and wine flavour (Bartwosky et al., 2002). It is usually

desired in red wines and rarely in white ones. Furthermore, LAB improve the microbial stability of wines (Moreno-Arribas and Polo, 2005), as their metabolisation of sugars, nutrients, malic acid, citric acid, amino acids and vitamins prevents the development of other spoilage bacteria (Volschenk et al., 2006).

LAB possesses the ability to metabolize free and sulphite-bound acetaldehyde to acetic acid and ethanol (Osborne et al., 2000).

There are generally three methods to encourage MLF in wines: spontaneous MLF as well as the adding of starter cultures or high cell concentration of MLF bacteria. The first two methods involve the growth of LAB, which is influenced by several parameters like alcohol concentration, SO_2, temperature and nutrients (Wibowo et al., 1985). LAB is typically found in wine and belongs to the species of the genera *Lactobacillus, Pediococcus* and *Oenococcus*. The third method is carried out with a high concentration of cells without the necessity of cell growth (Zhang, D. and Lovitt, R., 2006).

The use of starter cultures is the most applied method all over the world. The most popular species used as starter culture is *Oenococus oeni*, because it has the ability to survive in wines with high alcohol content and low pH value. Only *Oenococus oeni* is considered to complete a rapid and fruity fermentation. *Oenococus oeni* possesses an extracellular β (1-3) glucanase activity. Therefore, *Oenococus oeni* could accelerate the yeast autolysis by increasing the hydrolysis of the cell wall glucans (Guilloux-Benatier el al., 2000). *Oenococus oeni* produces volatile acidity during its growth phase. During malolactic fermentation, diacetyl (2.3-butanedione) is produced. The threshold of diacetyl is very low in wine (0.2 mg/l) (Martineau et al. 1995), and can be an unsTable product that may be reduced with *Oenococus oeni* to 2.3 butanediol (Bauer et al., 2004).

The next generation of starter cultures is considered to be *Lactobacillus plantarum. Lactobacillus plantarum* possesses different abilities: resistance to harsh conditions, β-glucosidase activity, and production of plantaricins (Du Toit et al., 2010; Sestelo et al., 2004). *Lactobacillus plantarum* strains are able to degrade biogenic amines such as putrescine, tyramine and histamine (Capozzi et al., 2012,). Ethanol, polyphenols and SO_2 may influence the ability of *Lactobacillus plantarum* to degradete biogenic amines (Garcia-Ruiz et al., 2001; Capozzi et al., 2012).

Other strains like pediococci can produce wines with off-flavours like: yogurt, butter, bitter and animal notes. The presence of polysaccharides such as β-D-glucan imparts viscous and “ropy” texture to wines. The production of these polysaccharides is almost exclusively performed by *Pediococcus* spp. (Bartwosky, 2008).

All samples without SO_2, underwent malolactic fermentation. The malolactic fermentation started spontaneously at all three temperature levels (10°C, 15°C, 20°C) (Table 32). A high pH value positively influences the development of lactic acid bacteria.

Figure 16 and Figure 17 show the plate counts made for each sample of Grüner Veltliner wines. On MRS medium the samples I and IV contained many more viable cells than the other sample. On BCM medium only sample IV contained more viable cells. Probably the strain of this sample can grow well on both media, whereas the strain of sample 1 grows better on MRS-agar. The two samples 1 and 4 presented different molecular profiles regarding Collado-PCR (Figure 20).

As reference strains “Viniflora CH35” (*Oenococus oeni)* and “Viniflora oenos” (*Oenococus oeni)* were chosen. The producer of both starter cultures is *Hansen (Denmark)*.

“Viniflora CH35” (*Oenococus oeni)* and “Viniflora oenos” (*Oenococus oeni)* provided from *Chr. Hansen (Denmark)* are the most popular starter cultures in the cellar, therefore it was assumed that the bacteria that performed the malolactic fermentation could originate from these two starter cultures.

After Cello PCR was performed, six isolates with different pattern were sent to sequencing (Figure19). As it can be observed from the rep-PCR and RAPD PCR patterns (Figure 18 to 24), the lactic acid bacteria isolated from all samples show the same pattern. Comparing the rep- and RAPD PCR patterns of all isolates from samples 1 to 6 with those of the starter culture Viniflora CH 35 and Viniflora Oenos, it can be assumed that the isolates originate from the starter culture Viniflora Oenos. *Oenococcus oeni* possesses the ability to adapt in wines with different conditions, depending on the composition of the medium the adaptation can take quite a long time. Due to selective pressure exerted by the environment the bacterial population can change its genetic structure (Di Cello et al., 1997) temporarily. However, the differences at strain level were possible to see with the RAPD or rep-PCR analysis. It is likely that lactic acid bacteria found in the Grüner Veltliner wines came from the equipment.

Table 58 shows the results of blast (Basic local alignment search tool) sequencing. According to this Table, all six isolates belong to the species *Oenococcus oeni.*

Another interesting fact is that SK1 (Viniflora CH35) was identified as *Lactobacillus plantarum* strain, although it should have been *Oenococcus oeni.* Therefore, it seems that the product was incorrectly labeled. A similar situation was found with the commercially starter culture Biostart Bianco SK3. Instead of O. *oeni*, when the starter culture SK3 was inoculated to MRST without ethanol Lact. brevis was detected (Michlamayr et al., 2009). *Lactobacillus plantarum* could not prevail, whereas O. *oeni. O. oeni* can adapt better to harsh conditions in wine than *Lactobacillus plantarum,* probably that´s why no other LAB were found.

Although the grape musts and wines had a high pH level, no bacteria of the genera *Pediococcus* or *Lactobacillus* were found. *Oenococcus oeni* are more resistant than pediococci or lactobacilli at pH levels below 3.4 (Davis et al., 1988). Wines with high pH values (above 3.5) are a favourable medium for the growth of bacteria from the species *Pediococcus* or *Lactobacillus.*

Sensory attributes of Grüner Veltliner sur lie wines

4.5.4 Influence of yeast strain and contact time

4.5.4.1 Vintage 2006

The sensory analysis of Grüner Veltliner wines, produced in 2006 was done after 5 years of bottle ageing with two different panels, one consisting of 13 tastes with experience and one consisting of 44 viticulture students. It was assumed that the wines with lees contact would have more ageing potential.

Expert tasters

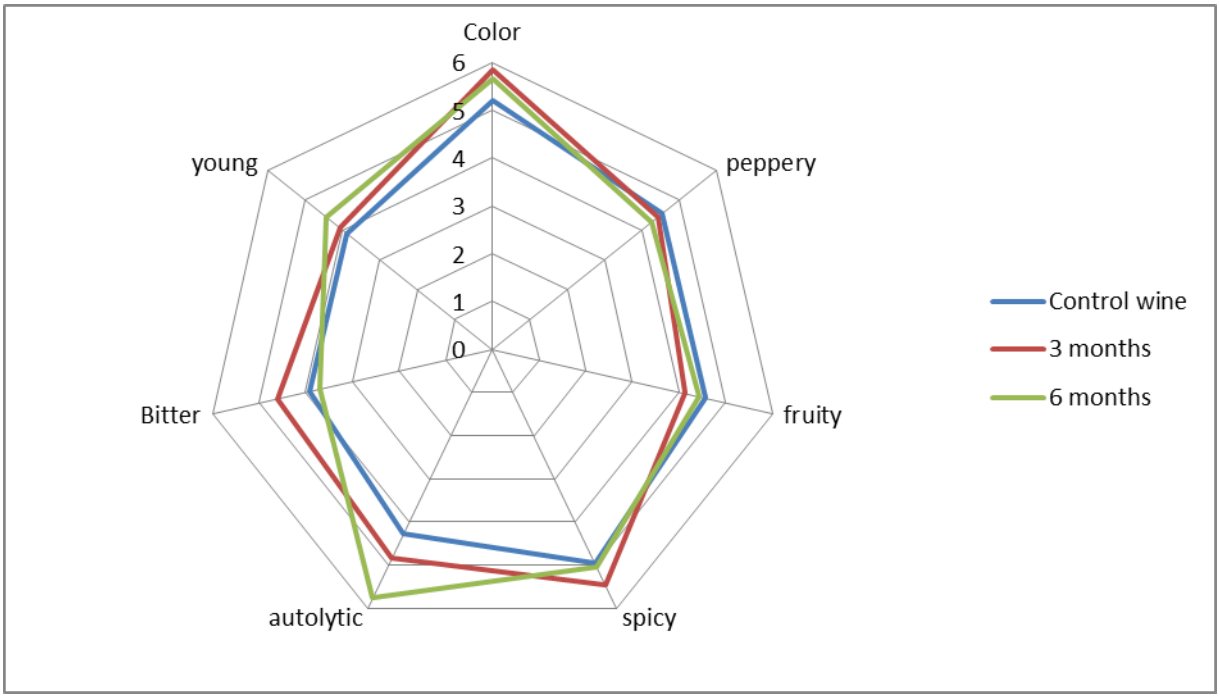

Figure 25: Aroma profile of Grüner Veltliner wines produced in 2006, with yeast Fermicru 4F9 at different stages of ageing on lees (expert tasters)

After 5 years of bottle ageing, as expected, the panelists did not find many differences between the wines. For the yeast Fermicru 4F9 (Figure 25), the tasters noted the descriptor “color” with almost the same intensity for the wines matured on lees, but with less intensity for the control wine. The descriptors “peppery” and “fruity” were marked with the same intensity for all the wines. o r the attributes “spicy” and “bitter” the wine with 3 months lees contact presented slightly more intensity than the other two wines. o r the attribute “young” the wine with 6 months lees contact showed slightly more intensity than the other two wines. The descriptor “autolytic” had the highest intensity in the wine after 6 months of lees contact.

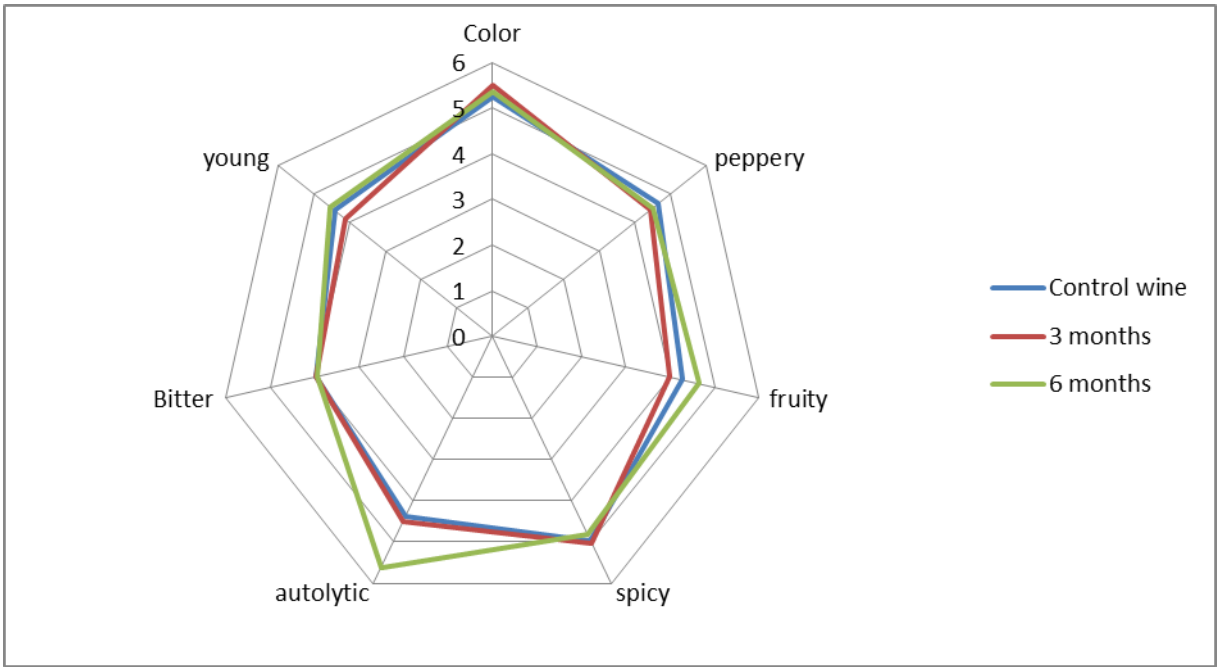

Figure 26: Aroma profile of Grüner Veltliner wines produced in 2006, with yeast EC 1118 at different stages of ageing on lees (expert tasters)

Figure 26 shows the aroma profile of Grüner Veltliner wines produced with the yeast EC 1118. The panelists noted the descriptors “color”, “peppery”, “spicy” and “bitter” with almost the same intensity. o r the attributes “fruity” and “young”, the wine matured on its lees for 6 months was noted with a slight more intensity than the other two wines, but with no significant differences. As for the yeast Fermicru 4F9, the autolytic character was marked with the highest intensity for the wine with 6 months lees contact. In this case the attribute “autolytic” was significantly influenced by the factor lees contact time.

From the comparison of the Figures 26 and 27 it can be noted that the contact period influenced the intensity of the descriptor “color” insignificantly. The attribute “peppery” decreased during the ageing time. The descriptor “fruity” decreased in the first 3 months of contact with lees for both yeasts. Afterwards, the intensity increased for the control wine and even more than the control wine for the yeast Fermicru 4F9 and for the yeast EC 1118. The “peppery” character was noted with more intensity than the control wine after 3 months of maturing on lees but after 6 months it decreased again. The differences were very slight. The attribute “autolytic”, which expresses the autolysis process, increased with the contact period. It can be noticed that after 3 months of ageing the control wine and the wine with 3 months lees contact presented the same intensity for the yeast EC 1118. Just after 6 months of maturing on lees the differences were significant. For the yeast Fermicru 4F9 the “autolytic” character presented after 3 months more intensity than the control wine. The yeast Fermicru 4F9 is suited to ageing on lees; this can explain the different results between the two yeasts. For the yeast e rmicru 4 9 the intensity of the descriptor “bitter” increased after the first period of lees contact, but in the next period decreased even below the intensity of the control wine. o r the yeast EC 1118 the descriptor “bitter” was always noted with the same intensity, therefore was not affected by the ageing time. The “young” character increased slightly with the ageing time for the yeast Fermicru 4F9, but for the yeast EC 1118 presented after 3 months a slight decrease, afterwards increased like the control wine, similarly as the descriptor “fruity”.

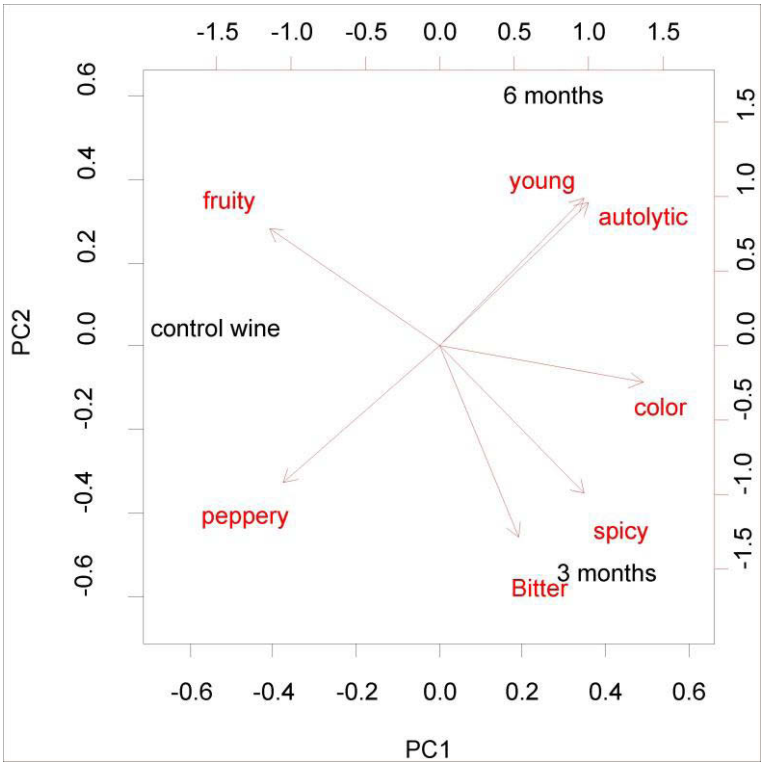

Figure 27: Principal component analysis of Grüner Veltliner wines produced in 2006, with yeast Fermicru 4F9 at different stages of ageing on lees (expert tasters)

Figure 27 shows the principal component analysis for the wines produced with the yeast e rmicru 4 9 made by the tasters with experience. The descriptors “autolytic” and “young” were very highly correlated. The vectors for the descriptors “autolytic” and “young” were negatively correlated with the vector for the descriptor “peppery”. The descriptor “spicy” was correlated with the descriptor “bitter” and negatively correlated with the descriptor ”fruity”.

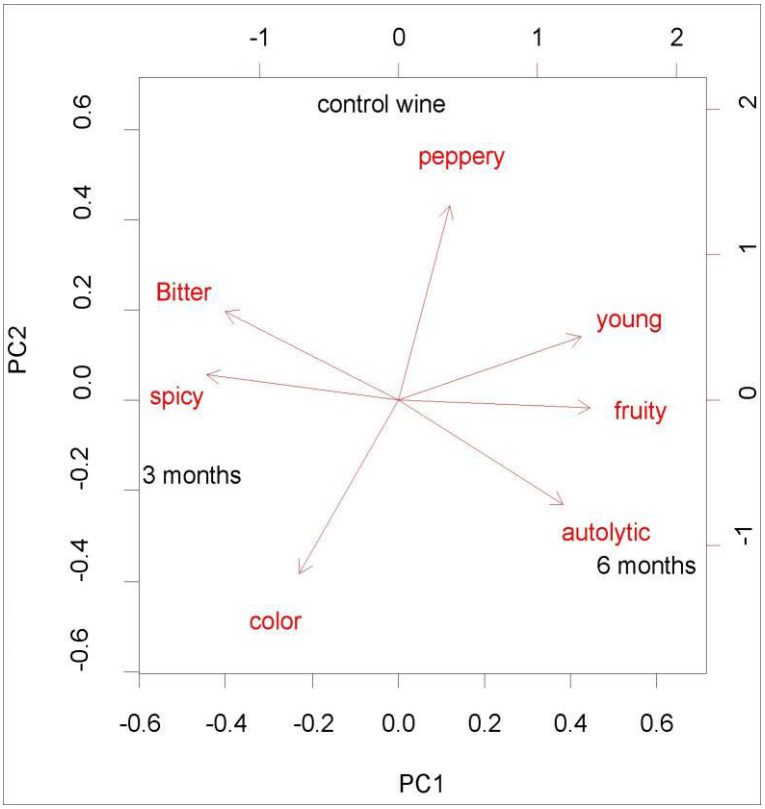

Figure 28: Principal component analysis of Grüner Veltliner wines produced in 2006, with yeast EC 1118 at different stages of ageing on lees (expert tasters)

Figure 28 shows the principal component analysis for the wines produced with the yeast EC 1118 made by the tasters with experience. The vectors for the attributes “autolytic”, “fruity” and “young” and were correlated and directed to the wine matured months on lees. The descriptor “autolytic” was negatively correlated with the attribute “bitter”. The attribute “bitter” was correlated with the attribute “spicy”. The descriptor “color” was negatively correlated with the descriptor “peppery”.

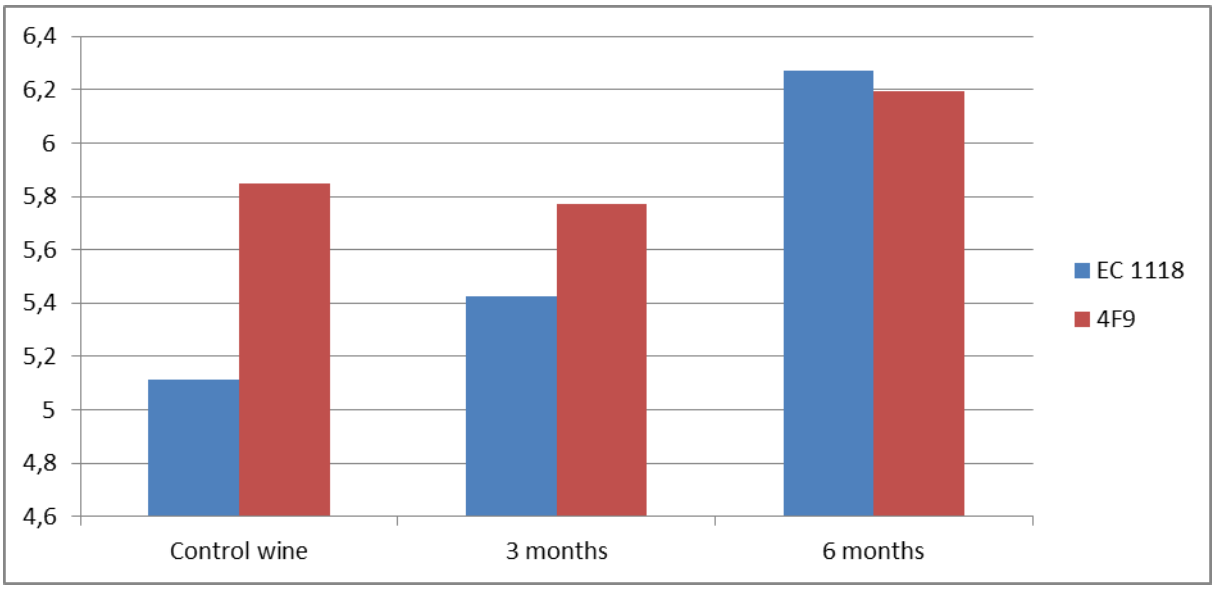

Figure 29: The overall sensory impression of Grüner Veltliner wines produced in 2006, with yeast Fermicru 4F9 and yeast EC 1118 at different stages of ageing on lees (expert tasters)

Figure 29 shows the results for overall sensory impression for the wines produced with the yeasts Fermicru 4F9 and EC 1118, made by the tasters with experience. The wine matured 6 months on lees was noted as the best wine for the both yeasts used.

Attributes	Yeast	Lees contact time	Yeast*Time
color	ns	Ns	Ns
peppery	ns	Ns	Ns
fruity	ns	Ns	Ns
spicy	ns	Ns	Ns
autolytic	ns	$p < 0.05$	Ns
bitter	ns	Ns	Ns
young	ns	Ns	Ns

Table 59: Sensory evaluation results of Grüner Veltliner wines produced with the yeasts 4F9 and EC 1118 and analysis of variance for each attribute (expert tasters)

Table 59 presents the results of the sensory data analysed with the Univariate analysis of variance (ANOVA). The factor lees contact time displays significant differences ($p < 0.05$) just for the descriptor ”autolytic“.

For the experienced tasting panels, just the autolytic character presented significant differences after 5 years of bottle ageing. It was not possible to demonstrate whether the wines matured on lees have more ageing potential, but it did show that the lees possess the ability to influence a wine character in such a significant way that it even can be noticed after a long period of bottle ageing.

Viticulture students

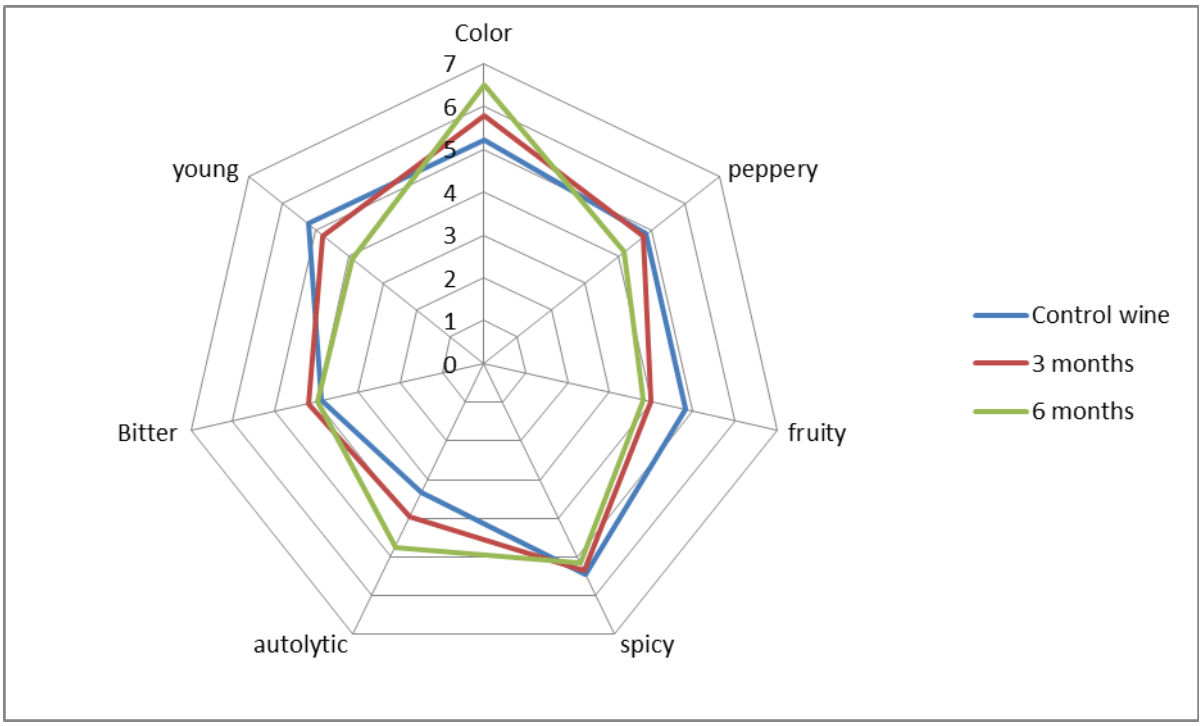

Figure 30: Aroma profile of Grüner Veltliner wines produced in 2006, with yeast Fermicru 4F9 at different stages of ageing on lees (44 viticulture students)

Figure 30 depicts the aroma profile of the wine produced with the yeast Fermicru 4F9 made by 44 viticulture students. The descriptor “color” was marked with the highest intensity for the wine with 6 months lees contact. The wine matured 3 months on lees was noted with less intensity for the descriptor “color”, but more intensity than the control wine. The attribute “peppery” was noted with the same intensity for the control wine and the wine with 3 months lees contact, but the wine with 6 months lees contact presented less intensity for this attribute. The descriptors “spicy” and “bitter” were marked with the same intensity for all the wines. The control wine presented the highest intensities for the attributes “fruity” and “young” and the wines matured on lees were marked with less intensities. The autolytic character was very well expressed after 3 months of ageing and the wine with 6 months lees contact was noted with the highest intensity.

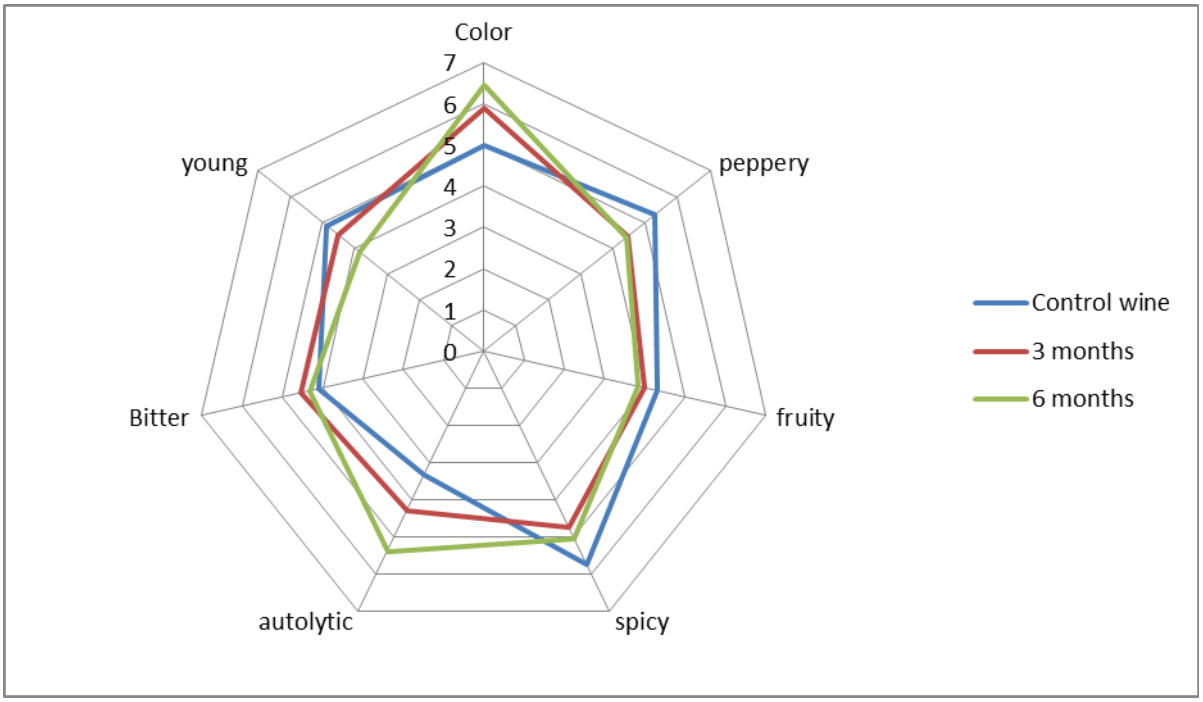

Figure 31: Aroma profile of Grüner Veltliner wines produced in 2006, with yeast EC 1118 at different stages of ageing on lees (44 viticulture students)

Figure 31 depicts the aroma profile of the wine produced with the yeast EC 1118 made by 44 viticulture students. The control wine showed the highst intensities for the attributes “peppery”, “fruity”, “spicy” and “young”. The wines matured on lees were marked with the same intensities for the descriptors “peppery”, “fruity” and “spicy”. All the wines produced with the yeast EC 1118 were noted with the same intensity for the descriptor “bitter”. Like the yeast Fermicru 4F9, the autolytic character was highly pronounced after 3 months and increased by time. The increase in autolytic character was correlated with a decrease for the attribute “young”.

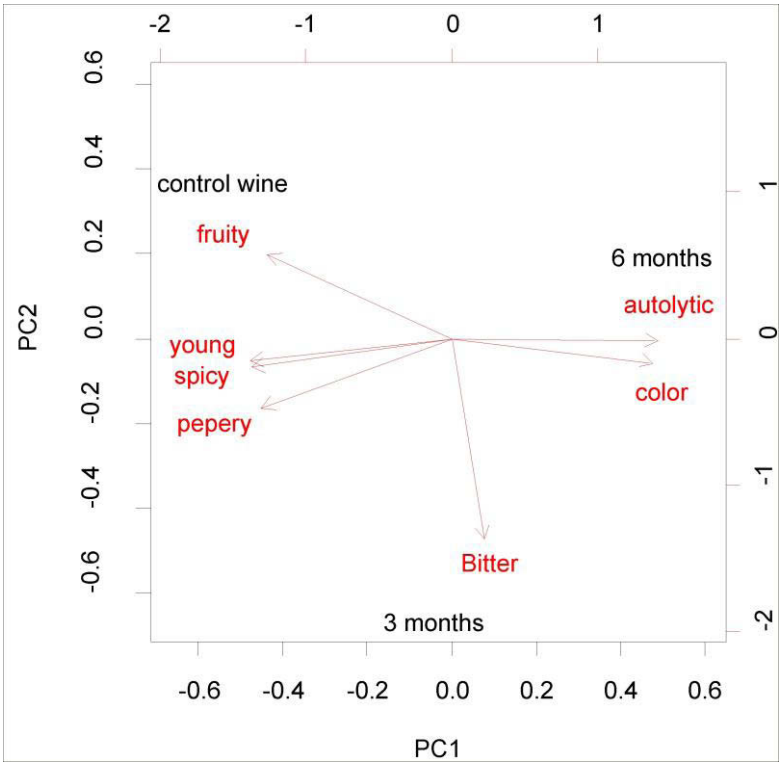

Figure 32: Principal component analysis of Grüner Veltliner wines produced in 2006, with yeast Fermicru 4F9 at different stages of ageing on lees (44 viticulture students)

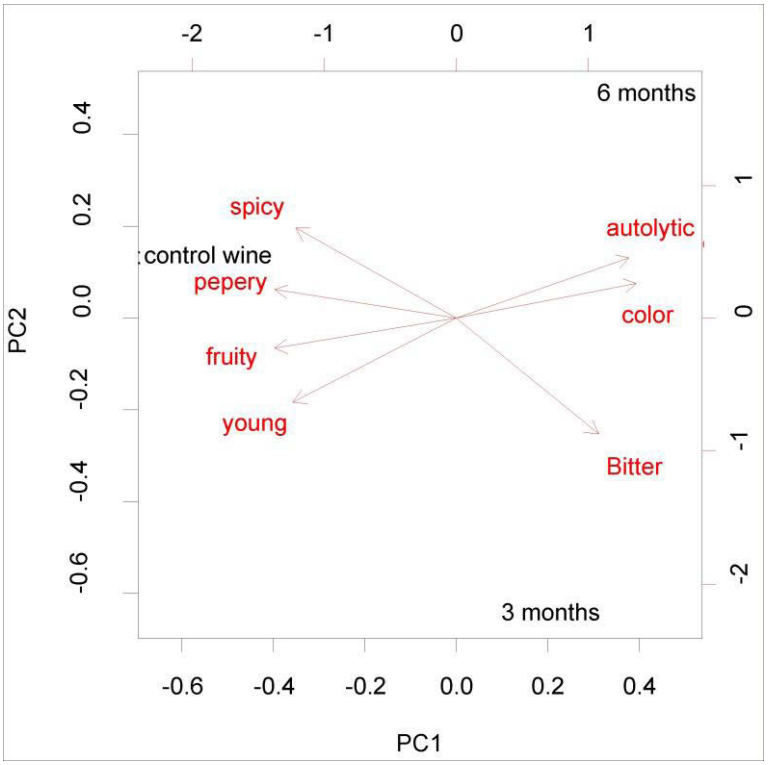

Figure 33: Principal component analysis of Grüner Veltliner wines produced in 2006, with yeast EC 1118 at different stages of ageing on lees (44 viticulture students)

Figures 32 and Figure 33 illustrate the principal component analysis for the wines produced with the yeasts Fermicru 4F9 and EC 1118 made by the viticulture students. For the both yeasts the descriptors “autolytic” and “color” were highly correlated, but the attribute “autolytic” was inversely correlated with the attribute “young”.

Attributes	Yeast	Lees contact time	Yeast*Time
color	ns	$p < 0.05$	ns
peppery	ns	$p < 0.05$	ns
fruity	ns	$p < 0.05$	ns
spicy	ns	$p < 0.05$	ns
autolytic	ns	$p < 0.05$	ns
bitter	ns	ns	ns
young	ns	$p < 0.05$	ns

Table 60: Sensory evaluation results of Grüner Veltliner wines produced with the yeasts Fermicru 4F9 and EC 1118 and analysis of variance for each attribute (44 viticulture students)

Table 60 presents the results of the sensory data analysed with the Univariate analysis of variance (ANOVA). The factor lees contact time displays significant differences ($p < 0.05$) for the descriptors “color”, “peppery”, “fruity”, “spicy”, ”autolytic“ and “young”. The yeast strain is a deciding factor because ageing on lees is a long-lasting process. Choosing a suitable yeast strain for sur lie ageing can reduce the maturation time. A specific yeast for sur lie will change the wine properties faster than a standard yeast.

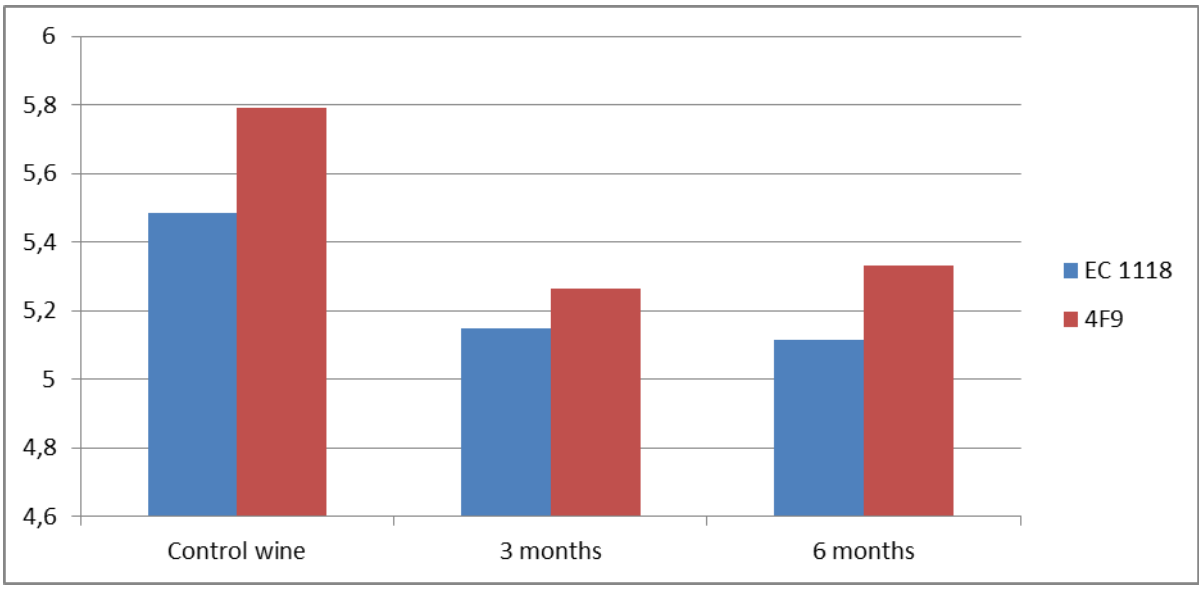

Figure 34: The overall sensory impression of Grüner Veltliner wines produced in 2006, with yeast Fermicru 4F9 and yeast EC 1118 at different stages of ageing on lees (44 viticulture students)

Figure 34 shows the results for overall sensory impression for the wines produced with the yeasts Fermicru 4F9 and EC 1118, made by 44 viticulture students. The control wine was noted as the best wine for both yeasts. The wines matured on lees were noted almost the same.

The results obtained with the viticulture students differ from the results obtained from the experts panel. For the tasters, with experience, the best wine was matured on lees for 6 months and autolytic was the only one attribute which presented significant differences. For the viticulture students the best wine was the control wine and they were able to find significant differences for the descriptors “color”, “peppery”, “fruity”, “spicy”, ”autolytic“ and “young”.

Separate processing of the data obtained at the tasting was necessary because of big opinion differences between the two panels, which clearly reflect the market situation. The different results can be explained with the help of a principal component analysis, which allowed to observe the correlation between attributes. The autolytic character was for the viticulture students a property that they did not prefer. They have associated the attribute “autolytic” with old, matured wines and the experience tasters have associated the attribute “autolytic” with young and fruity wines, therefore it is very important to know for which kind of customers the wines are produced.

4.5.4.2 Vintage 2007

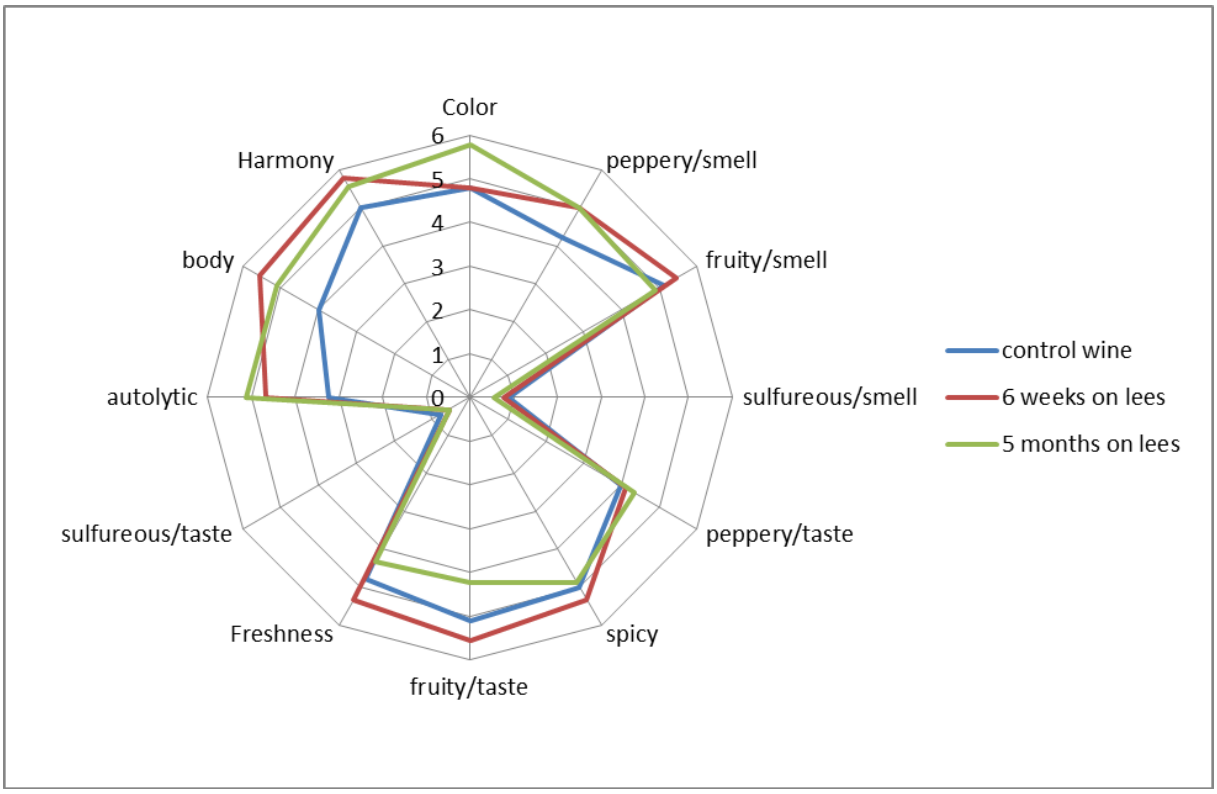

Figure 35: Aroma profile of Grüner Veltliner wines (vintage 2007) produced with yeast Oenofem Veltliner at different stages of ageing on lees

Figure 35 depicts the sensory profile for the wines produced with the yeast Oenoferm Veltliner. The wine matured six weeks on lees showed the highest intensities for the descriptors “fruity smell”, “spicy”, “freshness”, “body” and “harmony”. The autolytic character was expressed fairly well after six weeks on lees, but it increased by time, being more pronounced after five months on lees. The gain in autolytic character was accompanied by

an increase of color and a loss of “freshness”, “fruity taste” and “fruity smell”. The “peppery/taste” was marked in all wines at the same intensity.

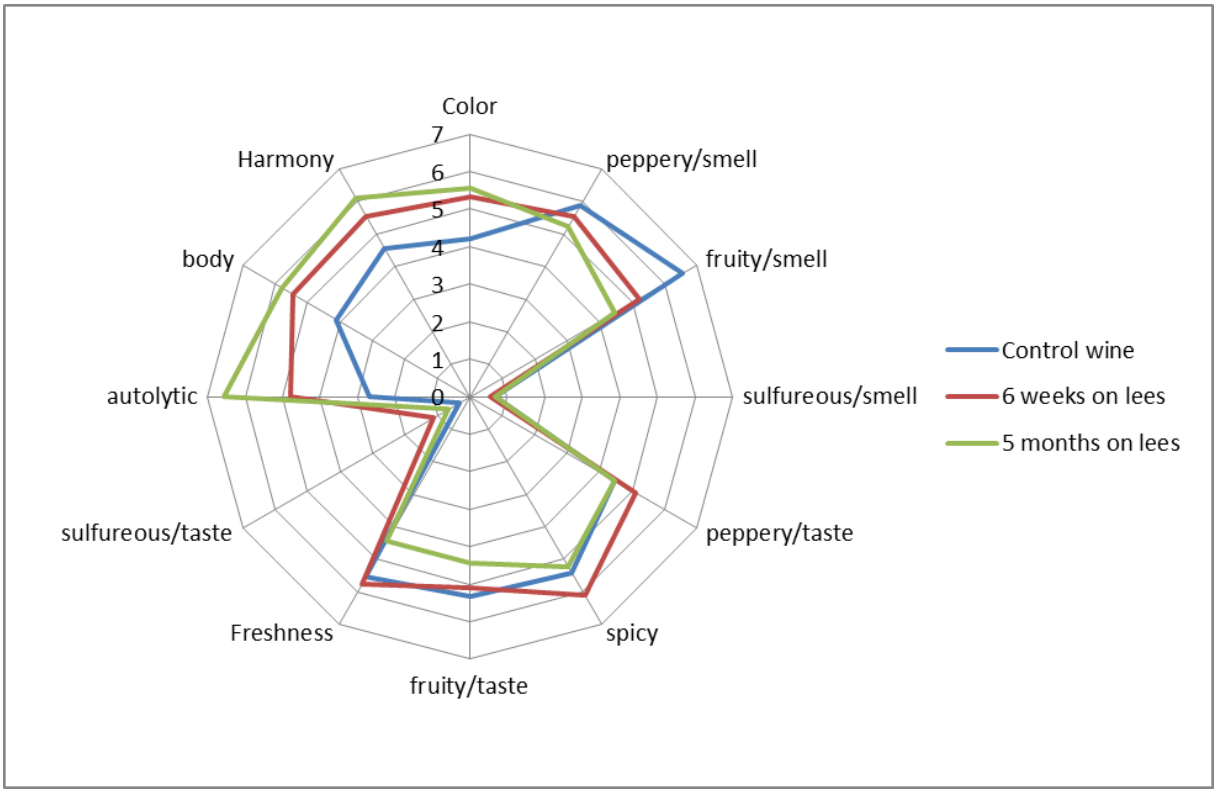

Figure 36: Aroma profile of Grüner Veltliner wines (vintage 2007) produced with yeast EC 1118 at different stages of ageing on lees

Figure 36 shows the sensory profile for the wines produced with the yeast EC 1118. In this case the descriptors “peppery/smell” and “fruity/smell” had the highest intensities in the control wine. In all wines the panelists marked the descriptors “peppery/taste” and “spicy” with the same intensity, but the intensities were slightly lower in the wine after five months lees contact. The “freshness” was marked with the same value for the control wine and the wine after six weeks on lees. However, after five months of lees the “freshness” decreased. The descriptors “autolytic”, “body” and “harmony” had the highest intensity in the wine after five months on lees. Color was of identical intensity in the wines matured on lees, but less pronounced in the control wine, which was early sulphited and kept in closed bowls.

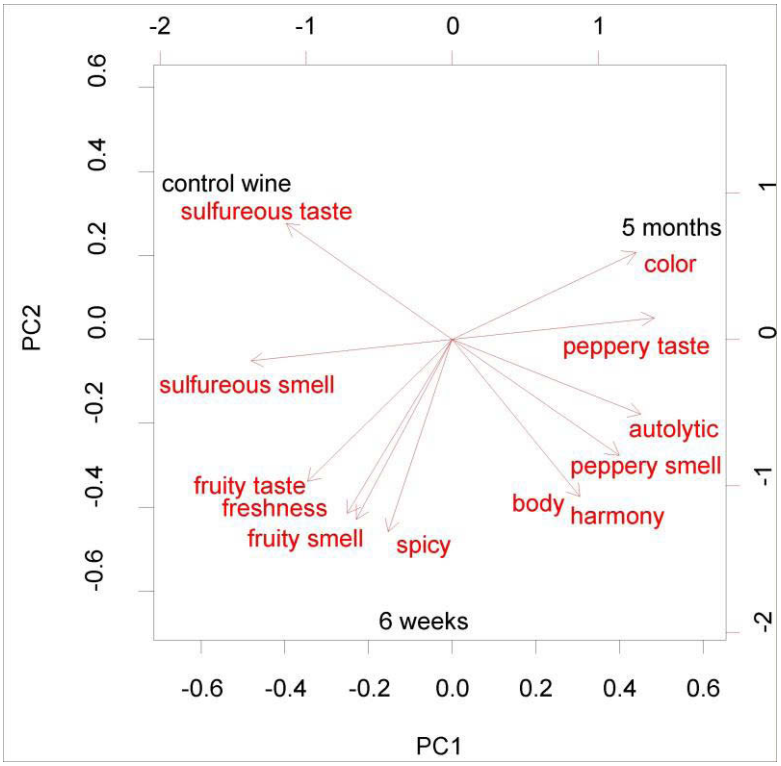

Figure 37: Principal component analysis of Grüner Veltliner wines (vintage 2007) produced with yeast Oenofem Veltliner at different stages of ageing on lees

Figure 37 depicts the principal component analysis of Grüner Veltliner wines (vintage 2007) produced with yeast Oenofem Veltliner at different stages of ageing on lees. The attributes “body” and “harmony” were very highly correlated, but negatively correlated with the attribute “sulphureous/taste”. The descriptor “peppery/smell” was as well negatively correlated with the descriptor “sulphureous/taste”. The attributes “freshness” and “fruity/smell” were highly correlated. The attribute “peppery/taste” was negatively correlated with the attribute “sulphureous/smell”.

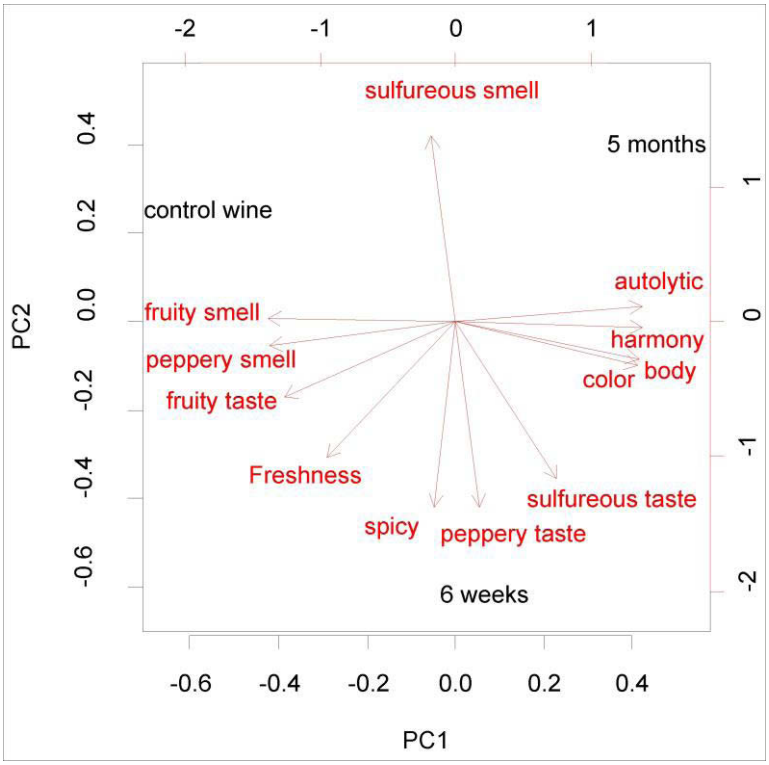

Figure 38: Principal component analysis of Grüner Veltliner wines (vintage 2007) produced with yeast EC 1118 at different stages of ageing on lees

Figure 38 presents the principal component analysis of Grüner Veltliner wines (vintage 2007) produced with yeast EC 1118 at different stages of ageing on lees. The attributes “color” and “body” were very highly correlated. The descriptors “autolytic” and “harmony” were as well highly correlated. The “peppery/taste” was negatively correlated with the attribute “sulphureous/smell”, the same situation like for the yeast Oenoferm Veltliner. The descriptor “fruity/smell” was negatively correlated with the descriptors “autolytic” and “harmony”.

Attributes	Taster	Yeast	Time	Yeast*Time
autolytic	$p < 0.05$	ns	$p < 0.05$	ns
color	$p < 0.05$	ns	$p < 0.05$	ns
freshness	$p < 0.05$	ns	$p < 0.05$	ns
fruity/smell	$p < 0.05$	ns	$p < 0.05$	$p < 0.05$
fruity/taste	$p < 0.05$	ns	ns	ns
harmony	$p < 0.05$	ns	$p < 0.05$	ns
body	$p < 0.05$	ns	$p < 0.05$	ns
peppery/smell	$p < 0.05$	$p < 0.05$	ns	ns
peppery/taste	$p < 0.05$	ns	ns	ns
sulphureous/smell	$p < 0.05$	ns	ns	ns
sulphureous/taste	$p < 0.05$	ns	ns	ns
spicy	$p < 0.05$	ns	ns	ns

Table 61: Sensory evaluation results and analysis of variance for each attribute (vintage 2007)

The sensory evaluation revealed differences between the control wines and the wines aged on lees. Differences regarding the influence of the individual yeast strains were also noted. The sensory data obtained from the panel was analysed with the Univariate analysis of variance (ANOVA) using the SPSS programme. The results showed that the factor time displays significant differences ($p<0,05$) for the attributes “autolytic”, “color”, “freshness”, “fruity/smell”, “harmony” and “body” (Table 61). If the ageing on lees is carried out without sulphur addition the factor time influences significantly the color intensity of wines. The factor time influences the autolytic character of wines which expresses the autolysis process. The gain in “autolytic” character was always accompanied by a loss of intensity for attributes “fruity”, “freshness” and “young”.

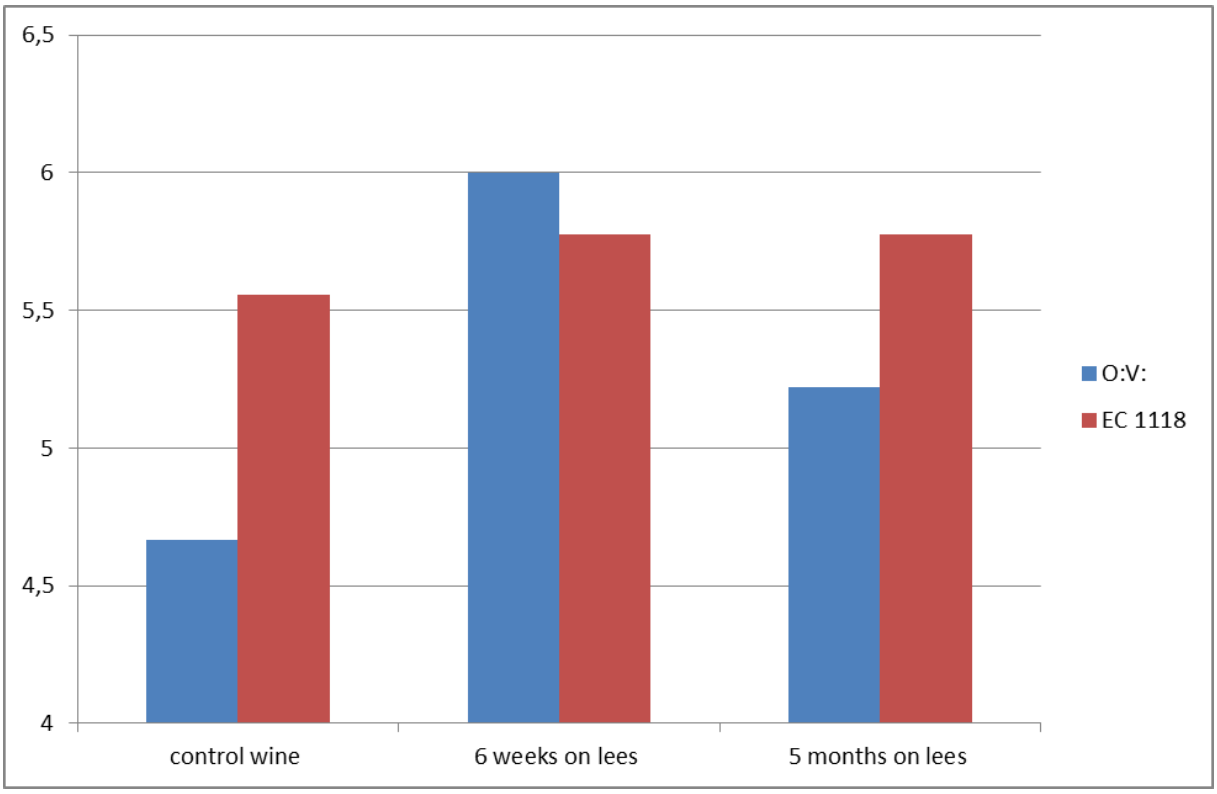

Figure 39: The overall sensory impression of Grüner Veltliner wines (vintage 2007) produced with yeast Oenoferm Veltliner and yeast EC 1118 at different stages of ageing on lees

Figure 39 depicts the overall sensory impression of Grüner Veltliner wines (vintage 2007) produced with yeast Oenoferm Veltliner and yeast EC 1118 at different stages of ageing on lees. The panel preferred the control wine produced with the yeast EC1118. For the both yeasts the wines maturated on lees were better rated than the control wines. For the yeast Oenoferm Veltliner, the most preferred wine was the wine with 6 weeks lees contact, but for the yeast EC 1118 the assessors did not find any difference in the overall sensory impression between the control and the treated wine.

4.5.5 Influence of qualitative and quantitative factors related to lees

4.5.5.1 Vintage 2008

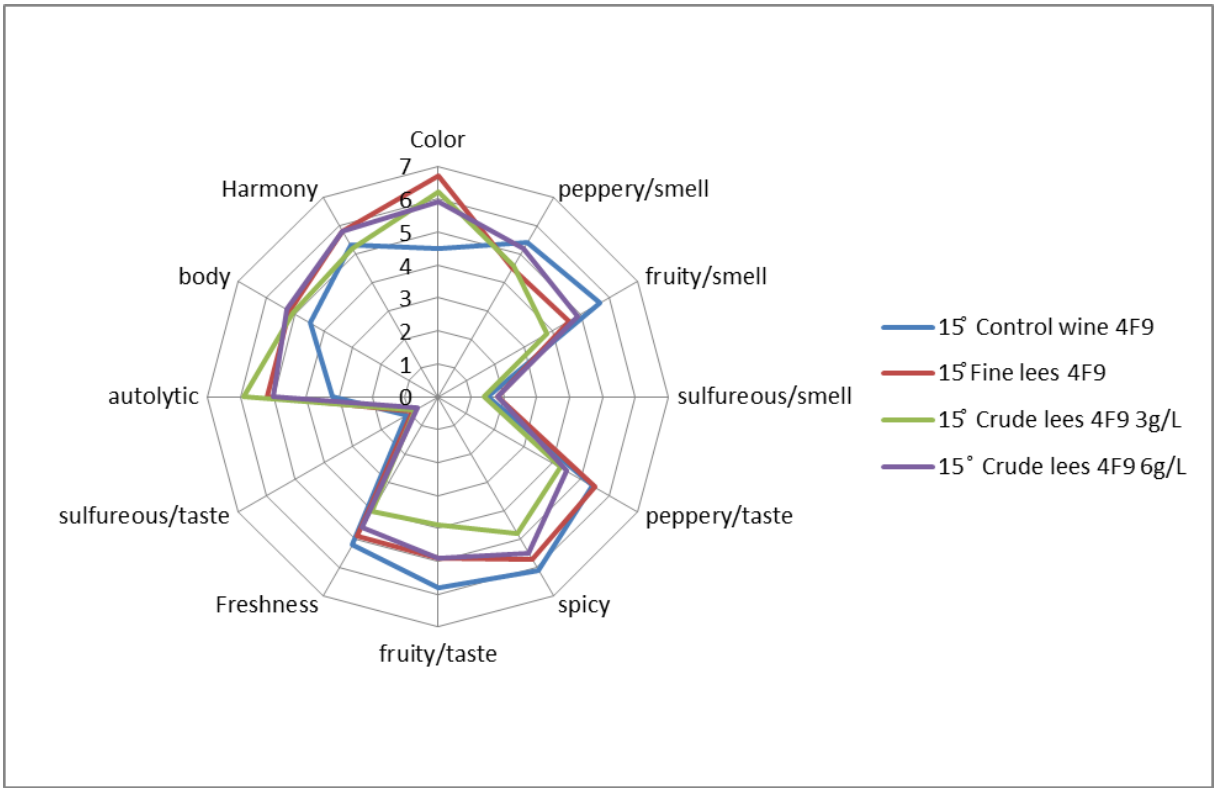

Figure 40: Aroma profile of Grüner Veltliner wines (vintage 2008) produced with Fermicru 4F9 15°C with different amounts of lees

Figure 40 presents the aroma profile of Grüner Veltliner wines (vintage 2008) produced with Fermicru 4F9 15°C with different amounts of lees. All the samples maturated on lees were assessed with higher intensity for the descriptor “color”. The descriptor “peppery/smell” had the highest intensity in the control wine and in the wine with 6g/l crude lees. The wine with fine lees and the wine with 3g/l crude lees were noted with the same intensity for the attribute “peppery/smell” but with a slight lower intensity than the other ones. The “fruity/smell” was marked with the highest intensity in the control wine. The “peppery/taste” was noted with the same intensity in the control wine and the wine with fine lees, the other two wines were marked with lower intensity. For the control wine, the wine with fine lees and the wine with 6 g/l crude lees the attribute “spicy” presented slight differences. The wine with 3 g/l crude lees was marked with much lower intensity for this attribute. The panel noted the descriptor “fruity taste” with highest intensity for the control wine. The wine with fine lees and the wine with 6 g/l crude lees were noted with the same intensity but the wine with 3 g/l crude lees was marked with much lower intensity for the attribute “fruity taste”. The descriptor “freshness” was rated with the lowest intensity in the wine with 3 g/l crude lees, in the other 3 wines the “freshness” was noted almost the same, more than the wine with 3 g/l crude lees. The “autolytic” character was fairly well expressed in all the wines with lees contact. The wine with 3 g l crude lees presented the highest intensity.The descriptor “body” presented for all the wines maturated on lees more intensity than the control wine. The sample fine lees

and the sample crude lees 6 g/l were noted with the highest intensity for the attribute “harmony”.

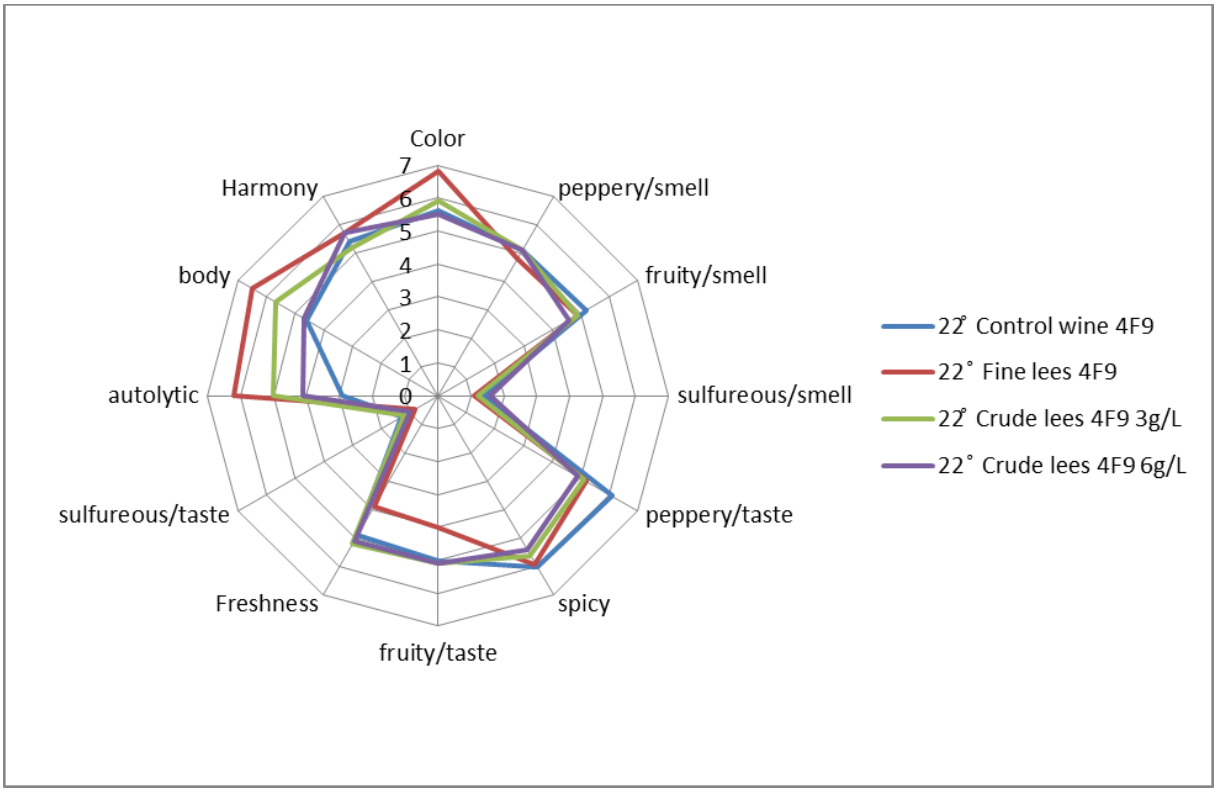

Figure 41: Aroma profile of Grüner Veltliner wines (vintage 2008) produced with Fermicru 4F9 22°C with different amounts of lees

The Figure 41 depicts the aroma profile of Grüner Veltliner wines (vintage 2008) produced with Fermicru 4F9 22°C with different amounts of lees. The attribute “color” was noted with the highest intensity in the wine with fine lees, between the other samples the attribute “color” presented small differences. The descriptors “peppery/smell”, “fruit/smell” and “sulphureous/smell” were assessed with the same intensity for all the samples. The panel noted the attribute “peppery/taste” with the highest intensity for the control wine, the sur lie samples were marked with the same intensity but lower than the control wine. The descriptor “spicy” was noted in all the wines with the same intensity. For the descriptors “fruity/taste” and “freshness” the wine with fine less was noted with lower intensity than the other wines. The control wine, the wines with 3 g/l and 6 g/l crude lees were marked for those two descriptors with the same intensity by the panel. The wine with fine lees presented the highest intensity for the attribute “autolytic”. The wines with crude lees were noted for this attribute with lower intensities. The “body” was well expressed in the wines with fine lees and crude lees 3 g/l, the sample with 3 g/l crude lees was marked with lower intensity than the sample with fine lees. The control wine and the wine with 6 g/l crude lees were noted with the same intensities. The descriptor “harmony” was assessed in all the samples with equal intensities.

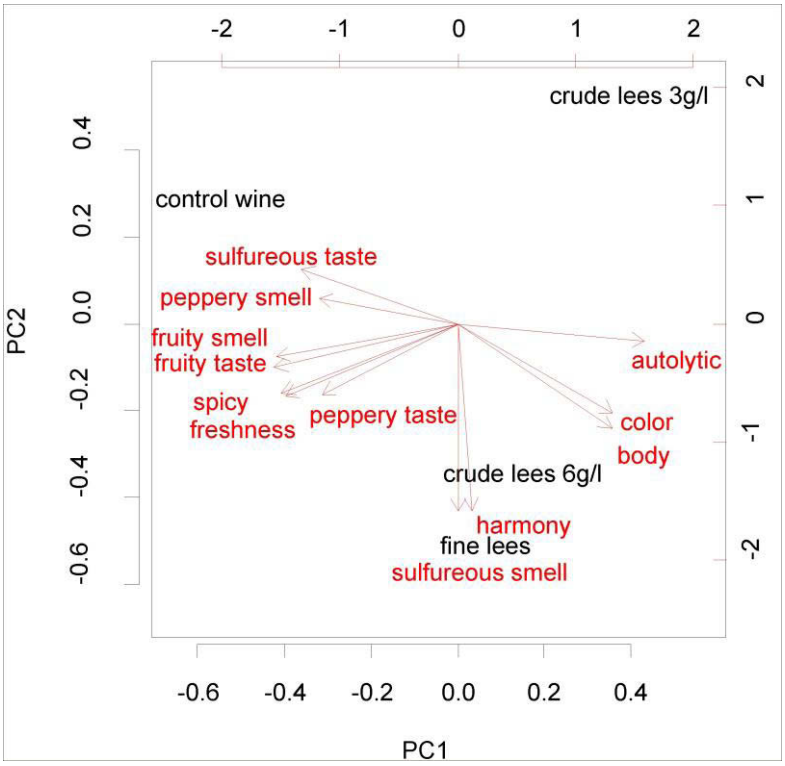

Figure 42: Principal component analysis of Grüner Veltliner wines (vintage 2008) produced with Fermicru 4F9 15°C with different amounts of lees

Figure 42 depicts the principal component analysis of Grüner Veltliner wines (vintage 2008) produced with Fermicru 4F9 15°C with different amounts of lees. The descriptors “color” and “body” were highly correlated and inversely correlated with the attribute “sulfureous/taste”. The attributes “spicy” and “freshness” were very highly correlated as well.

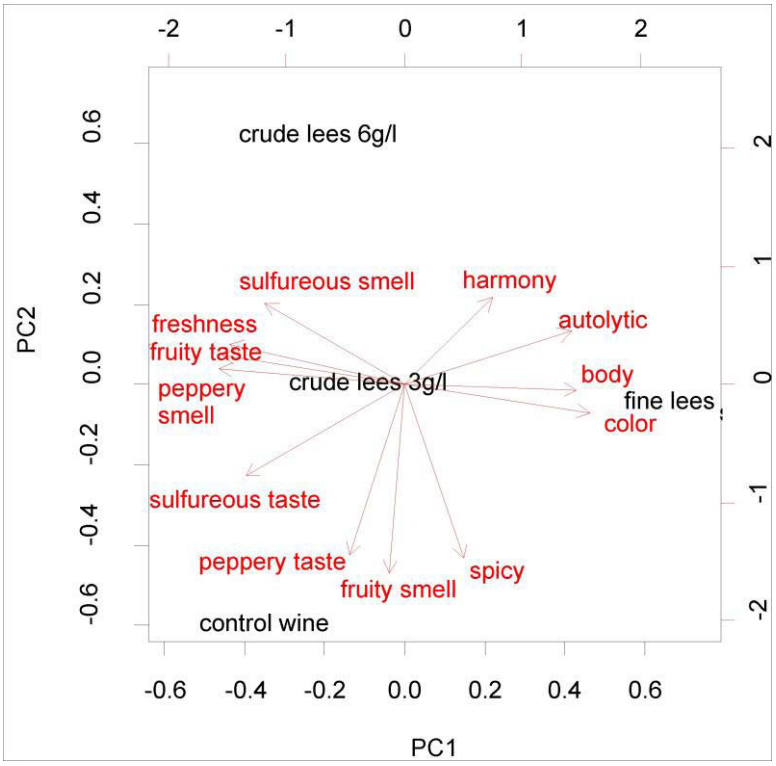

Figure 43: Principal component analysis of Grüner Veltliner wines (vintage 2008) produced with Fermicru 4F9 22°C with different amounts of lees

Figure 43 shows the principal component analysis of Grüner Veltliner wines (vintage 2008) produced with Fermicru 4F9 22°C with different amounts of lees. The descriptors “freshness”, “fruity/taste” and “peppery/smell” were very high correlated, but inversely correlated with the descriptors “body” and “color”. The attributes “peppery/taste”, “fruity/smell” and “spicy” were as well correlated but presented a lower correlation rate.

Attributes	Taster	Yeast amount	Temp	Yeast*Temp
autolytic	$p < 0.05$	$p < 0.05$	ns	ns
color	$p < 0.05$	$p < 0.05$	ns	ns
freshness	$p < 0.05$	ns	ns	ns
fruity/smell	$p < 0.05$	ns	ns	ns
fruity/taste	$p < 0.05$	ns	ns	ns
harmony	ns	ns	ns	ns
body	ns	$p < 0.05$	ns	ns
peppery/smell	$p < 0.05$	ns	ns	ns
peppery/taste	$p < 0.05$	$p < 0.05$	ns	ns
sulphureous/smell	$p < 0.05$	ns	ns	ns
sulphureous/taste	$p < 0.05$	ns	ns	ns
spicy	$p < 0.05$	ns	ns	ns

Table 62: Sensory evaluation results and analysis of variance for each attribute (vintage 2008)

Table 62 presents the results of the sensory data analysed with the Univariate analysis of variance (ANOVA). The factor yeast amount displays significant differences ($p < 0.05$) for the descriptors “autolytic”, “color”, “body” and “peppery smell”.

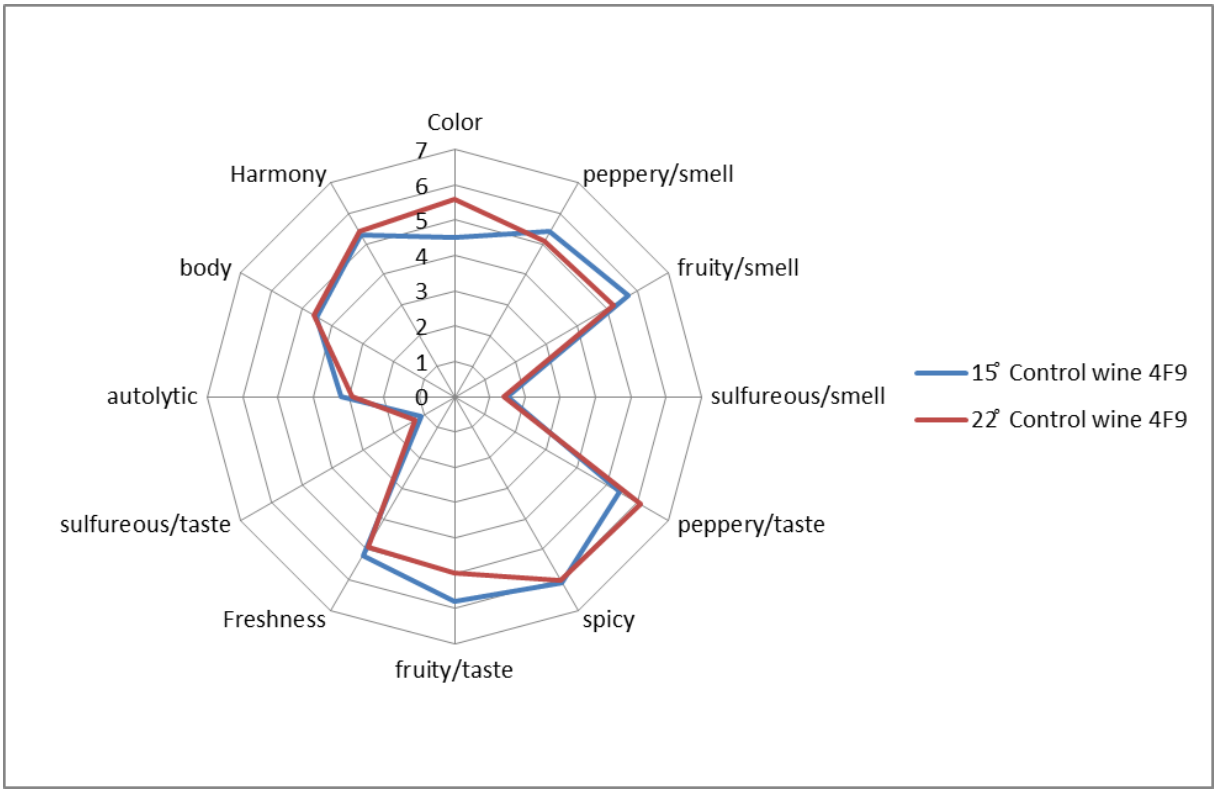

Figure 44: Aroma profile of Grüner Veltliner wines (vintage 2008, control wine) produced with Fermicru 4F9 at different temperature levels during ageing

After the fermentation the control wines were filtrated and sulphited. During storage the wines were kept at two different temperature levels, 15°C and 22°C. Figure 44 depicts the aroma profile of Grüner Veltliner wines (vintage 2008, control wines) produced with Fermicru 4 9 at different temperature levels during ageing. The attribute “color” presented more intensity in the wine kept at 22°C. The attributes “peppery/smell” and “fruity/smell” presented slightly more intensity in the wine stored at 15°C. The descriptor “peppery/taste” was assessed with more intensity for the wine stored at 22°C. The panel marked the wines with the same intensity for the descriptor “spicy”. The attributes “fruity/taste” and “freshness” were noted with more intensity in the wines kept at 15°C.

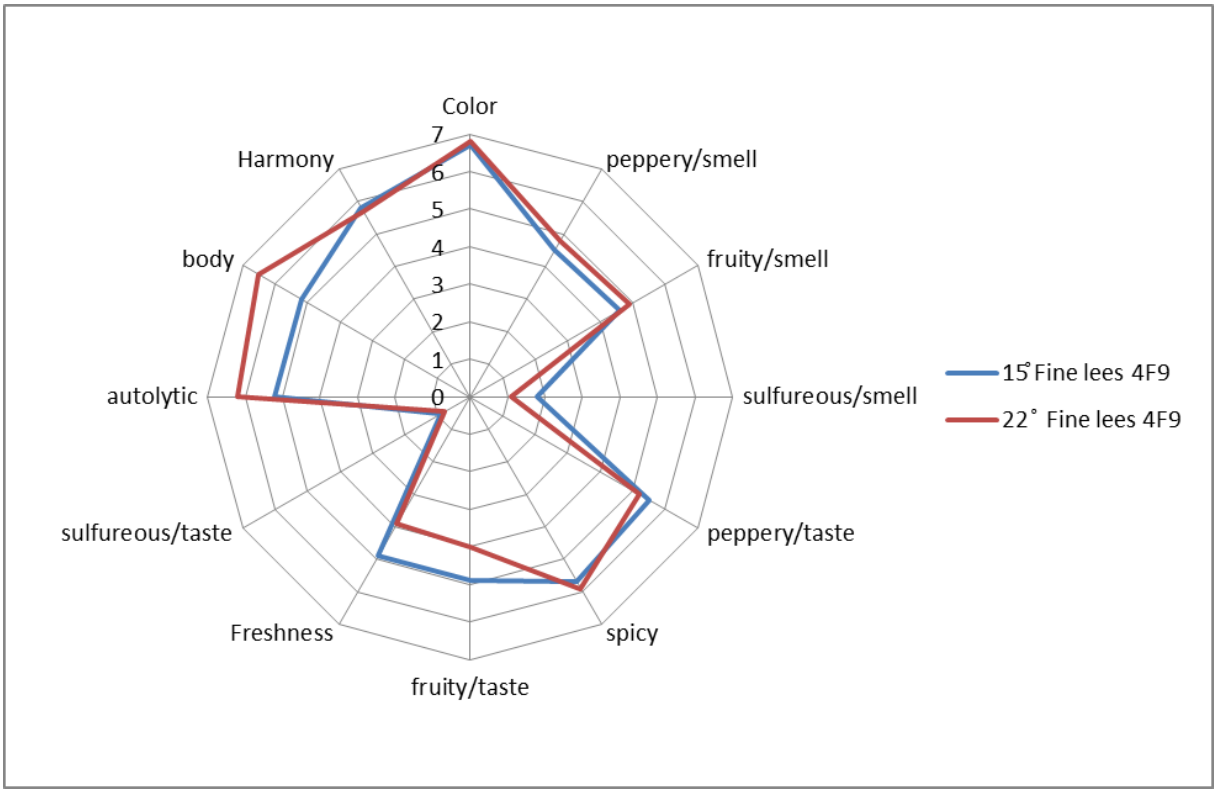

Figure 45: Aroma profile of Grüner Veltliner wines (vintage 2008, fine lees) produced with Fermicru 4F9 at different temperature levels during ageing

Figure 45 depicts aroma profile of Grüner Veltliner wines (vintage 2008, fine lees) produced with Fermicru 4F9 at different temperature levels during ageing. The tasters marked the attributes “color”, “peppery/smell”, “fruity/smell”, “peppery/taste” and “spicy” with the same intensity in the two wines. The descriptors “fruity/taste” and “freshness” were noted with more intensity in the wine with fine lees stored at 15°C. The attributes “autolytic” and “body” were marked with much more intensity in the wine with fine lees stored at 22°C.

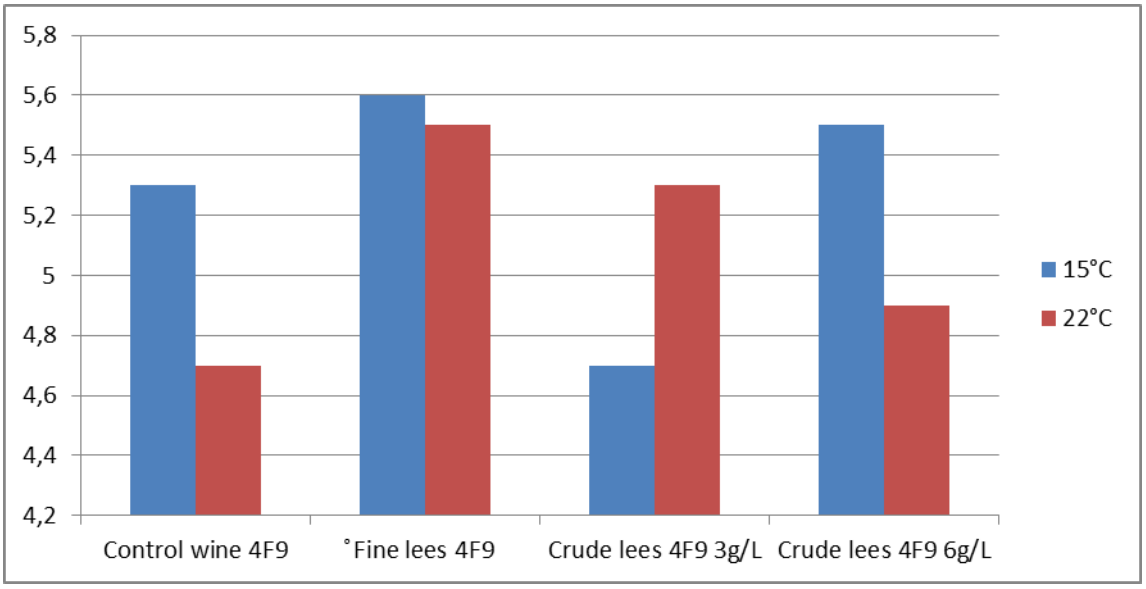

Figure 46: Overall sensory impression of Grüner Veltliner wines (vintage 2008) produced with yeast Fermicru 4F9 at 15°C and 22°C with different amounts of lees

Figure 46 presents the overall sensory impression of the wines produced with the yeast Fermicru 4F9 at 15°C and 22°C with different amounts of lees. At both temperature levels the most preferred wine was the wine with fine lees. The panel marked the control wine stored at 15°C much better than the control wine stored at 22°C. The wine with fine lees was noted almost the same at both temperature levels. The wine with 3 g/l crude lees stored at 22°C was preferred by the assessors. The wine with 6 g/l crude lees stored at 15°C was marked much better than the wine with 6 g/l crude lees stored at 22°C.

4.5.5.2 Vintage 2009

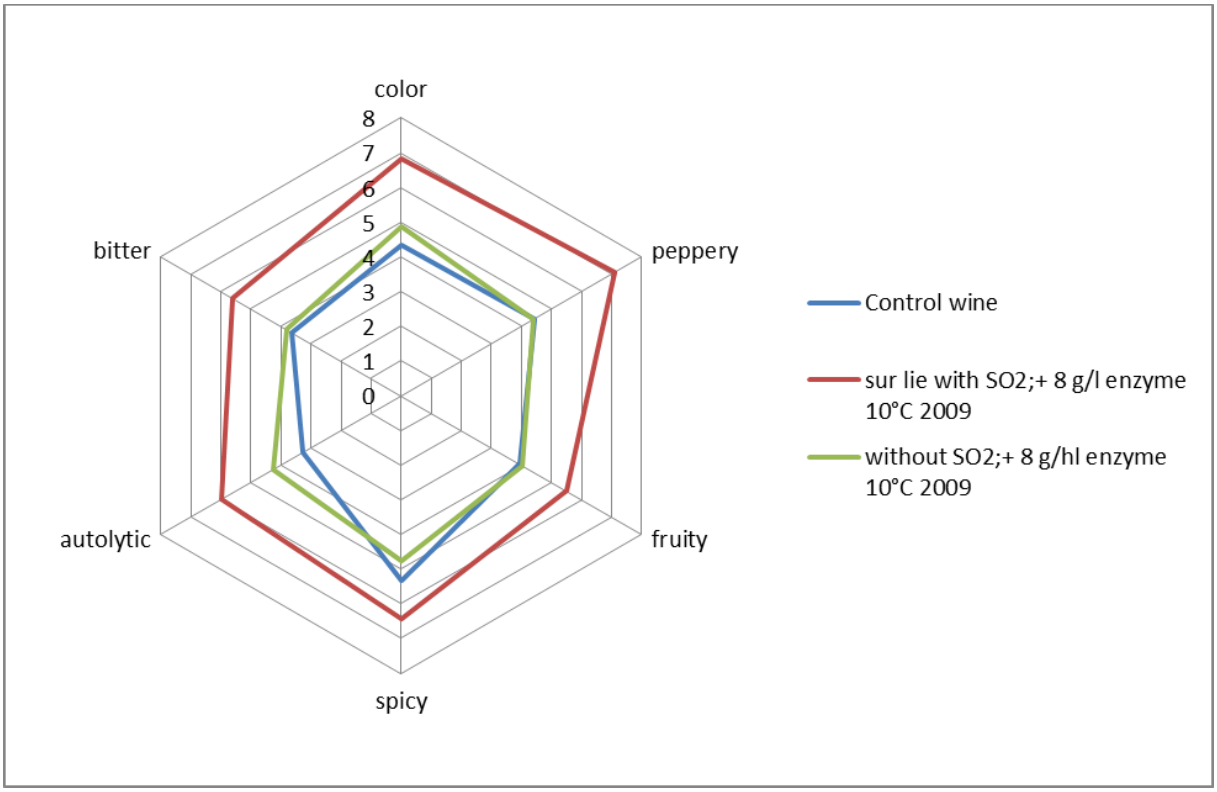

Figure 47: Aroma profile of Grüner Veltliner wines (vintage 2009) produced with the yeast Oenoferm Veltliner, 9 months lees contact at 10°C with and without sulphur addition

Figure 47 depicts the aroma profile of Grüner Veltliner wines (vintage 2009) produced with the yeast Oenoferm Veltliner, 9 months lees contact at 10°C with and without sulphur addition. The sur lie wine with SO_2 was marked by the assessors for the descriptor “color” with the highest intensity. The second wine for this descriptor was the sur lie wine without SO_2. The control wine was noted with the lowest intensity for the descriptor “color”. The attribute “peppery” and the attribute “fruity” were noted with the highest intensity in the sur lie wine with SO_2. The control wine and the sur lie wine without SO_2 were assessed with the same intensity but much lower than the sur lie wine with SO_2. The panel noted the sur lie wine with SO_2 with the highest intensity for the descriptor “spicy”. The control wine was noted for this attribute with slightly more intensity than the sur lie wine without SO_2, but much lower than the sur lie wine with SO_2. The autolytic character was very well expressed in both sur lie

wines, but the sur lie wine presented the highest intensity. The descriptor “bitter” was noted with the highest intensity in the sur lie wine with SO_2.

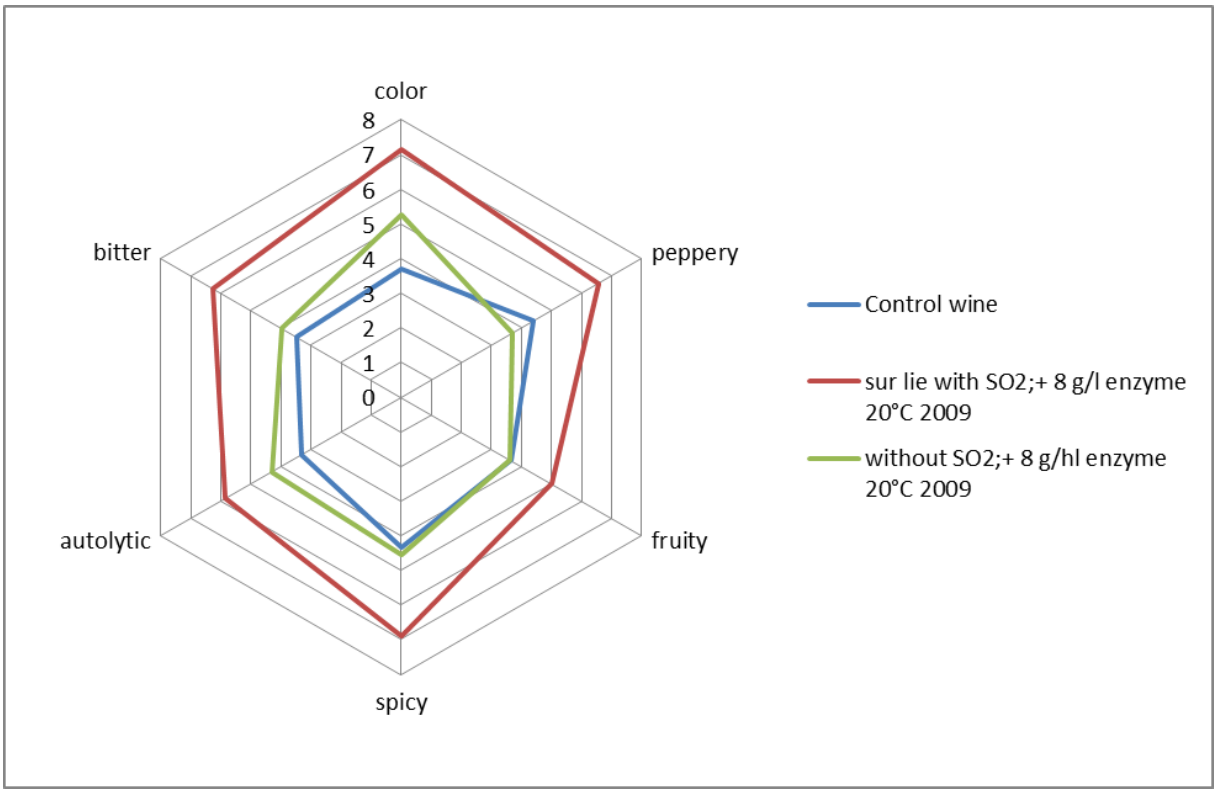

Figure 48: Aroma profile of Grüner Veltliner wines (vintage 2009) produced with the yeast Oenoferm Veltliner, 9 months lees contact at 20°C with and without sulphur addition

Figure 48 depicts the aroma profile of Grüner Veltliner wines (vintage 2009) produced with the yeast Oenoferm Veltliner, 9 months lees contact at 20°C with and without sulphur addition. The descriptor “color” was noted in the sur lie wine with the highest intensity, followed by the sur lie wine without SO_2. The control wine was marked with the lowest intensity. The attribute “peppery” presented the highest intensity in the sur lie wine with SO_2. The control wine and the sur lie wine without SO_2 were assessed with much lower intensities than the sur lie wine with SO_2, but the control wine presented slightly more intensity. For the attributes “fruity” and “spicy” the control wine and the sur lie wine without SO_2 were marked with the same intensity. The sur lie wine with SO_2 showed again the highest intensity for those two attributes. The “autolytic” character and the descriptor “bitter” were assessed with the lowest intensity in the control wine. The sur lie wine without SO_2 presented slightly more intensity than the control wine. The sur lie wine with SO_2 was marked with the highest intensity for the descriptors “autolytic” and “bitter”.

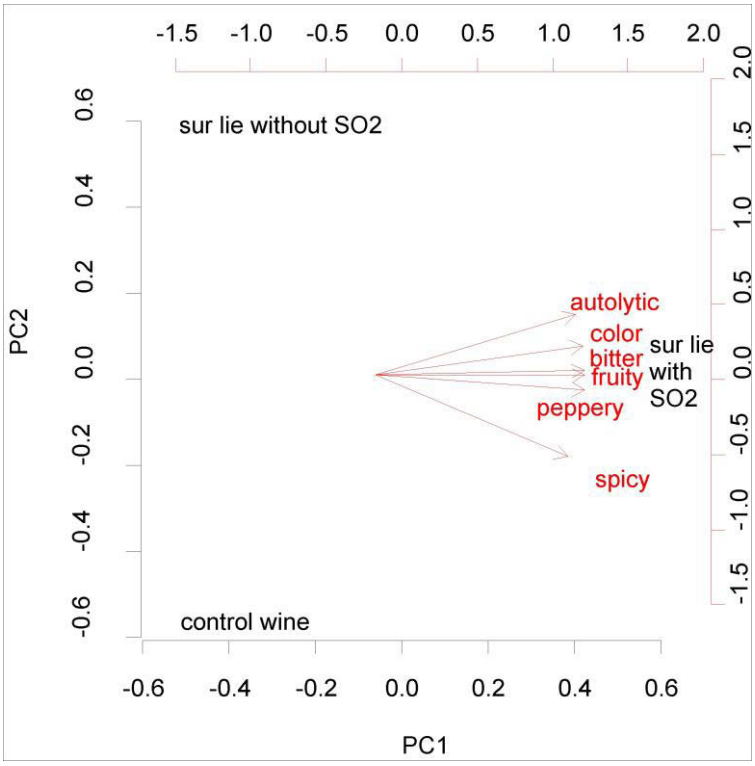

Figure 49: Principal component analysis of Grüner Veltliner wines (vintage 2009) produced with the yeast Oenoferm Veltliner, 9 months lees contact at 10°C with and without sulphur addition

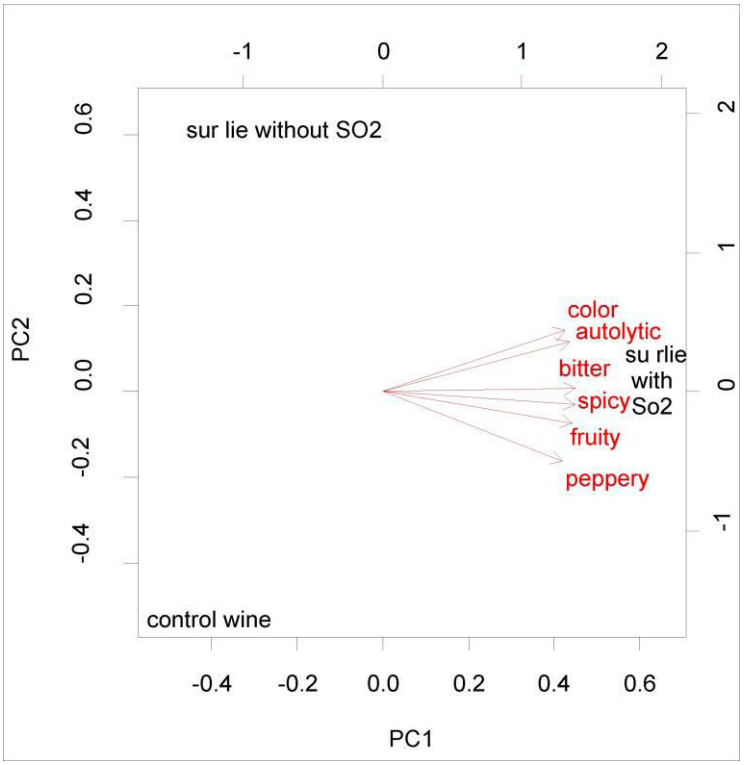

Figure 50: Principal component analysis of Grüner Veltliner wines (vintage 2009) produced with the yeast Oenoferm Veltliner, 9 months lees contact at 20°C with and without sulphur addition

Figures 49 and 50 show the principal component analysis of Grüner Veltliner wines (vintage 2009) produced with the yeast Oenoferm Veltliner, 9 months lees contact at different

temperature levels, with and without sulphur addition. All the vectors are directed to the sur lie wine with SO_2. This fact highlighted the preference of the tasters.

Attributes	Lees contact	Temp	SO_2
color	p < 0.05	ns	p < 0.05
peppery	p < 0.05	ns	p < 0.05
fruity	p < 0.05	ns	p < 0.05
spicy	p < 0.05	ns	p < 0.05
autolytic	p < 0.05	ns	p < 0.05
bitter	p < 0.05	ns	p < 0.05

Table 63: Sensory evaluation results and analysis of variance for each attribute (vintage 2009)

The sensory evaluation revealed differences between the control wines and the wines aged on lees, with or without SO_2. The sensory data obtained from the panel was analysed with the Univariate analysis of variance (ANOVA) using SPSS programme. The results showed that the factors lees contact and SO_2 display significant differences (p<0,05) for all the attributes (Table 63).

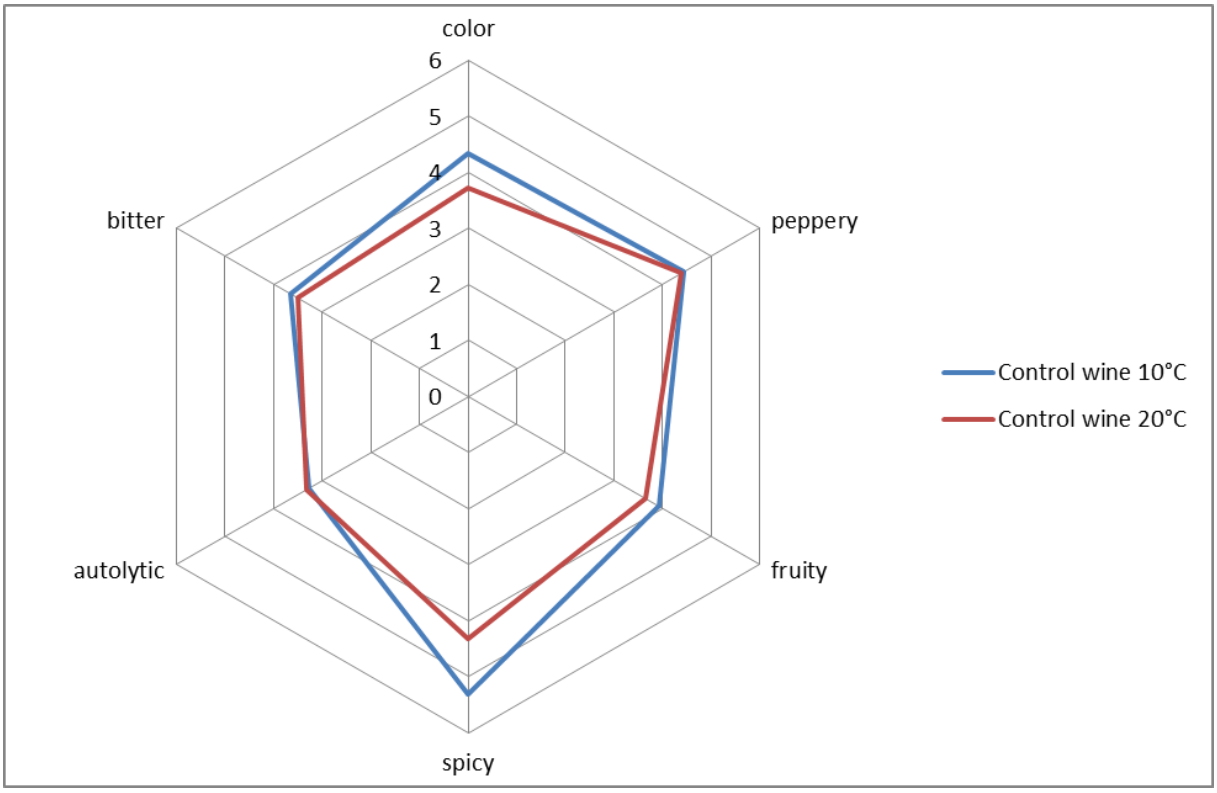

Figure 51: Aroma profile of Grüner Veltliner wines (vintage 2009, control wine) produced with the yeast Oenoferm Veltliner at different temperature levels

Figure 51 presents the aroma profile of Grüner Veltliner wines (vintage 2009, control wine) produced with the yeast Oenoferm Veltliner at different temperature levels. The attributes “color”, “fruity” and “spicy” presented more intensity in the control wine stored at 10°C for 9 months. The other attributes were marked with the same intensity.

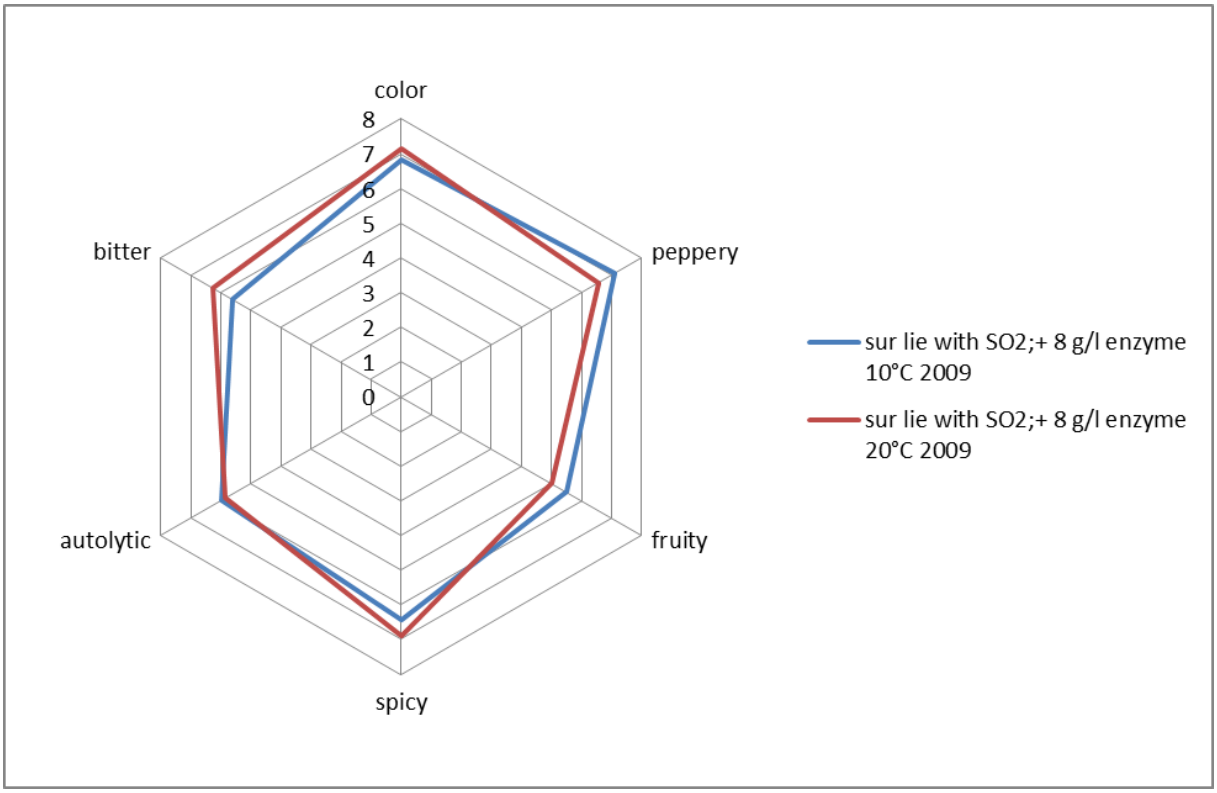

Figure 52: Aroma profile of Grüner Veltliner wines (vintage 2009, with sulphur addition) produced with the yeast Oenoferm Veltliner, 9 months lees contact at different temperature levels

Figure 52 presents the profile of Grüner Veltliner wines (vintage 2009, with sulphur addition) produced with the yeast Oenoferm Veltliner, 9 months lees contact at different temperature levels. The panel marked the descriptors “peppery” and “fruity” with more intensity in the sur lie wine with sulphur addition stored at 10°C. The attribute “bitter” was noted with more intensity in the sur lie wine with sulphur addition stored at 20°C. The other descriptors were noted with the same intensity.

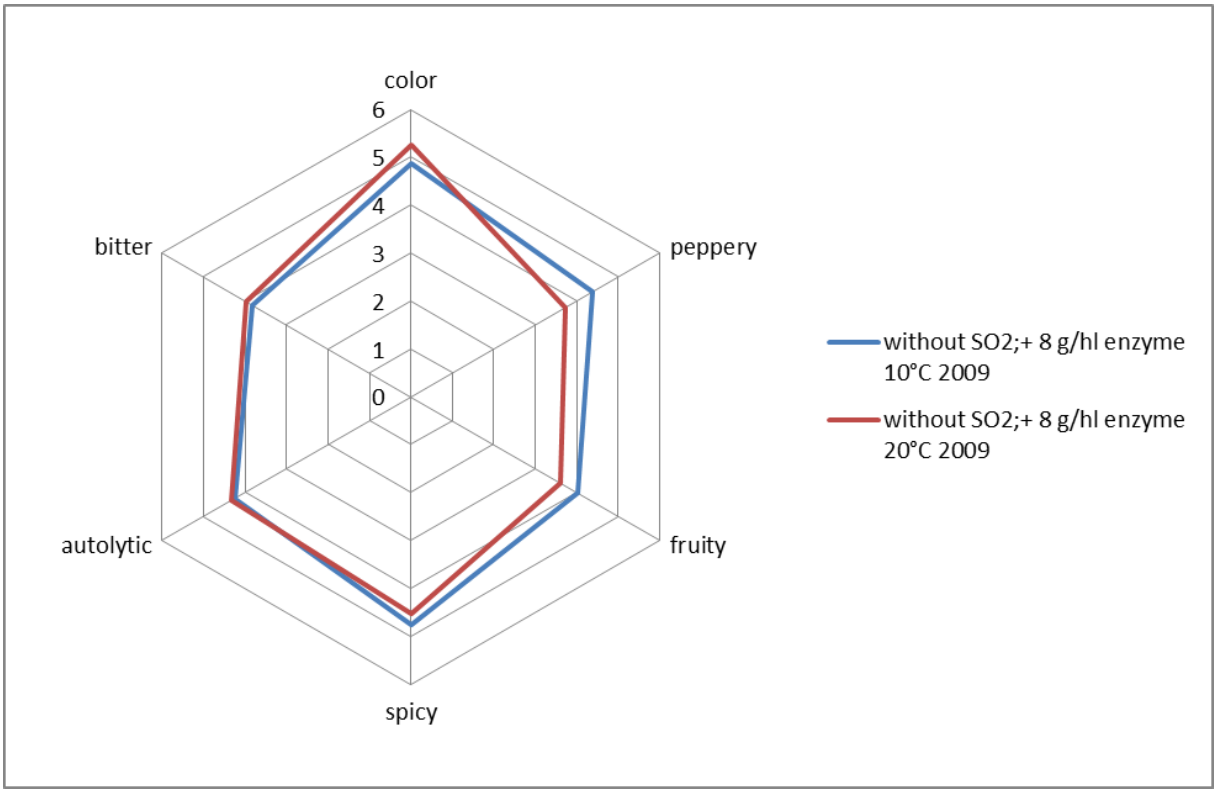

Figure 53: Aroma profile of Grüner Veltliner wines (vintage 2009, without sulphur addition) produced with the yeast Oenoferm Veltliner, 9 months lees contact at different temperature levels

Figure 53 depicts aroma profil of Grüner Veltliner wines (vintage 2009, without sulphur addition) produced with the yeast Oenoferm Veltliner, 9 months lees contact at different temperature levels. The sur lie wine without sulphur addition stored 9 months at 10°C was marked with more intensity for the descriptors “peppery” and “fruity”. The other attributes “spicy”, “autolytic”, “bitter” and “color” were noted with almost the same intensity.

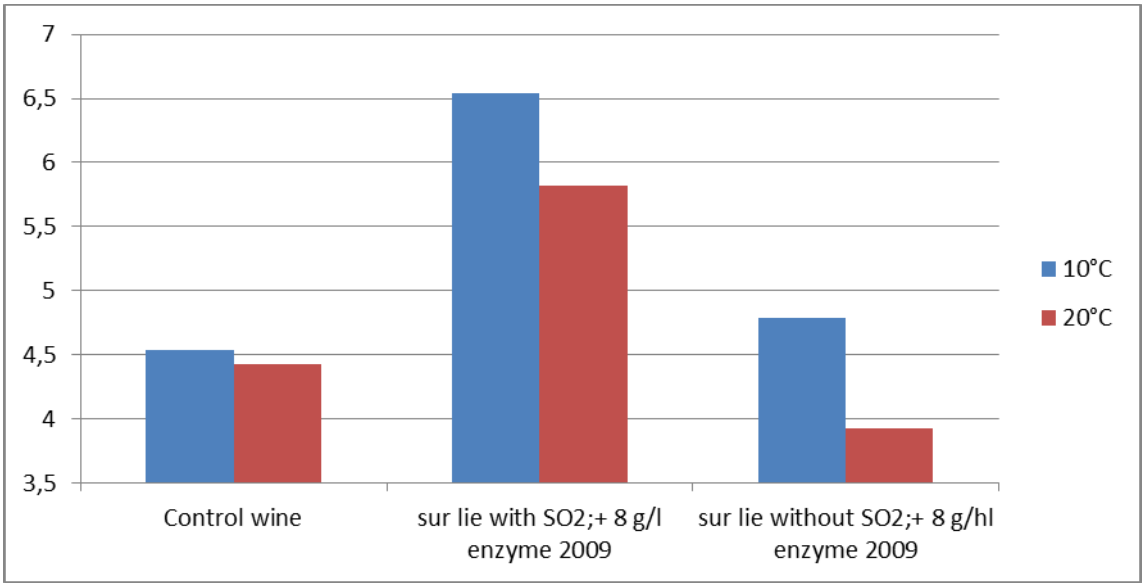

Figure 54: Overall sensory impression of Grüner Veltliner (vintage 2009) produced with the yeast Oenoferm Veltliner, 9 months lees contact at 10°C and 20°C with and without sulphur addition

Figure 54 depicts the overall sensory impression of Grüner Veltliner (vintage 2009) produced with the yeast Oenoferm Veltliner, 9 months lees contact at 10°C and 20°C with and without sulphur addition. The panel noted the control wines almost the same. The sur lie wines with sulphur addition were the most preferred wines. The tasters assessed the sur lie wine with sulphur addition, stored 9 months at 10°C as the best one. The sur lie wine without sulphur addition stored 9 months at 20°C was rated less than the control wine.

5. Discussion

Grüner Veltliner is a unique white wine grape variety. It can be produced in several different styles, from light fruity wines to powerful, full-bodied and complex wines. In the early 1960s Grüner Veltliner was known for its ability to produce higher yields. Reducing the yield and applying the progress of oenology from Grüner Veltliner grapes, it can produce world class wines. Grüner Veltliner is a cross of Traminer and St. Georgen. The spectrum of possible aromas is very complex, from Sauvignon Blanc wine styles to old-fashioned Grüner Veltliner wines.

The results obtained after the experiments carried out during four vintages reveals that the ageing on lees of Grüner Veltliner wines can create wines of a new type, offering the opportunity to diversify the product range.

The main sensory changes induced by the ageing on lees are: increase of body, harmony and complexity, the gain in autolytic character, the gain in color intensity and the loss of intensity for descriptors like fruity and freshness (the negative effects can be managed by sulfitation, temperature and duration of the contact with lees).

The increase of body and harmony was very well appreciated. The increase of autolytic character which is typical for the ageing on lees and the related loss of intensity for attributes like fruity, freshness and young are not always well approved.

The sensory changes induced by the ageing on lees are a result of compositional changes related to autolysis influenced by several factors, the most important are the duration of the lees contact time, yeast strain, yeast quality and amount, sulphur dioxide addition as well as temperature.

The autolytic character is well expressed after 3-6 months of lees contact time. With some yeast strains and maturation conditions the autolytic character can be noticed already after 6 weeks.

The yeast strain is an important factor when the wines are produced with usual technology: fermentation, racking, stabilization, filtration and bottling. In the experiments the sur lie ageing overcame the effect of yeast strain. During vintages 2006 and 2007 the influence of yeast strain in Grüner Veltliner sur lie wines was tested. The panel noted no significant differences among the wines regarding the effect of yeast strain.

Spicy and peppery the particular sensory attributes allocated to Grüner Veltliner wines are influenced by the sur lie ageing. Responsible for the distinctive aroma of Grüner Veltliner wines, peppery and spicy is the compound rotundone. Very high concentration of rotundone were found in Grüner Veltliner wines, concentrations higher than the sensorial threshold reported for red wines by a factor of between 4 and 17 (Mattivi et al. 2011). Rotundone was identified for the first time in Shiraz wine and in peppercorns. Rotundone is a sesquiterpene and by far the most powerful aroma compound, with an odor value in pepper on the order 50000-250000 (Wood, et al. 2008).

To elucidate the effect of ageing on lees on those specific attributes, further systematic experimental work is required, taking into account the evolution and involvement of rotundone. Reactions between rotundone and yeast cell wall are possible. One possible reaction can be between rotundone and mannoproteins located in the outer layer of the yeast

cell wall. At wine pH mannoproteins are negatively charged. An other possible pathway of rotundone modification can be the diffusing of rotundone molecule in the yeast cell wall to the protein layer. Proteins and particulary lipids from the yeast cell wall can react with terpens. Such a hypothesis has to be confirmed.

The experiments carried out during the vintage 2007, showed that for the wines produced with the yeast Oenoferm Veltliner the attribute peppery/smell was noted with higher intensity in sur lie wines compared to the control wine and the attribute peppery/taste was perceived with the same intensity by the panel. The attribute spicy was noted with almost the same intensity in all three wines. For the wines produced with the yeast EC 1118 the attribute peppery/smell was noted with almost the same intensity in all three wines but the attributes peppery/taste and spicy were noted with higher intensities in the sur lie wine maturated 6 weeks on lees. It can be assumed that the changes regarding the attributes peppery and spicy during ageing on lees are results between interactions of yeast lees and the compound rotundone. Perhaps during the fermentation some rotundone is adsorbed on the cell wall of the yeast and later during autolysis, when the cell walls are degraded, the absorbed rotundone is liberated back to the wine.

Vintage 2008, the wines stored at 15°C, the sur lie wine with 6g/l crude lees and the control wine were noted with the highest intensity for the attribute peppery/smell whereas peppery/taste was noted with the highest intensity in the sur lie wine with fine lees. The control wine presented the highest intensity for the attribute spicy. At 22°C no differences were observed for the attributes peppery/smell and spicy. The control wine presented the highest intensity for the attribute peppery/taste. Vintage 2009, the attributes peppery and spicy were noted with the highest intensity in the sur lie wines with SO_2 addition.

Another observation is that all the Grüner Veltliner sur lie wines with malolactic fermentation were noted with lower intensities for the attributes peppery and spicy. Perhaps the odor intensity of rontudone is influenced by the pH value. Malolactic fermentation increases the pH value of wine therefore the odor intensity of rontudone could be influenced by the malolactic fermentation.

The best results were obtained with ageing on fine lees independently on the temperature level.

As for the crude lees, the sensorial characteristics of wines were dependent on the temperature level: at 15°C the general impression and autolytic character were higher for the ageing on 6g/l crude lees whereas at 22°C better results were obtained with a lower amount of crude lees.

The slight initial sulfitation of wines allows a better evolution of their sensory features, prevents oxidation and inhibits the multiplication of bacteria. The sur lie wines with SO_2 produced in 2009 preserved their fruitiness and gained in peppery, spicy and autolytic.

In the absence of an appropriate level of free sulphur dioxide an increase of the color intensity was noticed as a result of oxidation and malolactic fermentation started spontaneously not only at the higher temperature levels, but also at the lower ones, being very hard to control, the fruitiness, freshness and balanced acidity were influenced.

The temperature is generally an important parameter during storage of wines; it accelerates or slows down most processes, including enzymatic ones. At lower temperature the wine preserves better its fruitiness, freshness and its potential to age. The same rule applies when it comes to ageing on lees. The wines aged on lees at lower temperature were preferred by

the tasters. The differences in freshness were more pronounced at 15°C in the wines matured on lees and the gain in body and autolytic character obtained at 22°C did not change the overall sensory impression.

The sur lie wine with SO_2 addition stored at 10°C produced in 2009 presented the best results by the overall sensory impression.

The wine treated with the product Batonnage plus Elevage had organoleptic attributes greatly differing from the sur lie wines. The wine treated with Batonnage plus Elevage presented high intensity for the attributes freshness and reduced odors. A direct comparation with sur lie wines was not meaningful. The added preparation can be compared with very short contact time with lees when some amino acids are extracted and the reductive character reinforces.

The autolysis produces strong sensory changes in the wines, the changes can still be observed after consecutive long bottle ageing periods.
Expert tasters and unexperimented ones perceive the sensory changes induced by the ageing on lees in a different manner. Most consumers do not find pronounced autolytic character very pleasant, so the ageing on lees should not be pushed too far.

Examination of wine samples

During the four vintages when the study was conducted the ethanol content of wines was different as a result of climatic conditions and harvest moment. The wines produced 2006 – had a high alcohol level: 14% vol; those from the vintage 2007 and vintage 2008 had moderate ethanol content: 12.7% vol and 12.4% vol respectively; for the vintage 2009 the ethanol concentration was 13.2% vol. All the wines produced for this study were dry, sugar level was below 2 g/l. Total acidity and pH were almost consistent in all four years. The wines that underwent malolactic fermentation showed a slight increase in tartaric acid concentration. Malolactic fermentation increases the pH value therefore increases the solubility of small potassium hydrogen tartrate crystals present in the lees. In the wines from the vintage 2008 samples with crude lees were setup, the increase of tartatic acid after malolactic fermentation was slightly higher in the sample with crude lees 3 g/l than in the sample with crude lees 6 g/l.
The contact with lees presented no significant influence on the general composition of wines, this is in agreement with Stuckey, et al (1991) who observed in Chardonnay wines no major changes in the pH and TA after a maturation period of five months and also with the results of Köhler et al. (2007).
The changes in pH, TA, malic acid and volatile acidity were provoked by malolactic fermentation.

Amino acids

The most abundant amino acids found in Grüner Veltliner wines during four years of study were: proline, arginine, alanine, ɣ aminobutyric acid, lysine and glutamic acid. Other authors (Soufleros et al., 2003) also observed that amino acids arginine, ɣ aminobutyric acid, lysine, alanine, glutamic acid and leucine were the most abundant in Greek white wines. Martinez-Rodriguez et al. (2002) found in the base wine for sparkling wine that the major amino acids were proline, glutamic acid, lysine and arginine.

Yeast strain

Vintage 2006 four yeast strains were used for the vinification and sur lie ageing. At the end of the sur lie process (6 months) the wines obtained with the yeast Fermicru 4F9 presented the highest concentration of free amino acids, followed by Oenoferm Veltliner, Lagerhaus Komplex and EC 1118 wines. For the yeast Oenoferm Veltliner the amino acid changes with the milieu are less important than for the other three yeasts. The yeast EC 1118, belonging to the specie *Saccharomyces bayanus*, absorbed the highest amount of amino acids during the fermentation. Taking into account the levels and the evolution of important amino acids during the ageing on lees the yeasts Lagerhaus komplex and Fermicru 4F9 have the ability to take up but also to give back the highest amounts of important amino acids. The following amino acids were significantly influenced by the yeast strain during the ageing on lees: serine, glutamic acid, glycine, glutamine, arginine, citrulline, proline, ɣ Aminobutyric acid, cysteine and ornithine.

During the vintage 2007 two yeast strains were used for vinification and sur lie ageing. At the end of the ageing period (5 months) the wine produced with the yeast Oenoferm Veltliner showed much more free amino acids than the one produced with the yeast EC 1118. The sum of amino acids without proline was 23% higher in the wine produced with Oenoferm Veltliner. All the amino acids were significantly influenced by the yeast with one exception ornithine. Here it should be considered that the yeasts EC 1118 and Oenoferm Veltliner are belong to different species (*Saccharomyces bayanus* and *Saccharomyces cerevisiae*) and that the results obtained during the experiments 2006 and 2007 regarding the uptake and release of amino acids are consistent.

It is important to note that the yeast strain influences the agumentation of essential amino acids in the wines during lees contact.

The results are slightly different from those obtained by Martinez-Rodriguez, et al. (2002) who reported that all the amino acids were significantly influenced by the yeast strain. In another study Martinez-Rodrigues et al. (2001) showed that the yeast strain significantly affected all the amino acids with two exceptions: methionine and asparagine. The induced autolysis was carried out in a model wine system. Perrot, et al. (2002) studied the effect of yeast strain on the nitrogen compounds released during induced autolysis in a model wine. They concluded that the yeast strain has no influence on the free amino acids released. Those findings disagree with Martinez-Rodriguez and the findings of this study.

Duration of lees contact

The effect of the ageing on lees time on amino acids was studied during vintages 2006 and 2007, but data from 2008 and 2009, with a single lees contact duration are also helpful compared with the control wine.
For the wines produced 2006 two ageing periods were used, 3 months and 6 months. After 3 months contact with lees the concentration of free amino acids increased. The same trend was registered after 6 months with same exceptions. The ageing time influenced significantly the free amino acids content except: glutamine, citrulline, alanine, ɣ aminobutyric acid, cysteine.
With the wines vintage 2007 the two ageing periods applied were 6 weeks and 5 months. The agumentation of free amino acids was even higher than in vintage 2006. Probably,

because of the different maturation level of grapes, the interaction with different yeasts and enzymes.
The duration of lees contact influenced significantly the concentration of all free amino acids which are used as nitrogen source by the yeasts.
The data obtained for the vintage 2008 and 2009 with 8 and 11 months lees contact showed an important increase of the amount of free amino acids compared to the control wine, whatever other conditions are used. The duration of lees contact is a very important factor related to the enrichment of amino acids.
Other authors have studied the release of amino acids during autolysis or induced autolysis. Alcaide-Hidalgo, et al. (2007) studied the influence of malolactic fermentation, postfermentative treatments and ageing on lees on the nitrogen compounds in red wines. His findings are in agreement with this study, all the free amino acids were significantly influenced by the ageing time. Martinez-Rodriguez, et al. (2002) studied the release of different nitrogen fractions in sparkling wines. All amino acids, except gamma aminobutyric acid were influenced by the ageing time. The release of nitrogen compounds during induced autolysis in a model wine system was studied by Martinez-Rodriguez, et al. (2001). All the analysed amino acids, except histidine were significantly influenced by the contact time. Other authors (Arizumi ı et al., 1994; Sato et al., 1997) also observed that aspartic acid, threonine, methionine, isoleucine, tyrosine, phenylalanine, histidine, and lysine are at least doubling in wines from Koshu region during storage of four months. It can be easily noticed that all those results reported by many authors have the same trend with some small differences, the ageing time displays significant influence on the free amino acids in wines with lees contact.

Enzyme addition

During the ageing on lees enzimatic disruption and degradation of cell walls occurs. Different enzimatic preparations were designed to accelerate these changes, to reduce the time of the sur lie ageing process.
The influence of enzyme addition was studied during two vintages: 2007 and 2009.
For the experimental wines vintage 2007 three enzymes were used. Between the three enzymes applied only small differences regarding amino acids concentration were noticed. In this study the most efficient enzimatic preparation was Vinoflow. The enzyme addition influenced the following amino acids: serine, histidine, valine, lysine, phenylalanine and tryptophan. During vintage 2009 one enzyme preparation was used. The most amino acids were not influenced by the enzyme addition. Only serine, asparagine and glycine were influenced by the enzyme addition. In these cases, with the chosen lees contact durations and temperatures, the natural enzimatic complex of lees acted fairly well. Probably shorter ageing periods should be applied in order to be able to point out the influence of enzymes on the concentration of amino acids in sur lie wines.

Lees quality and quantity

The influence of lees quality and quantity on amino acids concentration was tested at two temperature levels 15ºC and 22ºC on wines from the vintage year 2008.
The agumentation of amino acids was higher in sur lie wines with fine lees at both temperature levels. At 15ºC major differences were found between the wines with fine lees and wine with crude lees 3 g/l regarding the amino acids content. The concentration of serine, asparagine, glutamine, valine, lysine, isoleucine, phenylalanine and tryptophan was

double in the sur lie wine with fine lees. The same evolution was noticed at 22ºC with one exception being glutamine. As expected, the samples with crude lees 6 g/l showed higher concentration of amino acids than the samples with crude lees 3 g/l. All the free amino acids, except proline, methionine and ornithine were significantly influenced by the factor lees quality and quantity. Only proline showed higher concentration in the sur lie wines with crude lees at both temperature levels than in the sur lie wine with fine lees.
Koehler, et al. (2007), compared the effect of fine lees and whole crude lees on the amino acids concentration in sur lie wines produced from two grape varieties Silvaner and Grauburgunder (Pinot Gris). Both treatments influenced significantly the amino acids alanine, leucine, proline, asparagine, glutamine, phenylalanine, lysine, tyrosine, glycine, valine, methionine, histidine, isoleucine, tryptophan and cysteine but the wines with whole crude lees showed the highest concentrations of those amino acids.

Temperature and malolactic fermentation

The influence of temperature and malolactic fermentation was tested during vintage 2009. The sur lie wines stored at 22ºC with partially complete malolactic fermentation (0.6 g/l malic acid) presented after the ageing period higher concentration of amino acids than the sur lie wines stored at 15ºC with no malolactic fermentation. The level of free amino acids (without proline) in the control wines stored at 15ºC and 22ºC was almost the same. All free amino acids presented no major differences in concentration with two exceptions: aspartic acid was higher in control wine stored at 22ºC and lysine was higher in the control wine stored at 15ºC. The sum of free amino acids (without proline and hydroxyproline) in the sur lie wine with fine lees stored at 22ºC and partially completed malolactic fermentation was higher than in the sur lie wine with fine lees stored at 15ºC and partially complete malolactic fermentation. The concentration of aspartic acid, serine, glutamic acid, glycine, alanine, tyrosine, valine, ornithine, isoleucine, leucine, phenylalanine and tryptophan was higher in the sur lie wine with fine lees stored at 22ºC and partially complete malolactic fermentation. The concentration of asparagine and arginine was significantly lower in the sur lie wine with fine lees stored at 22ºC and partially complete malolactic fermentation. It is well known that arginine is used as a energy source. Arginine can be metabolized by lactic acid bacteria to form ornithine, CO_2 and ATP. In fact, the decrease of arginine level was correlated with the increase of the ornithine level. The same trend was observed in sur lie wines with crude lees 3 g/l and 6 g/l stored at 22ºC and partially completed malolactic fermentation. The sum of amino acids (without proline) was higher in the sur lie wines with crude lees 3 g/l and 6 g/l stored at 22ºC and partially completed malolactic fermentation than in sur lie wines with crude lees 3 g/l and 6 g/l stored at 15ºC and no malolactic fermentation. The difference can be explained by the fact that lactic acid bacteria possesses proteolytic activity which enriches the amount of free amino acids in wines and provides the cells with essential growth factors (Manca de Nadra, et al. 1997).
The following amino acids were significantly influenced by the temperature and malolactic fermentation: aspartic acid, serine, asparagine, glutamic acid, glycine, arginine, alanine, hyroxyproline, tyrosine, ornithine, isoleucine, leucine, phenylalanine and tryptophan.

Temperature

The amino acids content of control wines produced in 2009 is also affected by the storage temperature. Higher amounts are preserved at 10°C, the decrease between 15°C and 20°C

is less important. The rates of decrease are more important between 10°C and 15°C (losses of 52% for leucine, 45% for hydroxyproline, 39% for proline, 27% for glutamic acid). Between 15°C and 20°C these losses showed lower rates (between 1-15%), except for hydroxyproline (37%).
Temperature influences in a positive manner the liberation of amino acids. Even if there are other reactions reducing the amount of amino acids, increases of amino acids content with the increase of temperature were noticed. The increase of amino acids released with temperature is different for each compound. The most amino acids have a different correlation concentration-temperature. Important increases with temperature during sur lie ageing are noticed for lysine, glutamine and valine.

During the ageing on lees of the 2009 vintage wines three different temperature levels were used: 20ºC, 15ºC and 10ºC. The control wine stored at 10ºC presented the highest concentration of free amino acids. The other two control wines showed almost the same concentration of free amino acids. All amino acids except gycine and ornithine showed higher concentration in the control wine stored at 10ºC. It is assumed that some degradation reactions of amino acids were slower at this temperature level.
The sur lie wine with SO_2 and enzyme addition stored at 20ºC showed the highest concentration of free amino acids after the ageing period. The amino acids serine, asparagine, glycine, valine, lysine, isoleucine, leucine, phenylalanine and tryptophan presented higher concentration in this experimental wine. The same evolution was observed in the sur lie wines with SO_2 and no enzyme addition, the sample stored at 20ºC presented the highest concentration of free amino acids. The 11 months evolution on lees with a slight SO_2 protection lead to higher amino acid levels at 20°C compared to 15°C and 10°C, unaffected of the addition of exogenous enzymes.
In the sur lie wines without SO_2 and enzyme addition, minor differences were observed regarding the total concentration of free amino acids. The sum of amino acids was almost the same at all three temperature levels. In the sur lie wines without SO_2 and no enzyme addition the highest concentration of free amino acids was found in the sample stored at 20ºC. All amino acids except aspartic acid and alanine showed higher concentration in sur lie wine without SO_2 and no enzyme addition stored at 20ºC. As expected the agumentation of amino acids in sur lie wines stored at 20ºC was higher than at lower temperatures. The differences in total concentration of amino acids between 15ºC and 10ºC storing temperature were not so evident. Sato, et al. (1997) observed an increase of amino acids content about two times higher in the wine stored at 20ºC in contact with lees than the wine stored at 10ºC.
The temperature influenced significantly the following amino acids: serine, aspragine, glycine, glutamine, tyrosine, valine, ornithine and lysine.

SO_2 and malolactic fermentation

During the experiments on vintage 2009 wines which were not sulfitated underwent malolactic fermentation at all temperature levels. By comparing sulfitated samples with their not sulfitated ones it can evidentiate the effect of malolactic fermentation.
In all the sur lie wines with no SO_2 addition malolactic fermentation started spontaneously. The following amino acids were higher in all the sur lie wines with malolactic fermentation: serine, glutamic acid, threonine, alanine, tyrosine, lysine, isoleucine, leucine, phenylalanine and tryptophan. The level of total free amino acids was in most sur lie wines without SO_2

addition (with malolactic fermentation) higher than in sur lie wines with SO_2 addition. Only one situation was found to be different: at 20°C, the sur lie wine with SO_2 and enzyme addition showed after the ageing period a slight higher sum of free amino acids than the sur lie wine without SO_2 (with malolactic fermentation) and enzyme addition.
An increase of amino acids due to the assumed proteolytic activity of lactic acid bacteria was expected. The results meet the expectations of this study, but it some different patterns for individual amino aicds were noticed. The most amino acids have higher levels in the samples were malolactic fermentation took place, but their increase is temperature related. Serine for instance is increasing with malolactic fermentation more at 10°C and 15°C (by 31% and 38%) other amino acids (glutamic acid, isoleucine, tryptophan, lysine) show a maximum increase at 15°C (around 30%).
Arginine and ornithine should be judged separately as bacteria are able to transform one to the other. And indeed, the most important increase was noticed for ornithine at 10°C. For ornithine other pathways should be taken into account. At 20°C inspite of the decrease of arginine, the amount of ornithine did not increase, but it decreased.
Arginine was the only amino acid which was higher in the all sur lie wines with SO_2 addition, and can be a source of energy for some bacteria in a complete medium. Arginine cannot be taken up by the cell in the absence of a fermenTable sugar (Liu and Pilone, 1998). The metabolism of arginine was different at all three temperature levels. In sur lie wines with SO_2 addition stored at different temperature levels the concentration of arginine was between 89 and 105 mg /l and the concentration of ornithine was between 24 and 14 mg/l. In sur lie wines without SO_2 addition (with malolactic fermentation) stored at different temperature levels the concentration of arginine were between 7 and 60 mg/l and the concentration of ornithine was between 5 and 82 mg/l, therefore arginine was consumed by lactic acid bacteria and converted to ornithine. The consume of arginine was much higher at 10°C than at 15°C and 20°C; the amount of ornithine presented the same trend, at 10°C much more ornithine was produced than at 15°C and 20°C. Two possible explications are for the differences in consumption/production of arginine and ornithine: *O. oeni* strains differ in their ability to metabolize arginine or at low temperature the energy demand is higher therefore the bacteria have to metabolize more arginine. The consumtion of arginine leads to the production of ornithine, citrulline, carbamyl phosphate and ammonia (Liu, et al. 1994). Those reactions can influence positively or negatively the properties of wines. Ornithine has a inhibitory effect on the growth of *Hansenula minuta* (Mayer, et al. 1973), therefore can improve biological stability of wines. Citrulline is precursor of ethyl carbamate and ammonia increases the pH value and thus the risk of growth by spoilage bacteria (Mira de Orduna, et al. 2000).

Higher alcohols, methanol and ethyl acetate

The higher alcohols contribute to the sensorial complexity of wines, but higher amounts of these compounds can be detrimental for wine quality.

Yeast strain

The influence of yeast strain on the concentration of higher alcohols, ethyl acetate and methanol in Grüner Veltliner wines was analysed during vintages 2006 and 2007. The concentration of 1-propanol, isobutanol, isopentanol and 1-hexanol was significantly influenced by the yeast strain applied. Those results are in accordance with Herjavec, et al.

(2003) who investigated the influence of different yeast strains on the aroma substances in Chardonnay wines. Other authors (Wondra and Berovic, 2001; Lema, et al. 1996; Aragon, et al. 1998; Delteil, et al. 1992; Longo, et al. 1992) also concluded that the yeast type used for the alcoholic fermentation influences the quantity of higher alcohols therefore, the yeast strain influences the quality of wines.

Ageing time

The effect of ageing time on the concentration of higher alcohols, ethyl acetate and methanol in Grüner Veltliner wines was tested during vintages 2006 and 2007. No major changes were observed during sur lie process. The factor ageing time displayed no significant effect on the evolution of 1-hexanol, 1-propanol, ethylacetat, isobutanol and methanol. During both vintages the concentration of isopentanol decreased after long periods of ageing on lees. The evolution of isopentanol (isoamyl alcohol) was significantly influenced by the ageing time. Bueno et al. (2006) studied the effect of contact time with lees on volatile composition of Airen and Macabeo wines. He reported a significant increase in concentration for propanol, isobutanol, isoamyl alcohol, 2-phenylethanol, 1-butanol, 2-butanol, 1-hexanol and Z-3-hexenol due to the contact with lees in Airen wines. In Macabeo wines he reported a decrease in concentration for those compounds. The results are in contrast with the results obtained in Grüner Veltliner wines during ageing on lees. 1-Butanol and 2-butanol were not detectable in Grüner Veltliner wines whereas Bueno et al. (2006) detected high concentration (8000 µg/l). Bautista et al. (2006) studied the effect of contact with lees on the volatile composition of white wines. His results are in accordance to the findings obtained in Grüner Veltliner wines. 2-Butanol was not detected and the concentration of methanol, 1-propanol and isobutanol were very similar during ageing on lees.

Lees quality and quantity

During vintage 2008 the influence of lees quality/quantity on higher alcohols, ethyl acetate, ethyl lactate and methanol was analysed. None of those compounds presented significant behavior in relation to lees quality/quantity.

Enzymes addition

The enzyme addition during sur lie ageing was during vintages 2007 and 2009 tested. The compounds higher alcohols, ethyl acetate, ethyl lactate and methanol presented no significant behavior under the influence of this factor.

Temperature and malolactic fermentation

In Grüner Veltliner sur lie wines only ethyl lactate showed significant increase due to malolactic fermentation and higher temperature. The concentration of ethyl lactate was significantly lower in sur lie Grüner Veltliner wines stored at lower temperature with no malolactic fermentation. The level of ethyl lactate was above its taste threshold of 60-110 mg/l (Dittrich, 1987). Ethyl lactate enhances the body of wines (Henick-Kling, 1993).

Temperature

The influence of temperature on higher alcohols, ethyl acetate, ethyl lactate and methanol was tested during vintage 2009. The concentration of 1-propanol, ethyl lactate and isobutanol changed under the influence of temperature during ageing on lees. The levels of ethyl lactate presented major differences. At low temperature the concentration of ethyl lactate was 3 times lower than at high temperature. The formation of the ester is influenced by the temperature indirectly the first step is the formation of lactic acid. At low temperature the concentration of ethyl lactate was below its taste threshold.

SO_2 and malolactic fermentation

1-propanol, ethyl acetate, ethyl lactate and isobutanol were influenced by the factor SO_2/MLF. The concentration of 1-propanol and isobutanol was higher in the samples with malolactic fermentation stored at low temperature. At high temperature the levels of 1-popanol and isobutanol were almost the same in the control wine and in different sur lie wines. The concentration of ethyl acetate and ethyl lactate was at all temperature levels higher in the sur lie wine with malolactic fermentation.

Biogenic amines

Biogenic amines are organic bases mainly formed by decarboxylation of amino acids or by amination and transamination of aldehydes and ketons (Maijala, et al. 1993; Silla Santos, 1996). Amines like histamine and tyramine are important for the blood pressure and the nervous system. Polyamines are necessary for growth of bacteria, therefore amines have an essential role in living cells (Lonvaud-Funel, 2001), but amines can display negative effects on humans like headaches and change in blood pressure. Those effects are amplified by ethanol directly or indirectly inhibiting the enymes responsible for the degradation of these substances (Maynard and Schenker, 1996).

The levels of biogenic amines were analysed in Grüner Veltliner wines with or without lees contact, with or without malolactic fermentation, from two vintages 2008 and 2009. The total concentration of biogenic amines was low in both vintages, but during vintage 2009 some changes were observed during the ageing on lees.

Factors that influence the presence of biogenic amine in wine:

Wine storage temperature in a reducing environment such as bottle, has a slight influence on the content of biogenic amine. Most changes in amines concentration were noticed during the 45 days of wine storage (Gonzalez, A.M and Azpilicueta, C. A., 2006). Wine ageing and acidity influence the content of biogenic amines in wines. Proestos, et al. (2008) found that wines with low acidity contained higher amounts of histamine. It appears that addition of complex bacterial nutrients after the fermentation could increase the production of biogenic amine. The use of complex nutrients in real grape must increase the histamine concentration and the use of complex nutrients in synthetic grape must increase the concentration of putrescine and cadaverine. Therefore it is recommended to use bacterial nutrients in combination with commercial starter cultures that do not produce biogenic amine (Smit et al., 2012). Those results contrast with Marques at al. (2008) who reported that viticultural region, grape varieties and storage on lees influence the wine amines content but the use of alcoholic fermentation and malolactic activators does not influence the biogenic amines concentration.

During this study it was not wanted to stimulate malolactic fermentation, on the contrary. But the amino acids, released during ageing on lees, stimulated MLF.

During the ageing on lees spontaneous malolactic fermentation took place in wines having insufficient SO_2 protection. The lactic bacteria involved in this process were identified as *Oenococcus oeni* strains. Lactic acid bacteria, including *Oenococcus oeni* are known for their different ability to decarboxilate amino acids and to produce amines.

It was tried to inhibit malolactic fermentation during vintage 2008 by adding the enzyme preparation lysozyme to the sur lie wines, but the action of lysozyme was temporarily. Probably the bacterial population was too high by the time when the enzyme was added, therefore malolactic fermentation was only delayed. Probably it would have been better to add lysozyme already to the must in order to keep the bacterial population low and than again during sur lie ageing. The use of lysozyme or the use of lysozyme in combination with slight sulfitation does not guarantee absolut protection against malolactic fermentation for long periods of time.

Vintage 2008

The content of phenylethylamine, isopentylamine and putrescine after 8 months of lees contact was almost identical for all the treatments applied. No significant changes were observed after the ageing period. These results are in agreement with Marques et al. (2008), who reported that levels of histamine, isoamylamine, phenylethylamine and putrescine in red wines were not influenced by the factor lees contact after two months of lees contact. The levels of cadaverine and tyramine were higher in the wines stored on lees. After six months of lees storage Marques et al. (2008) observed a slight increase of tiramine and a decrease of cadaverine. In Grüner Veltliner wines cadaverine and tyramine were not detected.

Vintage 2009

No significant differences were observed between the control wine and the sur lie wines with SO_2 additon (without malolactic fermentation) regarding biogenic amines content. As expected the sur lie wines without SO_2 (with malolacitc fermentation) showed higher levels of biogenic amines. Malolactic fermentation had a strong effect on the concentration of putrescine. Putrescine presented the highest concentration in the sur Grüner Veltliner wines with malolactic fermentation. Those results are in agreement with Martin-Alvarez et al. (2006) and Alcaide-Hidalgo at al. (2007). The source of putrescine in the red wines appears to be the malolactic fermentation, as opposed to white wines, where the initial concentration of the musts is the source (Herbert et al. 2005). There were indeed putresceine in all wines, including the control wines. The amount of putresceine increased very much in wines with no SO_2 protection and consequently malolactic fermentation. The putresceine levels were higher in the wines at 15°C, almost twice compared to 20°C, probably due to the different bacteria strains involved in the malolactic fermentation (see Chapter 5.1.4.3).

6. Conclusion and future perspectives

General considerations

This study is based on comprehensive vinification trials and on an observation period of four years and represents the first systematic investigation on the impact of the sur lie method on Grüner Veltliner wines.

The obtained results further indicate that the on lees maturation process can be regarded as an appropriate tool for producing Grüner Veltliner wines as it allows to obtain wines of different styles reflecting some high degree of novelty and diversity.

It could be shown that during the ageing on lees changes take place in terms of wine composition and sensory features, depending on different technological conditions.

When producing a high quality Grüner Veltliner sur lie wine the winemaker should pay attention to some conditions and technological factors: temperature during on lees ageing (not more than 14°C), type of container where the wine is stored (steel tank or wood barrel), lees quality and quantity, depending on the type of wine required with malolactic fermentation or without malolactic fermentation, with sulfur addition or without sulfur addition. In addition, regular sensory assessments of the wine aged on lees are necessary in order to avoid some "over–yeasty" off-flavour.

There are no regulatory constraints regarding sur lie vinification. For example, stirring the lees layer, the addition of enzymes and mannoproteins, yeast cell walls, or the addition of lees from another tank are permitted.

Analytical parameters and effects of the vinification method

The most important changes during the maturation on lees were noticed in the amino acids content and in the intensity of sensorial attributes (spicy, peppery, freshness, fruitiness and autolytic).

Amino acids such as proline, arginine, alanine, ɣ aminobutyric acid, lysine and glutamic acid were identified as being the most abundant ones in Grüner Veltliner wines. In fact, their levels correlate with the sur lie duration. This observation was consistent with earlier findings for other varieties and further depends on the individual yeast strain applied. In particular, the yeast strain used influences the concentration of higher alcohols in Grüner Veltliner wines, although during the lees contact time no major changes were observed regarding these metabolites.

The content of biogenic amines was very low in Grüner Veltliner wines, even in sur lie wines with malolactic fermentation. Only putrescine level increased significantly in the sur lie wines produced with malolactic fermentation stored at high temperature levels.

In the presence on lees, the following factors are of crucial impact on wine quality during maturation of Grüner Veltliner: SO_2 addition, temperature, contact time, lees quality, the individual properties of yeast strains and the wine matrix. The duration of lees contact time and corresponding ageing conditions largely determine the nature of the final product.

It should be taken into consideration that lees contact promotes malolactic fermentation, by offering nutrients for bacterial growth. If ageing on lees is carried out without the addition of sulphite, malolactic fermentation is very hard to control; it may start spontaneously at temperatures above 10°C.

The presence of crude lees did not reveal negative results as stated on other wine varieties. However, the quantity of free amino acids in the sur lie wines aged on crude lees was lower than in the sur lie wines aged on fine lees, but no other remarkable difference was noticed. If the must is well clarified (to less than 50 NTU), there is no problem in applying the sur lie method even in the presence of crude lees.

There are different enological products on the market that claim to allow a reduced duration of lees contact time. In this study enzyme preparations containing betaglucanase activity and being accepted for wine treatments did not shorten significantly the process.

Refining agents such as Battonage and Elevage (yeast cell walls) were not able to impart sensory features comparable with the sur lie wines. Other preparations should be tested in future trials.

Grape varieties may differ to some large extent; therefore, the wines matrices are very different and several parameters influence the autolysis process. Taking into account this fact, the present findings cannot be directly transferred to any other type of wine without prior testings.

Sensory attributes

The sensory properties of the wine are constantly changing during its evolution on lees, by e.g., gain in more body, harmony and complexity, but all the modifications are correlated with a decrease in freshness and fruitiness, more pronounced at higher temperatures. The ageing on lees modifies the sensory properties of a wine in such a powerful way that it can be noticed with different intensities even after long periods of bottle ageing. The significant differences provoked by lees contact were registered by both experienced and not experienced tasters.

The autolysis character as a result of a long-lasting process can be considered as a particular feature of the wines aged on lees for longer periods.

“Spicy and peppery” the particular sensory attributes allocated to Grüner Veltliner wines are influenced by the sur lie ageing. As rotundone could be responsible for these sensory attributes, patterns of its possible involvement were suggested to be confirmed by future studies.

Future research needs and practical impact

Taking into account the possible role of rotundone-related sesquiterpene content in the development of typical attributes in Grüner Veltliner wines, more research is needed to understand the impact of sur lie ageing. Studies are ongoing to clarify this approach.

From the industrial point of view, the obtained results of this study may help the winemaker on the one hand to select the suited yeast strain preparation for “sur lie” ageing of wines and

on the other hand to apply appropriate conditions in order to produce wines responding to the desired requirements.

If the winemakers decide to apply the sur lie maturation method they have to take into account several factors and questions. Among these, the following are of major relevance: which type of Grüner Veltliner wine they want to produce, the intensity of the autolytic character, when will the wine be launched on the market, if malolactic fermentation is desired or not, the parameters of the ageing on lees process depend on these demands. Among the major advantages resulting from the sur lie method, the generation of a new type of wine, the evolution of new sensory characteristics, the diversification of product range due to the enrichment of essential amino acids may be mentioned. On the contrary, a too much pronounced autolysis character can be observed, if the duration of lees contact is too long, or, last but not least, unwanted malolactic fermentation and potential production of biogenic amines may take place.

Regarding economic aspects, there are no major costs resulting from sur lie ageing. Higher costs only are relevant if new barrique barrels are used. As autolysis usually is a long lasting process, the wine produced in this manner needs longer time until its launch on the market. Thus, wineries acting under time pressure would not prefer the sur lie ageing principle.

7. Literature

Alcaide-Hidalgo, M., Victoria Moreno-Arribas, M., Martin-Alvarez, J. and Polo C. M. (2007) Influence of malolactic fermentation, post fermentative treatments and ageing with lees on nitrogen compounds of red wines. Food Chemistry 103, 572-581.

Alexandre, H. and Guilloux-Benatier, M. (2006) Yeast autolysis in sparkling wine – a review. Australian Journal of Grape and Wine Research 12, 119-127.

Aragon, P., Atienza, J. and Climent, D. (1998) Influence of clarification, yeast type, and fermentation temperature on the organic acid and higher alcohols of Malvasia and Muscatel wines. Am. J. Enol. Vitic., Vol. 49, No. 2, 211-219.

Arizumi, K., Suzuki, Y., Kato, I. Yagi, Y., Otsuka, K. and Sato, M. (1994) Winemaking from Koshu variety by the sur lie method: Change in the content of nitrogen compounds. Am.J. Enol. Vitic., Vol. 45, No. 3, 312-318

Arrieta, M. P. and Prats-Moya, M. S. (2012) Free amino acids and biogenic amines in Alicante Monastrell wines. Food Chemistry 135, 1511-1519.

Babayan, T.L. and Berzukov, M.G. (1985) Autolysis in yeast. Acta Biotechnologia, 2, 129–136.

Bartwosky, E.J. (2008) Bacterial spoilage of wine and approaches to minimize it. Letters in applied microbiology, 149-156.

Bartwosky, E.J., Costello, P. and Henschke, P.A. (2002) Management of the malolactic fermentation-wine flavour manipulation. Aust. & N.Z. Grapegrower & Winemaker 461a, 7-8, 10-12.

Bauer, R. and Dicks, L. M. T (2004) Control of malolactic fermentation in wine. A review. S. Afr. Enol. Vitic., Vol. 25, 74-88.

Bautista, R., Fernandez, E. and Falque, E. (2006) Effect of the contact with fermentation-lees or commercial-lees on the volatile composition of white wines. Eur. Food Res. Technol. 234, 405-413.

Bueno, J.E., Peinado, R. A., Medina, M. and Moreno, J. (2006) Effect of short contact time with lees on volatile composition of Airen and Macabeo wines. Biotechbol. Lett. 28, 1007-1011.

Busto, O., Guasch, J. and Borrull, F. (1996). Biogenic amines in wine: A review of analytical methods. Journal International des Sciences de la Vigne et du Vin, 30, 85–101.

Capozzi, V., Russo, P., Ladero, V., Fernandez, M., Fiocco, D., Alvarez, A.M., Grieco, F. and Spano, G. (2012) Biogenic amines degradation by *Lactobacillus plantarum:* toward a potential application in wine. Frontiers in Microbiology Vol. 3, 122, 2.

Caridi, A., Galvano, F., Tafuri, A. and Ritieni A. (2006) Ochratoxin A removal during winemaking. Enzyme Micrib. Technol. 40(1), 122-126.

Carrascosa, A. V., Martinez-Rodrigues, A., Cebollero, E. and Gonzalez, R. (2011) Saccharomyces yeast II: Secondary fermentation. In Carrascosa, A. V., Munoz, R., and Gonzalez, R. (Eds) Molecular wine microbiology, 43-51. Academic Press, Elsevier.

Cebollero, E. and Golzalez, R. (2006) Induction of autophagy by second-fermentation yeast during elaboration of sparkling wines. Applied and environmental Microbiology, June, 4121-4127.

Charpentier, C. (2010) Ageing on lees (sur lie) and the use of speciality inactice yeasts during wine fermentation. In Reynolds, A. G. (Eds.), Manageing wine quality, 164-182. Philadelphia: Woodhead Publishing.

Charpentier, C. and Freyssinet, M. (1989) The mechanism of autolysis in wine. Yeast, 5, 181-186.

Charpentier, C., Aussenac, J., Charpentier, M., Prome, J. C., Duteurtre, B. and Feuillat, M. (2005) Release of nucleotides and Nucleotides during yeast autolysis: Kinetics and potential impact on flavor. J. Agric. Food Chem. 53, 3000-3007.

Coeuret, V., Dubernet, S., Bernardeau, M., Gueguen, M., and Vernoux, J.P. (2003) Isolation, characterisation and identification of lactobabilli focusing mainly on cheeses and other dairy products. Lait 83, 269-306.

Collado, M.C., Moreno, Y., Cobo, J.M. and Hernandez, M., (2006) Microbiological evaluation and molecular characterization of bifidobacteria strains in commercial fermented milks. Eur. Food Res. Technol. 1–2, 112–117.

Costantini, A., Garcia-Moruno, E. and Victoria Moreno-Arribas, M. (2009) Biochemical Transformations Produced by Malolactic Fermentation. In Moreno-Arribas, M.V. and Polo, M.C. (Eds.), Wine Chemistry and Biochemistry, 61-80. New York: Springer.

Davis, C., Wibowo, D., Fleet, H. and Lee, T. (1988) Properties of wine lactic acid bacteria: Their potential enological significance, Am. J. Enol. Vitic., Vol. 39, 137-142.

Delteil, D. and Jarry, J.M. (1992) Characteristic effects of two commercial yeast strains on Chardonnay wine volatiles and polysaccharide composition. Austral. NZ Wine Indust. J. 7(1), 29-33.

Di Cello, F., Bevivino, A., Chiarini, L., Fani, R., Paffetti, D., Tabacchioni, S. and Dalmastri, C. (1997) Biodiversity of a *Burkholderia cepacia* population isolated from the maize rhizosphere at different plant growth stages, 4485-4493.

Dittrich, H.H. (1987) Mikrobiologie des Weines. Handbuch der Lebensmitteltechnologie, 2nd edition. Stuttgart: Ulmer.

Doco, T., Vuchot, P., Cheynier, V. and Moutounet, M. (2003) Structural modification of wine arabinogalactans during ageing on lees. Am. J. Enol. Vititc. 54:3, 150-157.

Dulpin, I. V. S., Mckinnon, B. M., Ryan C., Boulay, M., Markides, A. J. et al. (2000) *Saccharomyces* cerevisiae mannoproteins that protect wine from protein haze: Their release during fermentation and lees contact and a proposal for their mechanism of action. J. Argic. Food. Chem. 48, 3089-3105.

Du Plessis, H.W., Dicks, L.M.T., Pretorius, I.S., Lambrechts, M.G. and Du Toit, M. (2004) Identification of lactic acid bacteria isolated from South African brandy base wine. Int. J. Food Microbiol. 91, 19-29.

Du Toit, M., Engelbrecht, L., Lerm, E. and Krieger-Weber, S. (2010) Lactobacillus: the next generation of malolactic fermentation starter cultures-an Overview. Food and bioprocess Technology. Vol. 4, No. 6, 876-906 doi: *10*.1007/s11947-010-0448-8.

Ferrari, G., Meunier, J. M. and Feuillat, M (1987) Dosage des acides gras totaux du vin et des levures de vinification. Sciences des Aliments, 1, 61-76.

Feuillat, M., (2002) Yeast macromolecules: Origin, Composition and enological interest. Am. J. Enol. Vitic. 54:3, 211-2013.

Feuillat, M. and Charpentier, C. (1982) Autolysis of yeast in Champagne. Am. J. Enol. Vitic., Vol. 33, No. 1, 6-13.

Feuillat, M., Escot, S., Charpentier, C. and Dulau, L. (2000) Elevage des vins rouges sur lie fines. Interet des interactions entre polysaccharides de levures et polyphenols du vin. Rvue des Oenologues, 98, 17-18.

Fiechter, G., Pavelescu, D., Mayer, H.K. (2011) UPLC analysis of free amino acids in wines: Profiling of on-lees aged wines. Journal of Chromatography B, 1361-1366.

Fleet, G.H. (1993) the microorganisms of wine-making – isolation, enumeration and identification. In Wine Microbiology and biotechnology ed. Fleet, G.H., 1-26. Switzerland: Harwood Academicar Publisher.

Gabriel, K., R. (1971) The biplot graphical display of matrices with application to principal component analysis. Biometrika 58, 453–467.

Garcia-Moruno, E., Sanlorenzo, C., Boccaccino, B. and Di Stefano, R. (2005) Treatment with yeast to reduce the concentration of ochratoxin A in red wine. Am. J. Enol. Vitic. 56, 73-76.

Garcia-Ruiz, A., Gonzalez-Rompinelli, E. M., Bartolome, B. and Moreno-Arribas, M. V. (2011) Potential of wine-associated lactic acid bacteria to degrade biogenic amines. Int. J. Food Microbiol. 148, 115-120.

Gonzalez, A. M. and Azpilicueta, C. A. (2006) Amine concetrations in wine stored in bottles at different temperatures. Food Chemistry 99, 680-585.

Gonzalez, A. M. and Azpilicueta, C. A. (2006) Influence of lees contact on evolution of amines in Chardonnay wine. Journal of Food Science 71, Nr. 9, 544-548.

Guilloux-Benatier, M., Pageault, O., Man, A. and Feuillat, M. (2000) Lysis of yeast cells by *Oenococcus oeni* enzymes. J. Ind. Microbiol. Biotech., 25, 193-197.

Henick-Kling, T. (1993) Malolactic fermentation. In Wine Microbiology and biotechnology ed. Fleet, G.H., 289-326. Switzerland: Harwood Academicar Publisher.

Herbert, P., Cabrita, M. J., Ratola, N., Laureano, O. and Alves, A. (2005) Free amino acids and biogenic amines in wines and musts form the Alentejo region. Evolution of amines during alcoholic fermentation and relationship with variety, sub-region and vintage. J. of Food Engineering 66, 315-322.

Herjavec, S., Podgorski, V., Redzepovic, S. and Mirosevic, N. (2003) The influence of some commercial *Saccharomyces cerevisiae* strains on the quality of Chardonnay Wines. Food Technol. Biotechnol. 41, 77-81.

Herraiz, T., Huang, Z. and Ough, C. S. (1993) Amino acids and ethyl esters of amino acids in sparkling and “sur lie” wines. Ital. J. o od Sci., No. 1, 11-20.

Jacobson, J. L. (2006) Other residual reducing sugar methods. In Introduction to wine laboratory practices and procedures. Springer Science+Business Media, Inc., 133-134. New York, USA.

Karagiannis, A. and Lanaridis, P. (1999) The effectof various vinification parameters on the development of several volatile sulphur compounds in Greek white wines of the cultivars Batiki and Muscat Hamburg. Am. J. Enol. Vitic., 50, No. 3, 334-342.

Klis, M. F., Mol, P., Hellingwerf, K. and Brul, S. (2002) Dynamics of cell wall structure in *Saccharomyces cerevisiae.* FEMS Microbiology Reviews 26, 239-256.

Koehler, H. J., Gessner, M. and Herrmann J. (2007) Einfluss des Hefekontaktes und einer Zweithefegabe auf den Wein. Das deutsche Weinmagazin 22.

Lema, C., Garcia-Jares, C., Orriols, I. and Angulo, L. (1996) Contribution of *Saccharomyces* and non-*Saccharomyces* population to the production of some components of Albarina wine aroma. Am. J. Enol. Vol. 47, No. 2, 206-216.

Leroy, M., Charpentier, M., Duteurtre, B., Feuillat, M. and Charpentier, C. (1990) Yeast autolysis during champagne ageing. Am. J. Enol. Vitic. Vol. 41, No. 1, 21- 28.

Lick, S. (2003) Review: Typing systems for lactobacilli. Milchwissenschaft 58, 5/6.

Liu, S. Q. and Pilone, G. J. (1998) A review: Arginine metabolism in wine lactic acid bacteria and is practical significance. Journal of Applied Microbiology, 84, 315-327.

Liu, S. Q., Pritchard, G. G., Hardman, M. J. and Pilone, G. J: (1994) Citrulline production and ethyl carbamate (Urethane) precursor formation from arginine degradation by wine lactic acid bacteria *Leuconostoc oenos* and *Lactobacillus buchneri*. Am. J. Enol. Vitic., 45, no. 2, 235-242.

Lodisch, H., Berk, A., Zipursky, S.L., Matsudaira, P., Baltimore, D. and Darnell, J.E. (2001): Molekulare Zellbiologie. 4. Auflage. Spektrum Akademischer Verlag. Heidelberg; Berlin.

Longo, E., Velazquez, J. B., Sieiro, C., Cansado, J., Calo, P. and Villa, T. G. (1992) Production of higher alcohols, ethyl acetate, acetaldehyde and other compounds by 14*Saccharomyces cerevisiae* wine strains isolated from the same region (Salnés, N.W. Spain). Wold J. Microbio. and Biotech., Vol. 8, 5, 539-541.

Lonvaud-Funel, A. (2001) Biogenic amines in wines: role of lactic acid bacteria. FEMS Microbiology Letters 199, 9-13.

Lubbers, S., Leger, B., Charpentier, C. and Feuillat, M. (1993) Essai colloides protecteurs d'extraits de parois de levures sur la stabitite tartrique d'un vin modele. J. Intern. Sci. VIgne Vin 27, 13-22.

Lupski, J.R. and Weinstock, G.M. (1992) Short, interspersed repetitive DNA sequences in prokaryotic genomes. J. Bacteriol. 174, 4525-4529.

Lurton, L., Segain, J.P. and Feuillat, M. (1989) Etude de la proteolyse au cours de l' autolyse de levures en milieu acide. Sciences des Aliments 9, 111-124.

Maijala, R. L., Eerola, S. H., Aho, M. A. and Hirm, J. A. (1993) The effect of GDL-induced on the formation of biogenic amines in meat. J. Food Prot. 50, 125-129.

Manca de Nadra, M.C., Farias, M.E., Moreno-Arribas, M.V., Pueyo, E. and Polo, M.C. (1997) Proteolytic activity of *Leuconostoc oenos*, Effect on proteins and polypeptides from white wine. FEMS Microbiology Letters 150, 135-139.

Marques, A. P., Leitao, M. C. and San Romao, M. V. (2008) Biogenic amines in wines: Influence of oenological factors. Food Chemistry 107, 853-860.

Martineau, B., T. Henick-Kling and T. E. Acree. (1995) Reassessment of the influence of malolactic fermentation on the concentration of diacetyl in wines. Am. J. Enol. Vitic. 46, 385-388.

Martin-Alvarez, P.J., Marcobal, A., Polo, C. and Moreno-Arribas, M.V. (2006) Influence of technological practices on biogenic amine contents in red wines. Eur. Food Res. Technol. 222, 420-424.

Martinez-Rodriguez, A. J. and Polo, M. C., (2000) Characterization of the nitrogen compounds released during yeast autolysis in a model wine system. J. Agric. Food. Chem. 48, 1081-1085.

Martinez-Rodriguez, A. J., Carrascosaa, A. V., Martin-Alvarez, P. J., Moreno-Arribas, V. and Polo, M. C. (2002) Influence of the yeast strain on the changes of the amino acids, peptides and proteins during sparkling wine production by the traditional method. J. Ind. Micro. & Biotech. 29, 314-322.

Martinez-Rodriguez, A. J., Carrascosaa, A. V. and Polo, M. C. (2001) Release of nitrogen compounds to the extracellular medim by three strains of *Saccharomyces cerevisiae* during induced autolysis in a model wine system. Int. J. of Food Mirco. 68, 155-160.

Maynard, L.S. and Schenker, V.J. (1996) Monoamine-oxidase inhibition by ethanol in vitro. Nature, 196, 575-576.

Mattivi, F., Caputi, L., Carlin, S., Lanza, T., Minozzi, M., Nanni, D., Valenti, L and Vrhovsek, U. (2011) Effective analysis of rotundone at below-threshold levels in red and white wines using solid-phase microextraction gas chromatography/tandem mass spectrometry. Rapid Commun. Mass Spectrom. 25, 483-488.

Mayer, K., Pause, G., Vetsch, U., Künsch, U. and Temperli, A. (1973) Aminosäurengehalte in Verlaufe der Vinifikation einiger Rotweine. Mitt. Klosterneuburg 23, 311-40.

Michlmayer, H., Schümann, C., Barreira Braz da Silva, N.M, Kulbe, K.D. and A.M. del Hierro (2009) Isolation and basic characterization of a β-glucosidase from a strain of *Lactobacillus brevis* isolated from a malolactic starter culture. Journal of Applied Microbiology 108, 550-559.

Mira de Orduna, R., Patchett, L. M., Liu, S. Q. and Pilone, G. J. (2000) Growth and arginine metabolism of the wine lactic acid bacteria *Lactobacillus buchneri* and *Oenococcus oeni* at different pH values and arginine concentrations. Applierd and Environmental Microbiol., 1657-1662.

Moine-Ledox, V. and Dubourdieu D. (2002) Role des mannoproteines de levures vis a vis de la stabillisation tartrique des vins. Bull. OIV 75, 471-482.

Moreno-Arribas, M.V. and Polo, C. (2005) Winemaking microbiology and biochemistry: current knowledge and future trends. Crit. Rev. Food Sci Nutr., 45, 265-286.

Moreno, N. J. and Azpilicueta, C. A. (2007) Binding of oak volatile compounds by wine lees during simulation of wine ageing. LWT, 40, 619-624.

Osborne, J. P., Mira de Orduna, R., Pilone, G. J. and Liu, S. Q. (2000) Acetaldehyde metabolism by wine lactic acid bacteria. FEMS Microbiol. Letters., 191, 51-55.

Perez-Serradilla, J.A. and Luque de Castro, M.D. (2008) Role of lees in wine production: A review. Food Chemistry 111, 447-456.

Perrot, L., Charpentier, M., Charpentier, C., Feuillat, M. and Chassagne, D. (2002) Yeast adapted to wine: Nitrogen compounds released during induced autolysis in a model wine. J. Int. Micro. & Bitech. 29, 134-139.

Pozo-Bayon, M.A., Pardo, I., Ferrer, S., Moreno-Arribas, M.V. (2009) Molecular approaches for the identification and characterisation of oenological lactic acid bacteria. Afr. J. Biotechnol. 8, 3995-4001.

Pripis-Nicolau, L., Gilles de Revel, Bertrand, A. and Maujean, A. (2000) Formation of flavor components by the reaction of amino acid and carbonyl compounds in mild conditions. Journal of agricultural and food chemistry, Vol. 48, No. 9, 3761-3766.

Proestos, C., Loukatos, P. and Komaitis, M. (2008) Determination of biogenic amines in wines by HPLC with precolumn dansylation and fluorimetric detection. Food Chemistry 106, 1218-1224.

Pueyo, E., Martinez-Rodriguez, A., Polo, M. C., Santa-Maria, G. and Bartolome, B. (2000) Release of lipids during yeast autolysis in model wine system. Journal of agricultural and food chemistry, 48, 116-122.

Ribereau-Gayon, P., Dubourdieu, D., Doneche, B. and Lonvaud A. (2006) Lactic acid Bacteria in Handbook of Enology, The Microbiology of Wine and Vinifications, Vol. 1, 115-138.

Ribereau–Gayon, P., Glories, Y., Maujean, A. and Dubourdieu, D. (2006) Organic acids in wine. In Handbook of Enology: The chemistry of wine and stabilization and treatments. John Wiley & Sons, Ltd, Vol. 2, 3-49.

Rosi, I., Cheri, A., Domizio, P. and Fia, G. (1999) Production de macromolecules parietals de *Saccharomyces cerevisiae* au cours de la fermentation et leur influence sur la fermentation mololactique. Rev. Oenolog. Techn. Vitivinic. Oenologiq. 94, 18-20.

Salmon, J. M., Fornairon-Bonnefond, C., Mazauric, J. P. and Moutounet M. (2000) Oxygen consumption by wine lees: Impact on lees integrity during wine ageing. Food Chemistry 71, 519-528.

Sato, M., Suzuki, Y., Hanamure, K., Katoh, I., Yagi, Y. and Otsuka, K. (1997) Winemaking from Koshu variety by the sur lie method: Behavior of free amino acids and proteolytic activities in the wine. Am. J. Enol. Vitic., Vol. 48, No.1, 1-6.

Sestelo, A.B.F., Poza, M. and Villa, T.G. (2004) β-Glucosidase activity in a *Lactobacillus plantarum* wine strain. World Journal of Microbiology & Biotechnology 20: 633-637.
Silla Santos, M.H. (1996) Biogenic amines: their importance in food. International Journal of Food Microbiology 29, 213-231.

Smit, A. Y., Engelbrecht, L. and Maret du Toit (2012) Manageing your wine fermentation to reduce the risk of biogenic amine formation. Frontiers in Microbiology doi: 10.3389/fmicd.
Soufleros, E.H., Bouloumpasi, E., Tsarchopoulos, C. and Biliaderis, C.G. (2003) Primary amino acids profiles of Greek white wines and their use in classification according to variety, origin and vintage. Food Chemistry 80, 261-273.

Stuckey, W., Iland, P., Henschke, A. and Gawel, R. (1991) The effect of lees contact time on Chardonnay wine composition. International symposium on Nitrogen in Grapes and Wine.

Svec, P., Vancanneyt, M., Seman, M., Snauwaert, C., Lefebvre, K., Sedlacek, I. and Swings, J. (2005) Evaluation of $(GTG)_5$ – PCR for identification of *Enterococcus* spp. FEMS Microbiol. Letters, 247, 57-63.

Torresi, S., Frangipane, M.T., Anelli, G. (2011) Biotechnologies in sparkling wine production. Interesting approaches for quality improvements: A review. Food Chemistry 129, 1232-1241.
Torriani, S., Zapparoli, G. and Dellaglio, F., 1999. Use of PCRbased methods for rapid differentiation of *Lactobacillus delbrueckii subsp. bulgaricus* and *L. delbrueckii subsp. lactis*. Appl. Environ. Microbiol. 65, 4351–4356.

Tynkkynen, S., Satokari, R., Saarela, M., Mattila-Sandholm, T. and Saxelin, M. (1999) Comparation of ribotyping, randomly amplified polymorphic DNA analysis, and pulsed-field gel electrophoresis in typing of *Lactobacillus rhamnosus* and *L. casei* strains. Applied and Environmental Microbiol. Vol. 65, No. 9, 3908-3914.

Volschenk H., Vuuren van H.J.J. and Viljoen-Bloom M. (2006) Malic acid in wine: Origin, Functin and Metabolism during Vinification. S. Afr. Enol. Vitic., Vol. 27, No. 2.
Waters, E.J., Pellerin, P. and Brillouet, J.M. (1994) A Saccharomyces mannoprotein that protects wine from protein haze. Carbohyd. Polym. 23, 185-191.

Waters AccQ. Tag Chemistry Package (Instruction Manual), (1993) Waters Corp, Milford, MA, USA, April.

Weingartengrunderhebung Statistik Austria 2009.

Wibowo, D., Eschenbruch, R., Davis, C.R. Fleet, G.H. and Lee, T.H. (1985) Occurrence and growth of lactic acid bacteria in wine: A review. Am. J. Enol. Vitic. 36, 302-313.

Wondra, M. and Berovic, M. (2001) Analyses of aroma components of Chardonnay wine fermented by different yeast strains. Food technol. Biotechnol. 39, 141-148.

Wong, K. H., Aziz, S. A. and Mohamed, S. (2008) Sensory aroma from Maillard reaction of individual and combinations of amino acids with glucose in acidic conditions. International journal of food science and technology, 43, 1512-1519.

Wood, C., Siebert, T. E., Parker, M., Copone, D. L., Elsey, G. M., Pollnitz, A., P., Eggers, M., Meier, M., Vössing, T., Widder, S., Krammer, G., Sefton, M. A. and Herderrich, M. J. (2008) From Wine to pepper: Rotundone, an Obscure Sesquiterpene, Is a potent Spicy Aroma Compound. J. Agric. Food Chem. 56, 3738-3744.

Zhang, D. and Lovitt, R., (2006) Review Strategies for enhanced malolactic fermentation in wine and cider maturation. Chem. Technol. Biotechnol. 81:1130-1140.

8. Appendix

Tasting sheet – Vintage 2006

Datum:
Serie:
Koster:

Farbe

0 1 2 3 4 5 6 7 8 9 10

pfeffrig

0 1 2 3 4 5 6 7 8 9 10

fruchtig

0 1 2 3 4 5 6 7 8 9 10

würzig

0 1 2 3 4 5 6 7 8 9 10

hefig

0 1 2 3 4 5 6 7 8 9 10

bitter

0 1 2 3 4 5 6 7 8 9 10

Reife
Jung 0 1 2 3 4 5 6 7 8 9 10 Gereift

Gesamteindruck

0 1 2 3 4 5 6 7 8 9 10

Tasting sheet – Vintage 2007 / 2008

Datum:
Serie:
Koster:

Farbe

0 1 2 3 4 5 6 7 8 9 10

pfeffrig/Geruch

0 1 2 3 4 5 6 7 8 9 10

fruchtig /Geruch

0 1 2 3 4 5 6 7 8 9 10

schwefelig /Geruch

0 1 2 3 4 5 6 7 8 9 10

pfeffrig/Geschmack

0 1 2 3 4 5 6 7 8 9 10

würzig

0 1 2 3 4 5 6 7 8 9 10

fruchtig /Geschmack

0 1 2 3 4 5 6 7 8 9 10

Frische

0 1 2 3 4 5 6 7 8 9 10

schwefelig /Geschmack

0 1 2 3 4 5 6 7 8 9 10

autolytisch

0 1 2 3 4 5 6 7 8 9 10

Körper

0 1 2 3 4 5 6 7 8 9 10

Gleichgewicht/Harmonie

0 1 2 3 4 5 6 7 8 9 10

Andere Eindrücke - nennen

0 1 2 3 4 5 6 7 8 9 10

Gesamteindruck

0 1 2 3 4 5 6 7 8 9 10

Tasting sheet – Vintage 2009

Datum:
Serie:
Koster:

Farbe

0 1 2 3 4 5 6 7 8 9 10

pfeffrig

0 1 2 3 4 5 6 7 8 9 10

fruchtig

0 1 2 3 4 5 6 7 8 9 10

würzig

0 1 2 3 4 5 6 7 8 9 10

hefig

0 1 2 3 4 5 6 7 8 9 10

bitter

0 1 2 3 4 5 6 7 8 9 10

Säure

0 1 2 3 4 5 6 7 8 9 10

Gesamteindruck

0 1 2 3 4 5 6 7 8 9 10