GEISENHEIM INSTITUTE
GERMANY, APRIL 24, 2009

SENSORY DEVELOPMENT OF COOL-CLIMATE VARIETALS DURING WINE FERMENTATION

17

LALLEMAND

GEISENHEIM INSTITUTE, GERMANY, APRIL 24, 2009

SENSORY DEVELOPMENT OF COOL-CLIMATE VARIETALS DURING WINE FERMENTATION

PROCEEDINGS
OF

LES XXI^{es} ENTRETIENS SCIENTIFIQUES LALLEMAND

LALLEMAND

FOREWORD

This year, the *XXIes Entretiens Scientifiques Lallemand* focused on cool-climate varietals and how to understand their sensory development as well as the impact wine fermentation has on their properties. The meeting was held in collaboration with the Geisenheim Research Centre, which celebrated the 115th anniversary of the Geisenheimer Hefe-Reinzucht-Station. The meeting gathered some of the top scientists in the field to present this topic to an international and local crowd. It was also an opportunity to bestow the Lallemand award on the most deserving student at the Geisenheim Research Centre in the field of Wine Microbiology. Daniel Gerhards, a graduate student, was the proud recipient of this award for his work *The investigation of the metacaspase YCA1 for better detection of apoptosis in* Saccharomyces cerevisiae *during alcoholic fermentation of grape must*.

Professor Manfred Grossmann, head of the Microbiology and Biochemistry section at the Geisenheim Research Centre, opened the meeting with a comprehensive review of the diversity of current research at our host institution.

Dr. Chris Curtin, from the Australian Wine Research Institute, presented the first results of trials done on Chardonnay from Margaret River, a relatively cool region in Western Australia, fermented with different yeasts, and how that would impact the production of specific sensory compounds, including thiols, and consumer preference.

Based on the research at the DLR Rheinpfalz in Germany in the laboratory of Ulrich Fischer, their study on yeast and its role in *terroir* expression was presented. Their preliminary work showed that *terroir* goes beyond individuality – it is all about recognizable typicality, related to unique vineyards or regions – and the winemaker influences it via viti-vinicultural decisions.

Dr. Yves Le Fur, from the UMR FLAVIC, INRA-ENESAD – Université de Bourgogne group, echoed some of the same sentiment regarding *terroir* expression when he presented their work on the definition of the unique sensory space of Chardonnay wines by wine experts, by having wines defined by exemplary scores.

From the perspective of malolactic fermentation (MLF), Ramón Mira de Orduña, from Cornell University, presented on acetaldehyde, which is a small and highly reactive molecule that has chemical, sensory and microbiological significance in wine, although lately there has been controversy regarding its negative impact on human health.

MLF was also the focus of Tatjana Košmerl, from the University of Ljubljana in Slovenia. The work of her team showed that the use of different lactic acid bacteria starters not only had an impact on the course of MLF in Welsh Riesling and Sauvignon, but on their chemical composition as well.

The sensory impact of yeast and bacteria are now established facts, and as ongoing research results allow us to understand the mechanisms behind those processes winemakers can benefit from this information. The prime goal of our interest in research is to translate scientific results into improvements in wine quality, and our association with the Geisenheim Research Centre for this event reflects this cooperative spirit.

115TH ANNIVERSARY OF THE GEISENHEIM RESEARCH CENTRE

Prof. Dr. Manfred GROSSMANN

Forschungsanstalt Geisenheim/ Research Institute Geisenheim Fachgebiet Mikrobiologie und Biochemie/
Department Microbiology and Biochemistry Von-Lade-Strasse 1
D-65366 Geisenheim

The Geisenheim Pure Yeast Culture Centre: "Addicted" to Wine Science and Production for Generations

When the French scientist Louis Pasteur published his breathtaking research results about yeasts and bacteria found in wines, determining the quality of the winemaking process in the second half of the 19th century, he inspired researchers in many countries to follow his footsteps in the challenging field of wine microbiology.

It might be surprising to realize that **Hermann Müller**, now known as a breeder of the white grape variety "Müller Thurgau," was the first person in Geisenheim to start – in 1876 – isolating wine yeasts of different origins and checking their potential as pure yeast cultures for wine production. At the time, he was working in the newly established "Königliche Lehranstalt für Obst- und Weinbau Geisenheim" (Royal Education Centre for Pomology and Viticulture), inaugurated by the Prussian King in 1872.

Julius Wortmann, who followed Müller, established the "Geisenheimer Hefereinzucht Station" in 1894. However, looking back in history one has to realize that the wine people at that time were not ready for the sterile propagation of a small volume of liquid yeast into hundreds of litres of starter culture, or for controlling the course of fermentation by using a microscope. Moreover, the world economic crisis and two world wars dramatically hindered the development of yeast technology. Although wine microbiology as part of wine science has grown immensely since the 1950s, the ultimate breakthrough in the usage of pure yeasts occured when the drying process for fresh yeast was optimized to meet the needs of the wine industry.

At the Geisenheim Research Centre, our microbiology and biochemistry section is now one of a total of 13 sections. The premier advantage of Geisenheim is that we can work along the entire wine production chain, from the grape wine up to market research. Through this, we are often involved in interdisciplinary projects where, for example, a special microbiological research field (sluggish and stuck fermentations) is the focus, and other sections, like viticulture and wine technology, are colleagues, not rivals as in other projects. The type of project we work on that is most appreciated are those where international institutions or companies related to the wine business join us to solve current problems in the wine industry or to provide new methods using special microorganisms to meet market/ consumer demands.

Our research work in the microbiology and biochemistry section currently focuses on:

- Elucidating impact factors and microbial pathways delivering pleasant (thioles) or unpleasant aroma compounds in different quantities
- Developing new techniques, including molecular biology tools, to predict and control fermentations, as well as the resulting alcohol levels
- The interactions among different yeasts, and between yeasts and bacteria, and the resulting aroma profiles
- Classic breeding and genetic engineering of yeasts, and risk assessment studies.

Last but not least, it is our prime goal to transform scientific results into improvements in wine quality and the health of wine consumers. We are truly honoured to host the Lallemand technical conference in 2009.

CONTENTS

YEAST MODULATION OF COOL-CLIMATE CHARDONNAY SENSORY PROFILES AND CONSUMER PREFERENCES

Chris CURTIN, Brooke TRAVIS, Patricia OSIDACZ, Robyn KIEVIT, BELINDA BRAMLEY and Leigh FRANCIS

The Australian Wine Research Institute
P.O. Box 197, Glen Osmond SA 5064, Australia

Introduction

The term "quality" when applied to wine evokes a feeling that what you are about to taste will meet and hopefully exceed your expectations. If you limit the scope to a single Chardonnay wine style, experienced tasters can broadly agree on what these expectations are, even in blind tastings (Ballester et al. 2005). For such experienced tasters, it has been shown that neural processing in response to wine tasting occurs through regions of the brain implicated in working memory and behavioural response (Castriota-Scanderbeg et al. 2005). In other words, they are able to recall the intrinsic properties of a wine they consider of good quality while tasting a new wine. Inexperienced tasters, on the other hand, respond via brain areas involved in emotional processing (Castriota-Scanderbeg et al. 2005). The average consumer can be strongly influenced by such factors as branding and context (Lockshin et al. 2006), and a group of consumers will not categorize wines in the same way as a group of experts (Ballester et al. 2005). Nonetheless, intrinsic sensory attributes of Chardonnay certainly affect consumer preferences (Lattey et al. 2004), and sensory acceptability is a strong foundation for repeat purchase of wine (King et al. 2008a).

If both experienced tasters and consumers respond to the intrinsic sensory attributes of wine, the underlying wine composition must contain "quality" indicators. What then constitutes a Chardonnay that experienced tasters see as "true-to-style" and that consumers prefer?

A detailed study by Lorrain et al. (2006) extended the Chardonnay "concept" developed in Ballester et al. (2005), whereby compositional data were used to generate an aroma model for a good example of Burgundian Chardonnay. Wines previously considered intermediate in their typicality within this context were rated as good examples when as few as 10 aroma compounds were added. Thus an experienced taster's opinion of Chardonnay quality may be indicated by the relative concentrations of a subset of fermentation esters and volatile fatty acids, along with some grape-derived and grape-derived/yeast-modified compounds such as linalool, δ-decalactone, 4-vinylphenol and 4-vinylguaiacol.

Based on common observations of consumer behaviour, where preference is highly variable and driven by a range of influences (Lattey et al. 2007), it is reasonable to expect that only a subset of consumers would display preferences aligned to a single concept of quality such as this.

A broader approach to finding quality indicators involves multivariate statistical methods applied to compositional and sensory descriptive analysis data, which has proven successful in elucidating critical compounds either driving or masking aromas important in a sample set (Francis and Newton 2005). Extending this framework with subsequent consumer testing enables preferences to be mapped against sensory attributes and the compositional variables contributing to them – providing potential "quality" indicators for a larger proportion of the population.

A study by Smyth et al. (2004) applied this approach to a set of 20 unwooded Chardonnay wines that spanned

the sensory space observed in a preliminary tasting of 60 wines from various Australian winemaking regions. The goal was to elucidate the varietal character of Australian Chardonnay wines where oak treatment and malolactic fermentation influences were minimized. A large number (45) of volatile aroma compounds were accurately quantified using stable isotope dilution assays, yet only 23 were found to be above their sensory detection thresholds in at least one wine. Of these, fermentation-derived acetate- and ethyl-esters, along with a subset of higher alcohols and fatty acids, were most predictive of sensory characteristics differentiating the wines – floral, pineapple, and citrus. A greater proportion of consumers tested responded favourably to Chardonnay and Riesling wines exhibiting these characteristics (Lattey et al. 2004), thus quality indicators for Chardonnay could be considered to be predominantly yeast driven.

The role of yeast in modulating wine aroma and flavour is well established (for a detailed review see Swiegers et al. 2005), and it was recently demonstrated that the choice of yeast inoculum is sufficient to affect the level of polyfunctional thiols responsible for tropical fruit or box hedge flavours, and to influence consumer preferences for Sauvignon Blanc wine (King et al. 2008b). With a focus on quality in the eye of the consumer, the purpose of the present study was to demonstrate that yeast plays an important role in driving cool-climate Chardonnay aroma diversity, and that yeast-derived differences affect consumer preferences. A juice sourced from the premium cool-climate region of Margaret River in Western Australia was used for this study. This juice was selected because wines from this region can have a distinctive tropical fruit flavour that may be related to the influence of polyfunctional thiols.

Yeast influence on cool-climate Chardonnay aroma and flavour

A 2008 Margaret River Chardonnay juice (12.7° Baume, pH 3.4, TA 7.0 g/L, 19/64 g/L F/T SO_2) was used in a small-lot winemaking trial under controlled conditions as described in King et al. (2008b). This protocol mimics industrial fermentations, as the grape juice was sulphited and unfiltered, fermentations were conducted at relatively low temperatures (15°-18°C) and standard winemaking practices were used. Nine commercially-available active dry wine yeasts (ADY) known to differ in their propensity for forming aroma compounds were selected (Table 1) and inoculated into triplicate fermentation vessels according to the manufacturer's instructions.

The basic composition of each wine was within white wine specifications when analyzed at the time of bottling (Table 1). Significant differences ($p<0.05$) in wine composition were noted among the yeast treatments, although the degree of variation observed in most cases would not be expected to affect sensory properties. The possible exception was the slightly elevated residual sugar concentrations in the wines made with Melody.

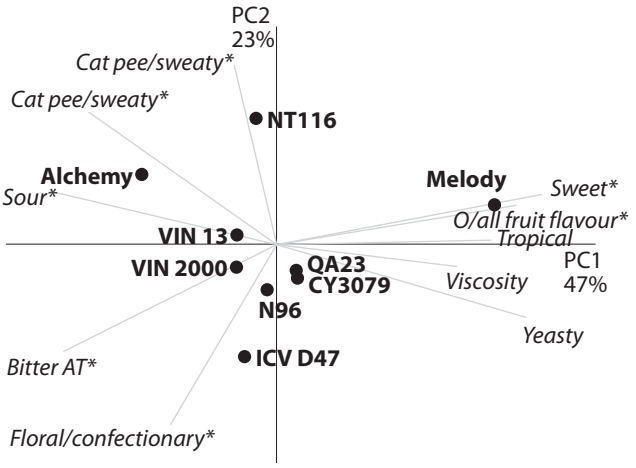
The wines were subjected to sensory descriptive analysis three months post-bottling. A panel of 10 assessors (six female) were convened for this study, all of whom are part of the AWRI's trained descriptive analysis panel.

All 27 samples (three fermentation replicates x nine treatments) were rated for 13 aroma attributes (12 defined terms plus "other") and nine palate attributes (eight defined terms plus "other") in duplicate presentations. An analysis of variance (ANOVA) revealed that eight of the 22 attributes rated were statistically significant ($p<0.05$), while an additional three had probability values of less than 0.1. Figure 1 shows the Principal Component Analysis (PCA) for the

Table 1. Results of standard chemical analysis

Treatment	Free SO_2 (ppm)	Total SO_2 (ppm)	pH	TA (g/L)	VA (g/L)	G+F (g/L)	Alcohol (%)
Alchemy I	27	162	3.4	6.8	0.4	0.5	13.9
N96	27	168	3.4	6.8	0.4	0.9	13.9
NT116	29	187	3.4	6.9	0.4	0.7	13.8
Vin13	26	164	3.5	6.5	0.3	0.7	13.9
Vin2000	27	169	3.5	6.9	0.5	0.7	13.9
QA23	26	164	3.4	7.0	0.4	0.4	13.9
CY 3079	27	186	3.5	7.0	0.6	0.4	14.0
ICV D47	27	161	3.5	6.7	0.3	0.8	14.0
Melody	27	144	3.5	6.6	0.5	1.4	13.8

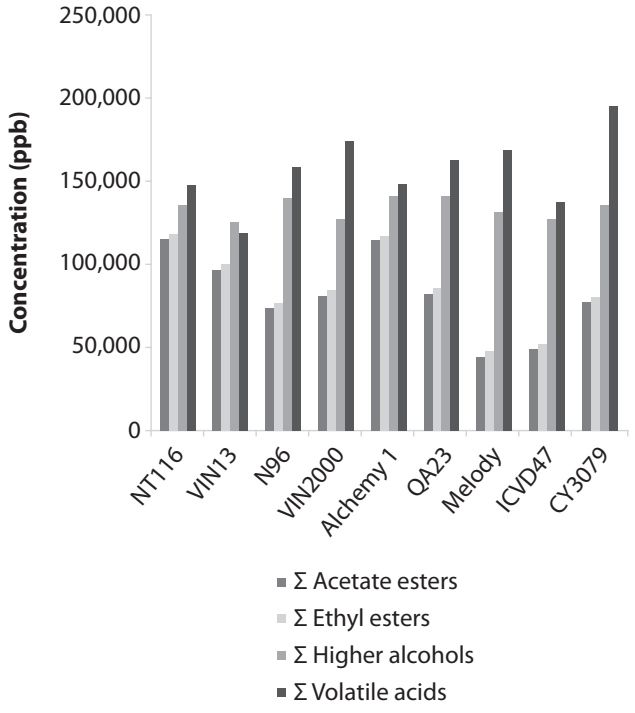
Figure 1. Principal component analysis biplot of mean sensory data for the nine yeast treatments (n=10 judges x 3 fermentation replicates x 2 presentation replicates)



*Significant ($p<0.05$), other attributes ($p<0.1$).

overall mean scores for each yeast treatment for the eight significant attributes and the three additional attributes of interest. The main separation of the wines along PC1 (47%) was on the basis of differences in sweetness, overall fruit flavour, tropical fruit and yeasty to the right, and sourness, bitter aftertaste and solvent to the left. Solvent scores were highly correlated with concentrations of acetate esters (see Figure 2) and specifically ethyl acetate concentrations (r=0.91). There was no correlation between solvent and volatile acidity.

Figure 2. Results of fermentation volatiles analysis

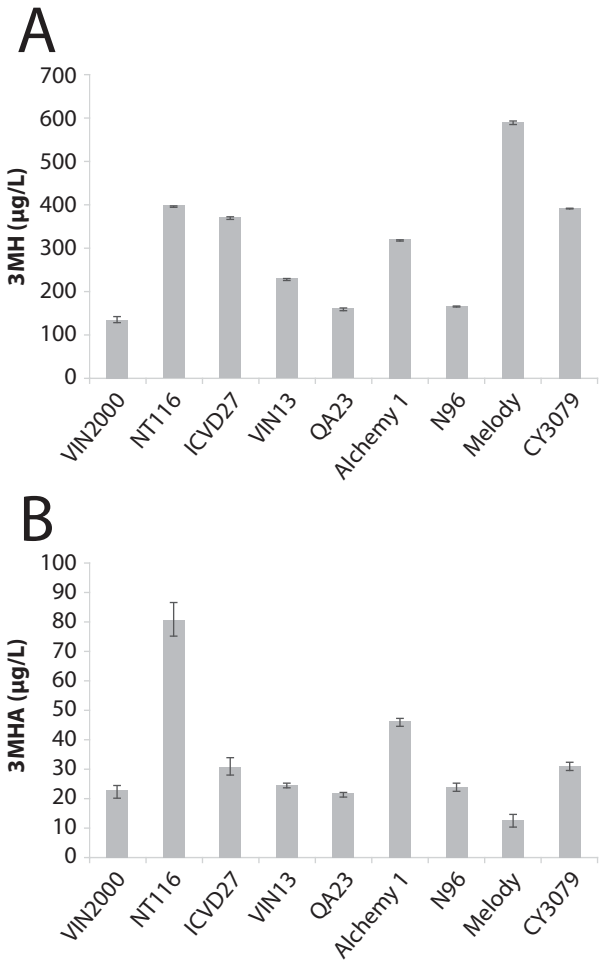


Interestingly, the attribute "sour" was found to be significantly different, even though the titratable acidity (TA) values of all the wines were very similar (Table 1). Sourness was inversely correlated to sweetness, thus it is possible that the slightly elevated residual sugar in wine made with Melody masked the perception of acidity. Wine made with Melody was also rated the highest in tropical and yeasty aromas, overall fruit flavour, sweet and viscosity. Again, the residual sugar may have contributed to the viscosity of the wine and given the impression of more fruit flavour. On the other hand, wild fermentations are known for their enhanced mouthfeel properties, and Melody, as a controlled simulation of such ferments, may be influencing wine composition outside of the compounds measured in this study. Further examination of volatile profiles revealed that Melody was indeed a good approximation of a wild fermentation – with the highest concentrations of 2-methylpropanol, ethyl-2-methylpropanoate, ethyl decanoate and ethyl dodecanoate, and the second highest concentration of 2-methylbutanoic acid in this study (data not shown). These compounds were reported by Varela et al. (2009) to be characteristic of a range of Chardonnay wines made using wild yeast.

The second separation on PC2 is attributed mainly to the cat pee/sweaty and floral/confectionary attributes. This separation explains approximately 23% of the variation among the wines. The wine made with NT116 had the highest level of cat pee/sweaty aromas, while Alchemy and Melody were also rated high in this attribute. Opposite to the cat pee/sweaty aroma on PC2 is the floral/confectionary aroma, which was rated highest in the wine made with ICV D47. This wine was also rated low in cat pee/sweaty aroma and highest in bitter aftertaste.

Cat pee/sweaty aroma is thought to be related to the polyfunctional thiol compound 3-mercaptohexyl acetate (3MHA), and it was evident that NT116 produced the highest concentration of this compound (Figure 3b). Overall there was a strong correlation between 3MHA concentration and mean panel scores for cat pee/sweaty (r=0.86). Despite 3MH being detected in all wines at concentrations well above the perception threshold of 60 µg/L (Figure 3a), it was interesting that no clear influence of 3MH on sensory characteristics was found. While low levels of 3MH have been previously reported in Chardonnay (Dubourdieu and Tominaga 2008), this is the first study, to our knowledge, where polyfunctional thiols have been shown to exert a dominant sensorial effect on Chardonnay aroma.

Figure 3. Results of volatile thiols analysis for 3MH (a) and 3MHA (b)
Samples were analyzed at the time of sensory analysis, from two representative fermentation replicates per treatment. Error bars depict standard deviation of duplicate analyses.



Can yeast be used to modulate consumer preferences for Chardonnay wine?

From an examination of the results of the sensory profiles and compositional data of the wines, a subset of seven wines was selected for a central location consumer test. Wines were selected based on the broadest sensory space. Wines excluded from the study due to their relative neutrality were made with N96 and VIN2000.

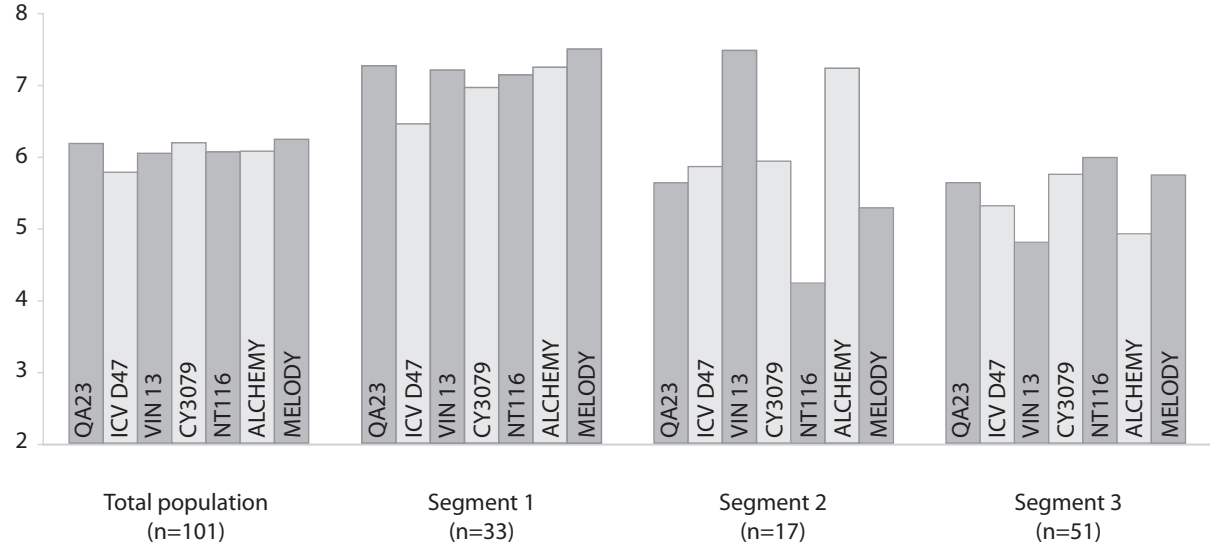
Consumers were recruited for the study who matched the following selection criteria: regular white wine drinkers (who drink white wine at least once per week and buy bottled wine in the AUS$10-$20 range from time to time); age 18-65; 50% males and 50% females; and living in Adelaide.

One hundred and one consumers evaluated all the wines on a blind basis with the wines presented one by one. Wines were presented in a randomized order in three-digit coded wine glasses at approximately 10°C. Consumers rated each wine for overall liking on a nine-point hedonic scale ("dislike extremely" to "like extremely"), followed by a five-point purchase intention question ("would definitely not purchase" to "would definitely purchase").

Figure 4 shows the mean liking scores obtained from the 101 consumers tested. No significant difference in hedonic ratings was found among the seven wines. However, the standard deviations of the consumer responses showed that there was a wide range of liking scores for each wine, indicating some consumers strongly liked some of the wines while others disliked them.

Cluster analysis identified three distinct segments in the population: consumers who liked the same wines are

Figure 4. Mean values of the overall liking results for the total population and the three consumer segments



grouped together. The first segment consists of 33% of the consumers who rated all wines with scores higher than 6, or "liked moderately." There was a significant difference (p=0.05) in the liking scores among the wines for the consumers in this segment. ICV D47 was the least preferred wine for this cluster, with Melody being the most liked and the other wines approximately equally liked. It is important to acknowledge that some yeast strains in this trial, such as ICV D47, are typically used in barrel fermentation where secondary characters are more important and it is desirable to limit the formation of fruity esters. Nonetheless, it is interesting to note that Melody, as an approximation of a wild fermentation, would find usage in contexts similar to ICV D47, despite their apparent differences in chemical profile and sensory attributes.

There was a high correlation coefficient (r=0.86) between overall liking scores of segment one and the total population, indicating that this segment's preferences were broadly representative of the total population. Segment one consumers were more experienced wine drinkers, being somewhat older as a group – 65% over 41 years old – and 80% had been drinking wine for more than 10 years. They also had higher incomes than the average consumers, with over 40% married with children, and a higher proportion of consumers had a post-graduate degree.

Segment two (17% of the consumers) and segment three (50% of the consumers) did not show significant differences in the liking scores among the wines, although closer examination reveals an interesting trend. The most liked and most disliked wines for these segments were those highest in ester concentrations and those rated highest in solvent. The key differentiator was wine made with NT116, which combines the solvent character with the 3MHA-related cat pee/sweaty attribute. It would seem that segment two consumers responded favourably to wine with high concentrations of esters, except when this was combined with high concentrations of 3MHA, whereas segment three consumers displayed the opposite tendency. This polarizing effect of polyfunctional thiols on consumer preferences was also observed by King et al. (2008b), indicating the potential to target consumer segments through choices made at the time of inoculation.

Conclusions

Beyond the expected differences in fermentation-derived esters, higher alcohols, and volatile fatty acids, we observed for the first time in Chardonnay a sensorially significant effect of yeast inoculum on polyfunctional thiol concentrations. Therefore, winemakers aiming to emphasize tropical fruit flavours in cool-climate Chardonnay can do so through the judicious choice of fermentation yeast. Importantly, the stylistic diversity possible through the application of different yeast inoculums was sufficient to affect consumer preferences for cool-climate Chardonnay.

References

Ballester, J., C. Dacremont, Y. Le Fur, and P. Etievant. 2005. The role of olfaction in the elaboration and use of the Chardonnay wine concept. *Food Quality and Preference* 16:351-359.

Castriota-Scanderbeg, A., G. E. Hagberg, A. Cerasa, G. Committeri, G. Galati, F. Patria, S. Pitzalis, C. Caltagirone, and R. Frackowiak. 2005. The appreciation of wine by sommeliers: a functional magnetic resonance study of sensory integration. *NeuroImage* 25(2):570-578.

Dubourdieu, D., and T. Tominga. 2009. Polyfunctional thiol compounds. M. V. Moreno-Arribas, and M. C. Polo (eds.). *Wine Chemistry and Biochemistry*. New York, USA. Springer. 275-293.

Francis, I. L., and J. L. Newton. 2005. Determining wine aroma from compositional data. *Aust. J. Grape Wine Res.* 11:114-126.

King, E., P. Osidacz, L. Francis. 2008a. Measuring consumer preference. *Wine Business Monthly* November 2008:47-49.

King, E., H. Swiegers, I. L. Francis, C. Curtin, S. Bastian, and I. S. Pretorius. 2008b. Yeast co-inoculation influences flavour and consumer preference of Sauvignon Blanc wines. *Aust. N.Z. Grapegrower Winemaker* 538:96-102.

Lattey, K. A., B. Bramley, I. L. Francis, M. J. Herderich, and S. Pretorius. 2007. Wine quality and consumer preferences: understanding consumer needs. *Australian and New Zealand Wine Industry Journal* 22(1):31-39.

Lattey, K. A., H. E. Smyth, N. E. D'Costa, B. K. Leibich, and I. L. Francis. 2004. Consumer acceptability and sensory properties of a set of commercial Australian Riesling and unwooded Chardonnay wines. R. Blair, P. Williams, and I. S. Pretorius (eds.). Twelfth Australian Wine Industry Technical Conference, Melbourne, Australia. Australian Wine Industry Technical Conference Inc., 319.

Lockshin, L., W. Jarvis, F. d'Hauteville, and J. P. Perrouty. 2006. Using simulations from discrete choice experiments to measure consumer sensitivity to brand, region, price, and awards in wine choice. *Food Quality and Preference* 17(3):166-178.

Lorrain, B., J. Ballester, T. Thomas-Danguin, J. Blanquet, J. M. Meunier, and Y. Le Fur. 2006. Selection of potential impact odorants and sensory validation of their importance in typical Chardonnay wines. *J. Agric. Food Chem.* 54:3973-3981

Smyth, H., D. Cozzolino, M. J. Herderich, M. A. Sefton, and I. L. Francis. 2004. Relating volatile composition to wine aroma: identification of key aroma compounds in Australian white wines. R. Blair, P. Williams, and I. S. Pretorius (eds.). *Proceedings of the Twelfth Australian Wine Industry Technical Conference*, Melbourne, Australia: Australian Wine Industry Technical Conference Inc., 31-33.

Swiegers, J. H., E. J.Bartowsky, P. A Henschke, and I. S. Pretorius. 2005. Yeast and bacterial modulation of wine aroma and flavour. *Aust. J. Grape Wine Res.* 11(2):139-173.

Varela, C., T. Siebert, D. Cozzolino, L. Rose, H. McLean, and P. A. Henschke. 2009. Discovering a chemical basis for differentiating wines made by fermentation with 'wild' indigenous and inoculated yeasts: role of yeast volatile compounds. *Aust. J. Grape Wine Res.* 15(3):238-248.

IMPACT OF YEAST AND *TERROIR* DIVERSITY ON THE SENSORY PROPERTIES OF GERMAN RIESLING

Ulrich FISCHER[1], Andrea BAUER[2], Stephan SOMMER[1], Sebastian GANSS[1], Hans-Georg SCHMARR[1], Sascha WOLZ[1] and Anette SCHORMANN[1]

[1] Department of Viticulture and Oenology, DLR-Rheinpfalz, Breitenweg 71, 67435 Neustadt/Wstr. Germany

[2] Department of Viticulture and Enology, University of California, Davis, CA 95616, California, U.S.A.

Abstract

The term "*terroir*," which originated in France, comprises the interaction of soil, climate and topography with the vines of a specific variety, and can be extended to the human impact by viticultural and oenological measurements. In order to study the sensory impact of *terroir*, 25 highly diverse vineyard sites were selected from the growing regions of Mosel, Nahe, Pfalz and Rheinhessen. For the vintages 2004 and 2005, sound Riesling grapes were harvested from these sites at optimal maturity. Grapes from each vineyard site were divided in half, with one half subjected to a standardized winemaking protocol, and the other half undergoing customary winemaking in the respective wine estate. Eight to 10 months after harvest, a descriptive analysis by 20 trained judges characterized the wines by one colour, 14 odours and five taste attributes. Wines from different vineyard sites yielded considerable variation. For example, wine made from Riesling grapes grown on a loamy loess soil with basalt stones was much more intense in its citrus, peach, mango and honey melon attributes than the wine produced from light-coloured sandstone, which was described as more sour with vegetative and mineral notes. Applying discriminant analysis, it was possible to group the five bedrock types (sandstone, loam loess, Rotliegendes, slate and limestone) according to their sensory properties, and identify the aroma notes typical for each bedrock type.

Although yeast is responsible notably for the central alcoholic fermentation, its role in *terroir* expression has so far been neglected. Examining the impact of site-dependent yeast flora on the sensory properties of wine, Riesling grapes were processed and fermented under customary conditions in wine estates or under sterile conditions in the pilot plant, excluding any yeasts from the cellar flora. Given the much greater sensory intensities found in spontaneous fermentations taking place in the wine estates, the yeast flora originating from the individual cellars appeared to predominate over the vineyard flora in terms of sensory composition. At 3%/vol. alcohol, the vineyard flora were partially removed from the fermenting vessels and transferred into the same sterile must. Sensory evaluation of the finished wines demonstrated a similar variation among the different vineyard flora versus two commercial yeast strains, which were also transferred at 3%/vol. alcohol into the sterile must. It seems that the cellar yeast *terroir* is more important than the vineyard *terroir*, which alone does not introduce more sensory variation than the use of commercial yeast starter cultures.

Sensory analysis of base wines and their subsequent sparkling wines suggested that secondary fermentation generally decreases green odour impressions and enhances those reminiscent of ripe fruit. The role of aroma precursors in Riesling and Chardonnay were investigated by adding precursor fractions, which were obtained from the respective varieties using an XAD-2 resin, to both base wines prior to fermentation. Due to precursor supplementation, a significant enhancement of colour, peach, elder blossom, green banana and green bean intensities was observed in the finished Riesling sparkling wine. For Chardonnay, colour, cantaloupe and grape juice perception was increased. Although base wines for sparkling wine production lack the degree of ripeness of normal still

wines, it could be demonstrated that the release of aroma compounds from precursors in the respective base wines partially explains the flavour changes observed during secondary fermentation. Therefore, the role of precursor fractions should be emphasized more when purchasing base wines, composing cuvées for sparkling wine production and selecting the most appropriate yeast strain.

Introduction

Addressing the topic of cool-climate viticulture and winemaking at a research conference in Germany, the first variety to come to everyone's mind is Riesling. It is not only the fact that worldwide plantation of Riesling is strongly dominated by Germany, comprising more than 60% of a total of 40,000 ha, it is also because Riesling is a cool-climate variety par excellence. In warmer climates, Riesling grapes tend to produce elevated concentrations of unpleasant volatile phenols or C_{13} norisoprenoids, which favour the early appearance of the bottle bouquet flavour, sometimes described as having a kerosene character (Winterhalter et al. 1990, Winterhalter 1991, and Marais et al. 1992), but also to the early formation of cracks in the thin berry skin, stimulating unwanted growth of *Botrytis cineria* and other rot fungi. During the past two decades, the styles of German Riesling wines available have broadened greatly. Increased consumer demand, especially in Germany, has increased the percentage of Riesling wines produced in a dry style. To respond to these changes in consumer preferences, much more rigid quality management – especially in the vineyards – was necessary, and increased sugar concentration in the grapes, due to the impact of a warmer climate, has contributed as well.

This paper will address three further winemaking aspects that are actively applied by German winemakers to improve not only wine quality, but to sharpen the stylistic expression of dry Rieslings from Germany and make them distinct from competing products as well. These aspects are:

- *Terroir*
- Yeast diversity
- Aroma precursors in the grape.

Terroir

To perform successfully in national wine markets in the context of growing competition, fuelled by the ongoing globalization of the wine trade, wines need a unique selling proposition (USP) that simultaneously attracts consumer interest. *Terroir*, the specific combination of a vineyard's soil composition, mesoclimate and topography in interaction with the vines, may serve as an excellent USP as these immutable factors are unique for local wine growing areas and cannot be imitated elsewhere, unlike grape varieties, winemaking technologies or yeast strains (Bauer and Fischer 2008). Although the geological and climatic characteristics of many vineyard sites have been described in the scientific literature (Fischer et al. 1999, Douglas et al. 2001, Bauer 2008), there is very little conclusive sensory interpretation of this natural diversity with regards to wine. However, consumers are strongly interested in how specific *terroirs* translate into sensory differences and how they can acquire an expertise in appreciating and even distinguishing different *terroir*s due to the sensory properties of the wines.

Materials and methods

In order to study the sensory impact of *terroir*, 12 highly diverse vineyard sites from 10 wine estates were selected in the German wine growing region of Pfalz. The substrates yielding the soils of these sites include limestone, sandstone, greywacke, basalt and breccias from the Rotliegend age (Lower Permian). One year later the research was extended to 13 additional sites in the growing regions of Mosel/Saar, Ahr, Nahe and Rheinhessen. These vineyards comprised further sites on limestone and Rotliegend material, as well as sites on slate and porphyry. In the vintages 2004 and 2005, sound Riesling grapes were harvested from these sites at optimal maturity, which was individually determined by the cooperating wine estates. One portion of grapes from each site was subjected to a standardized winemaking protocol, while the major portion underwent customary vinification at the respective wine estates. This division additionally allowed for evaluating the impact of individual winemaking on the wines' sensory properties. All wines were fermented with the addition of a selected yeast strain (Lalvin R-HST® Lallemand, Canada).

Eight months after harvest, 20 trained judges characterized the wines of the 2004 vintage by colour intensity, 14 odours (mineral, lemon/grapefruit, rhubarb, apple, peach, mango/passion fruit, cantaloupe, honey/caramel, smoky, floral, green grass/cucumber, box tree, green bean, buttery/bready/yeasty/sweaty) and five taste attributes (sweet, sour, harsh acidity, hard mouthfeel, bitter) in a descriptive analysis. For the 2005 vintage, two tasting panels were employed, consisting of 15 and 14 trained judges respectively, and the range of taste descriptors was extended by one more attribute (mineral taste), while one odour was dropped (green bean). The wines from the standardized vinification were sampled in triplicate, while those from the wine estates were evaluated in duplicate.

Impact of individual geological substrates

Confining *terroir* to the substrates from which the soils are derived is, from a scientific viewpoint, a gross simplification considering the great number of pedologic, topographic and climatic factors that impact the development of vines as well, therefore contributing significantly to the sensory properties of wines grown on specific sites. However, application of this simplification is now increasingly used in wine marketing as winemakers progressively replace the use of vineyard sites on bottle labels or price lists with the denomination of geological substrates or such bedrock types as slate, sandstone or limestone. Consequently, consumers expect geological substrates to have an impact on the sensory properties of wines. This demands the examination of the extent to which this consumer perception can be confirmed by sensory analysis.

Indeed, comparing the sensory profile of two *terroirs* in Figure 1, which are only 2 km apart and cultivated by the same estate, the two bedrock types had a highly significant impact on nine odour attributes and the four related to taste. The basalt bedrock type, with its higher loam content, yielded a Riesling with the stronger fruit character traditionally associated with German Riesling (lemon/grapefruit, apple, peach and rhubarb) as well as notes of exotic fruit, more related to higher content in thiols (mango/passion fruit, cantaloupe and box tree). The sandstone-derived soil of the Deidesheimer Kieselberg region, which is lighter and contains a substantial portion of cobbles, produces a Riesling with less fruit character, more green flavour and most distinct perception of stronger acidity, perceived as more harsh and with a harder mouthfeel.

In an opposite set-up, two wines were compared. The wines were produced from the same bedrock type Rotliegend, comprised of reddish breccias or slate material, but from two vineyard sites, which are located 150 km apart and differ strongly regarding their mesoclimate. According to Figure 2, both wines displayed a surprisingly high degree of similarity: only two odour attributes (rhubarb and apple) differed noticeably in their intensities. Among the taste attributes, only bitterness, harsh acidity and hard mouthfeel showed significant differences. This may be attributable to the 2%/vol. higher alcohol content of the wine from the Pfalz area, which is known to enhance bitterness (Fischer and Noble 1994). On the other hand, the majority of the sensory properties were equal in their intensities.

Figure 1. Sensory profiles of the bedrock types sandstone (Kieselberg) and basalt (Pechstein), both vinified at the Bassermann-Jordan estate (n = 20 judges x 3 repetitions)

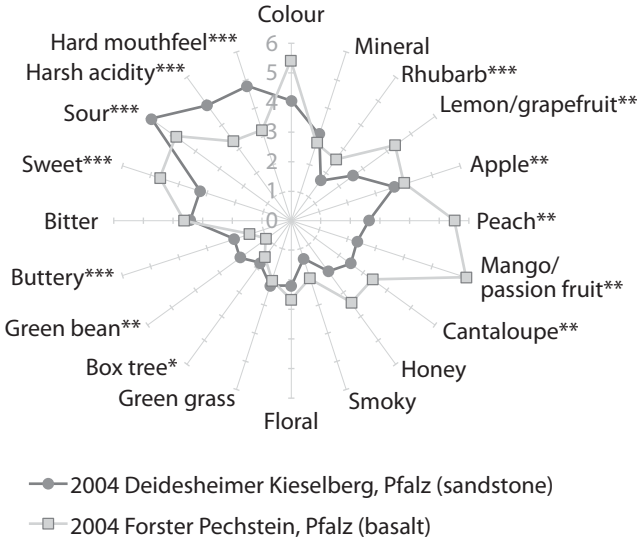
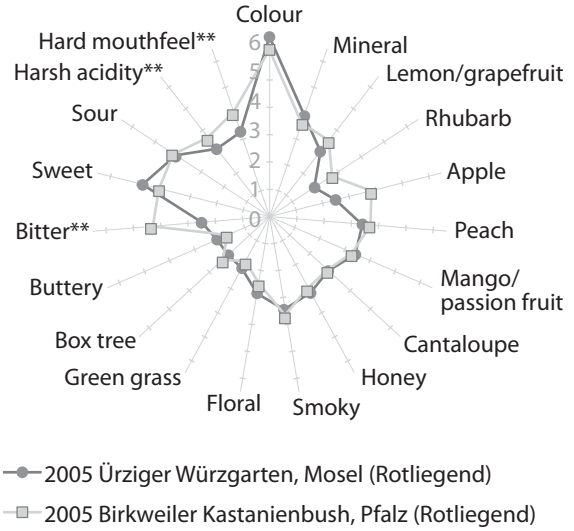


Figure 2. Sensory profiles of Riesling from two Rotliegend *terroirs*, vinified at the experimental cellar (n = 13 judges x 3 repetitions)



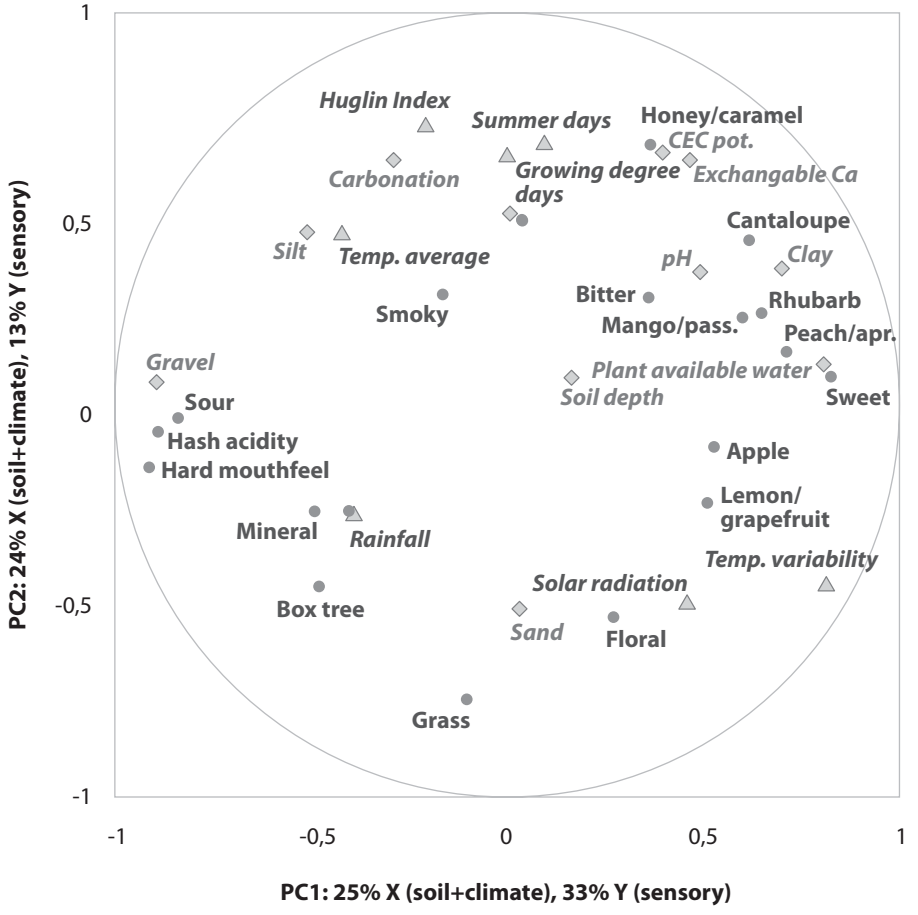
In conclusion, two vineyards differing in their geological substrate produced two very distinct wines, although they are located in close proximity, had nearly identical mesoclimatic conditions and were managed with same viticultural regime belonging to the same wine estate. At the same time, two vineyards of the same bedrock type in great distance and mesoclimatic diversity (Pfalz versus Mosel), showed very similar sensory profiles. According to single comparisons of different bedrock types, it seems reasonable to conclude they have a significant impact on the sensory properties of Riesling wines produced from grapes grown in these vineyards.

Applying an ANOVA to all wines of the 2004 vintage, the type of geological substrate proved to have a larger impact on the sensory properties of the wines than vinification (Bauer 2008): 12 out of 20 attributes differed significantly between the substrates, including the fruity notes with the exception of lemon/grapefruit, the vegetative notes with the exception of green grass/cucumber, and all the taste descriptors with the exception of bitter. In the 2005 vintage, however, only four taste attributes showed a significant variation between the substrates, presumably due to the atypical dry and hot weather conditions during the ripening period in 2005. However, it was still possible for both vintages to clearly distinguish between the different kinds of substrate according to the wines' sensory properties, through discriminant analysis, and to identify typical aroma notes for each type of substrate. The wines from basalt were perceived as markedly fruity-aromatic with high intensities in cantaloupe, peach/apricot, mango/passion fruit, lemon/grapefruit and smoky. The wines from limestone also expressed an intensive fruity-sweet aroma, particularly regarding mango/passion fruit and peach/apricot. They had well-balanced acidity on the palate, combined with a smooth mouthfeel. The wines from sandstone, as well as those from greywacke, could be recognized through their distinctive, harsh acidity and hard mouthfeel. They only had subtle fruity-sweet aromas, but showed a marked vegetative character. In contrast, the wines from the Rotliegend substrate showed high intensities in honey/caramel, cantaloupe, rhubarb, peach/apricot and mango/passion fruit odours, and on the palate expressed well-balanced acidity and a smooth mouthfeel. The wines from slate were characterized by acidity, and apple, citrus and vegetative notes. There was only one wine from porphyry that also had distinct acidity with higher intensities in the mineral, lemon/grapefruit, apple and peach/apricot odours. However, individual wines differed from these sensory characterizations, particularly in 2005.

Modelling sensory properties with pedological and meteorological data

Extensive soil analyses were conducted for the test sites of the Pfalz, and meteorological data from weather stations installed nearby or right on site were evaluated. These datasets were correlated with the intensities of the sensory attributes for the 2004 vintage through a Partial Least Squares-Regression (PLS). The PLS yielded a three-dimensional model, which accounted for 75.6% and 52% of explained variance in the pedo-meteorological (X) and sensory (Y) datasets. The PLS showed a distinct separation between the sour and vegetative attributes, the fruity-sweet attributes, and the floral and fruity-fresh (lemon/grapefruit, apple) attributes. The sour and vegetative attributes were associated to the contents of sand and gravel, and also to precipitation during the ripening period. The fruity-sweet attributes were correlated with the clay and exchangeable calcium, soil pH, plant available water (PAW), potential cation exchange capacity ($CEC_{pot.}$), Growing Degree Days (GDD), number of summer days (daily maximum temperature > 25°C), and the Huglin Index. For the floral and fruity-fresh attributes, the model

Figure 3. PLS-Regression of environmental parameters and intensity of sensory attributes of 21 Riesling wines of vintage 2004 (n = 20 judges x 3 or 2 repetitions)



showed associations to PAW, sand content, solar radiation, and the cumulated day-night temperature variability during the ripening period.

Conclusions regarding *terroir*

ANOVA and discriminant analysis showed a clear impact of *terroir* on the sensory properties of German Riesling, despite vintage and winemaking influences. Sensory patterns could be seen, and they varied depending on the geological substrate the wines originated from. In a PLS, 52% of the sensory variance could be modelled with pedological and meteorological data. Research on the subsequent vintages is being conducted, in order to critically review the above observations.

Yeast diversity

As already discussed, the French term *terroir* comprises the interaction of soil, climate and topography with the vines of a specific variety, and may be extended to the human impact by viticultural and oenological measurements. The impact of yeast however, although responsible for the central alcoholic fermentation, is being neglected or at least not defined in this traditional definition. This is surprising, as numerous studies investigated the composition of the yeast flora on the grapes and their dynamic change during fermentation with regard to diverse production areas, different viticultural management systems and the impact of the individual cellar flora of wine estates or even newly built wineries (Constanti et al. 1997, Pretorius et al. 1999 and Beltran et al. 2002). On the grape surface non-*Saccharomyces* strains are predominant, such as *Hanseniaspora uvarum*, *Candida stellata*, *Metschnikowa pulcherima*, *Pichia membranifaciens* and *Torulaspora delbrueckii* while *Saccharomyces cerevisiae* and *bayanus* could not be found at all (Sturm et al. 2002) or only by using sufficient amplification methods (Martini et al. 1996). In grape juice however, increased temperature and maceration on the skin lead to increased cell counts of *S. cerevisiae* (Köhler et al. 1995, Mendes Ferreira et al. 2001). This could be viewed as an indication that the cellar flora contribute primarily to the presence of *Saccharomyces* in the juice or fermenting wine, while the vineyard is mainly a source for yeast.

The dynamic change of yeast species during fermentation has been the subject of numerous studies, and it has been clearly demonstrated that non-*Saccharomyces* species are active only during the first quarter of a spontaneous fermentation. Increasing ethanol levels is the major selection force to favour the dominance of *Saccharomyces* during ongoing fermentation. However, typical yeast-derived aroma compounds, such as esters and higher alcohols, are formed mainly during the first quarter of fermentation, which is dominated by the non-*Saccharomyces* species (Dittrich and Grossmann 2005), which also show higher ß-glycosidase activities, liberating grape-derived bound aroma compounds (Mendes Ferreira et al. 2001). It is surprising how few sensory studies have been conducted to describe the specific impact of spontaneous fermentations (Egli et al. 1998, Soden et al. 2000). In order to facilitate some new results, this research will use specific sensory methods to address the questions of how spontaneous fermentation contributes to sensory properties of Riesling wines and if it is reasonable to speak not only of the *terroir* of bedrocks, soil types, mesoclimate and inclination, but also of a yeast-derived *terroir* expression.

Material and methods

Spontaneous versus inoculated fermentation in wine estates

In cooperation with wine estates from the Pfalz region, in 2003 identical Riesling grape musts were fermented by inoculation of 30 g/hL of the yeast strain Lalvin R-HST® (Riesling Heiligensteins Lallemand, Canada), or by spontaneous fermentation. Chemical data of these two grape musts formed the base for the fermentation curves and sensory profiles displayed in Table 1, as well as their subsequent treatment in the juice stage. Due to the extraordinarily hot and dry weather in 2003, pH adjustment with

Table 1. Composition of two grape musts and their subsequent treatment in the juice stage

	Deidesheimer Mäushöhle	Deidesheimer Kieselberg
picking date	26-09-2003	14-10-2003
juice density (sugar gradation)	1.086 (86°Oe)	1.095 (95°Oe)
sugar content	200 g/L	221 g/L
pH at harvest	3.4	3.55
titratable acidity at harvest	6.6 g/L	5.1 g/L
acidification with tartaric acid	1.5 g/L	1.5 g/L
pH after acidification	3.1	3.3
titratable acidity after acidification	8.2 g/L	6.5 g/L
SO_2 at juice stage	none	none
juice treatment:		
gelatine	20 g/hL	20 g/hL
active charcoal	30 g/hL	30 g/hL
bentonite	none	300 g/hL
diammonium hydrogen phosphate	80 g/hL	50 g/hL
yeast inoculum (Lalvin R-HST®)	30 g/hL	30 g/hL

tartaric acid was allowed in Germany at the juice and wine stage. In order to lower the high pH values of 3.40 and 3.55 at harvest, 1.5 g/L of tartaric acid was added. In January 2004 a volume of 25 litres was obtained from the wine estates, and further winemaking and bottling was done under standardized conditions.

Spontaneous fermentations with and without cellar flora

In 2004, spontaneous fermentations from five different vineyards were conducted in cooperating wine estates and in the experimental cellar of the DLR-Rheinpfalz using the same grapes. To investigate the impact of the combined vineyard and cellar flora versus the vineyard flora alone, grapes for the latter trial were harvested with sterile gloves into sterilized containers. All winemaking equipment was sterilized prior to contact with the grapes, must or wine, using either a 70% ethanol solution or 0.2% acetic acid solution. In both cases, grapes were not sulphited, vitamin B1 was added and 300 mg/L of diammonium hydrogen phosphate was added after fermentation had started. Further winemaking followed the same protocol as the 2003 trials.

Comparison of vineyard yeast flora versus actve dry yeast in the same pasteurized must

When spontaneous fermentations at the experimental cellar, which excluded the impact of any cellar flora, reached 3%/vol. ethanol, an aliquot was removed and centrifuged under gentle conditions. The yeasts obtained from those vessels, representing the vineyard flora of five vineyards, were used for inoculating the same Riesling grape juice, which was sterilized directly after harvest. In parallel, the same sterilized juice was inoculated with active dry yeast strains (Siha 7, Begerow, Langenlonsheim, Deutschland; Lalvin R-HST®, Lallemand, Canada), which are commonly employed to ferment Riesling in Germany. Further winemaking followed the protocol described above.

For the 2003 wines, a panel of 19 trained judges, consisting of seven females and 12 males aged 25 to 60, evaluated the overall 10 wines on April 20 and 21, 2004. Wines were assessed in duplicate and samples were served in a completely randomized design. Wines were served in DIN tasting glasses (DIN 10960, Schott-Zwiesel, Zwiesel, Germany) filled with 30 mL of wine at a temperature of 12°C, closed by a plastic lid. Based on six out of 10 wines, the most relevant and discriminating odour and taste attributes were selected by the consensus of five experts. Physical standards were made fresh for both days and each judge had access to the whole set of standards during the sessions. Administration of the assessment and data acquisition was accomplished by using the FiZZ-for Windows Version 2.00D Software (Biosystems, Cantenou, France). Sensory intensities were recorded using 10 cm unstructured scales, anchored by "none" on the left side and "strong" on the right. Odour attributes were assessed monadically, while taste attributes were served in a non-monadic way in order to prevent fatigue of the oral senses. A three-way analysis of variance was conducted as a mixed model, where judges were treated as random effects, while wines and replications were fixed effects (ANOVA, PC-SAS Version 9.12, SAS Institute, Cary, NC, USA).

Results and discussion regarding yeast diversity

Spontaneous versus inoculated fermentation in wine estates

According to winemakers, the risk of spontaneous fermentation is outweighed by the observation that, in most cases, these wines show superior sensory properties. They are described as more complex, having more exotic fruit flavours and better representing the *terroir* where the grapes were grown. In order to test this opinion in an objective way, several sensory profiles obtained from yeast-inoculated and spontaneously fermented wines were compared. Indeed, the sensory properties in Figure 5 could be viewed as superior, due to the slightly higher intensities observed for some flavour attributes typical for Riesling, such as apple, peach, pineapple and mango/passion fruit.

Figure 4. Sugar degradation in an inoculated versus spontaneous fermentation of a 2003 Deidesheimer Mäushöhle Riesling

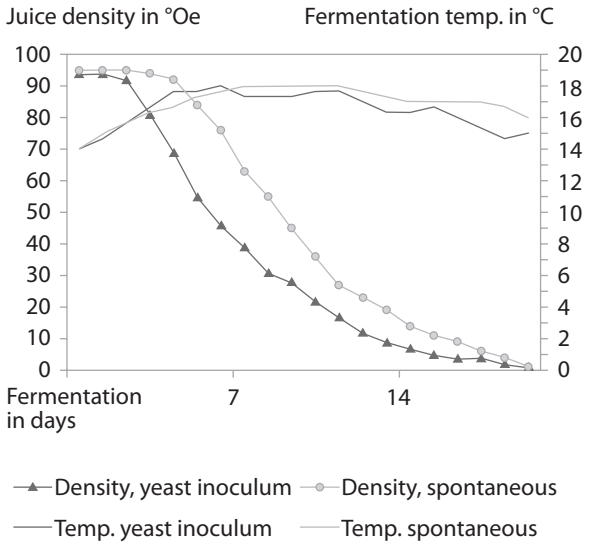
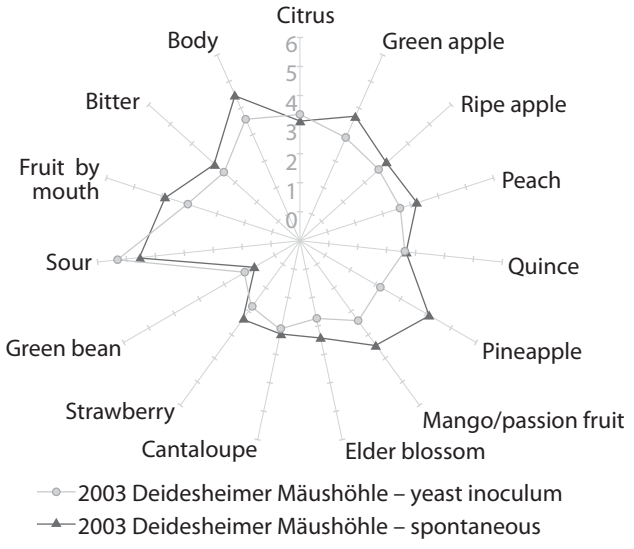
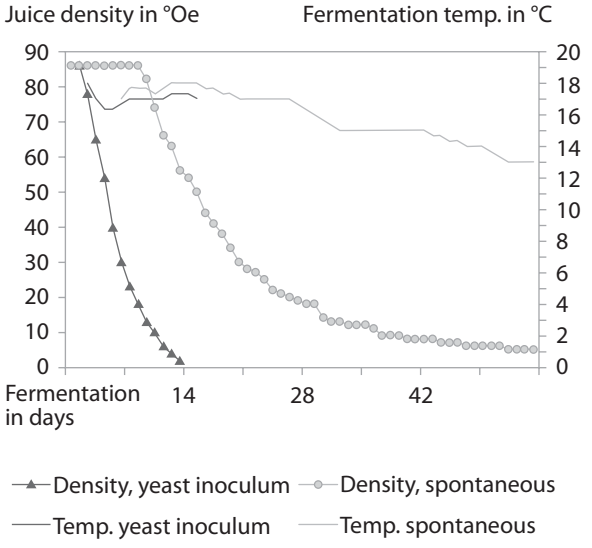


Figure 5. Sensory profiles of an inoculated versus spontaneous fermentation of a 2003 Deidesheimer Mäushöhle Riesling (n = 19 judges x 2 repetitions)



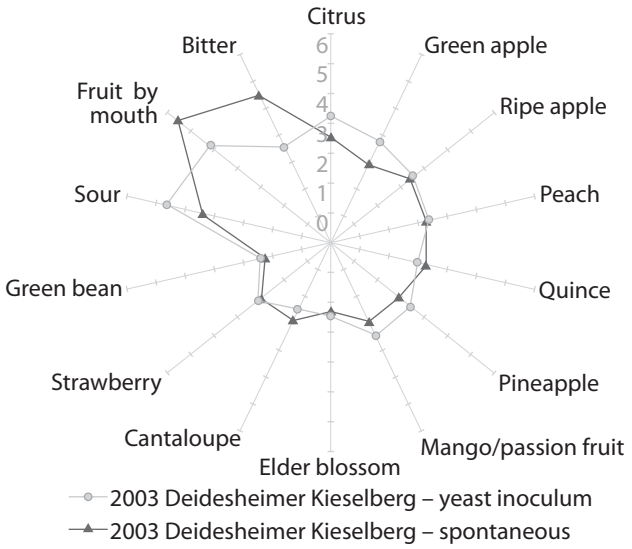
However, examining the sensory profiles in Figure 7, it was the yeast-inoculated wine which showed at least slightly higher intensities of lemon, green apple, pineapple and mango/passion fruit. Further on, the wine fermented spontaneously had a higher bitterness and a lower sourness score, not appreciated in Riesling.

Figure 6. Sugar degradation in an inoculated versus spontaneous fermentation of a 2003 Deidesheimer Kieselberg Riesling



For one possible explanation for these non-reproducible sensory results, it is worth examining the respective fermentation kinetics. In the case of the spontaneous fermentation with its well liked sensory profile in Figure 5, it took only five days to start the fermentation (Figure 4). Although the sugar degradation was slightly slower, the spontaneous fermentation finished in the same 20 days

Figure 7. Sensory profiles of an inoculated versus spontaneous fermentation of a 2003 Deidesheimer Kieselberg Riesling (n = 19 judges x 2 repetitions)



as the inoculated fermentation. In contrast, the spontaneous fermentation which led to the less desired sensory profile in Figure 7 started fermenting only after 14 days. Due to the slow and prolonged fermentation, the wine temperature fell to 13°C and fermentation stopped before the required sugar level for dry wines (less than 9 g/L) was reached. Concurrently, spontaneous malolactic fermentation took place and only when the pH in the juice stage was lowered, was a major build-up of acetic acid prevented. Similar sensory changes could be observed in two other spontaneous fermentations, which took longer than 35 days to ferment to dryness. Thus, it appears advisable to support the spontaneous yeast flora by not sulphiting the juice, adding sufficient yeast nutrients and raising the temperature to 20°C, to achieve the desired aroma profile and minimize the risk of stuck fermentation.

When the results of this experiment were presented to the group of participating winemakers and their colleagues nines months after the grape harvest, 70% preferred the wines fermented with inoculated yeast. Spontaneously fermented wines were criticized for having an exaggerated aroma, especially due to exotic fruit flavours supported by a lack of sourness. However, a consensus was reached that particular spontaneously fermented wines may serve as valuable blending partners for the Riesling wines fermented by the Riesling Heiligenstein yeast strain.

Sensory profiles of wines fermented by vineyard flora alone versus vineyard combined with a cellar flora

In 2003 we observed clear sensory differences between wines fermented by active dry yeast and spontaneous yeast flora. The next question to address during the 2004

vintage was if the yeast flora present on the grapes in the vineyard are responsible for the sensory properties obtained or if the flora present in the wine cellar had a significant impact as well. For this purpose, we excluded any cellar flora by harvesting grapes under sterile conditions, as well as during the entire winemaking process. Thus, the only possible microorganisms facilitating alcoholic fermentation were solely derived from the grapes and vineyard. In contrast, when the same grapes were processed as usual in participating wine estates, both vineyard and cellar yeasts were involved in conducting spontaneous fermentation.

Sensory evaluation of these experimental wines revealed very distinct differences between the wines fermented spontaneously in the experimental cellar versus the wine estate. For both vineyard sites, the Deidesheim Kieselberg in Figure 8 and the Köngisbacher Idig in Figure 9, the wines fermented in the wine estate were perceived as much more fruity (citrus, apple, peach, passion fruit and cantaloupe) and floral (elder blossom). For sourness, body, mouthfeel and mineral odour, no clear trend could be observed. The wines fermented under sterile conditions at the experimental cellar showed elevated intensities only for bitterness. According to these results, the yeast flora derived from the cellar have a very valuable impact, because the desired fruity and floral attributes were enhanced only when both cellar and vineyard flora could work together. On the other hand, when the cellar flora were completely excluded rather neutral wines was produced without the desired flavours.

The stronger sensory impact of the cellar flora could be rationalized by the better survival conditions for *Saccharomyces* species in the damp atmosphere of a wine cellar at moderate temperatures and free of the detrimental impact of ultraviolet light. Although the same grape material was utilized for spontaneous fermentation in both the wine estates and the experimental cellar, it could be speculated that grape processing, including skin maceration, took longer in the wine estates, which may have facilitated earlier and faster reproduction of vineyard flora. Concurrently, harvesting only small amounts within a short time and processing the grapes rapidly at the experimental cellar may have reduced the contact of the grape juice and skin surface of the berries, and hindered the complete transfer of yeast into the must.

Sensory diversity due to different spontaneous yeast flora and active dry yeast in the same pasteurized grape must

A final experiment addressed the question: How much sensory variation is due to different yeast flora from diverse vineyard sites and does this sensory diversity outweigh the diversity obtained by using different active dry yeasts? For this purpose, a yeast inoculum was harvested at 3%/vol. alcohol from the spontaneous fermentations, which were produced under sterile picking and processing conditions. In order to exclude the impact of varying juice compositions, including different levels of aroma precursors, these vineyard-derived yeasts were inoculated into the same pasteurized Riesling must of the same 2004 vintage. Concurrently, two yeast strains commonly employed for Riesling were inoculated into the same Riesling substrate.

Figure 8. Sensory profiles of vineyard flora alone versus vineyard and cellar flora fermenting a 2004 Deidesheimer Kieselberg Riesling (n = 15 judges x 2 repetitions)

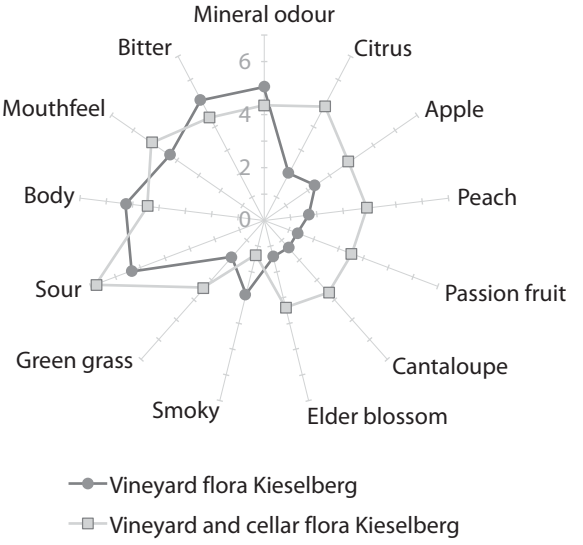
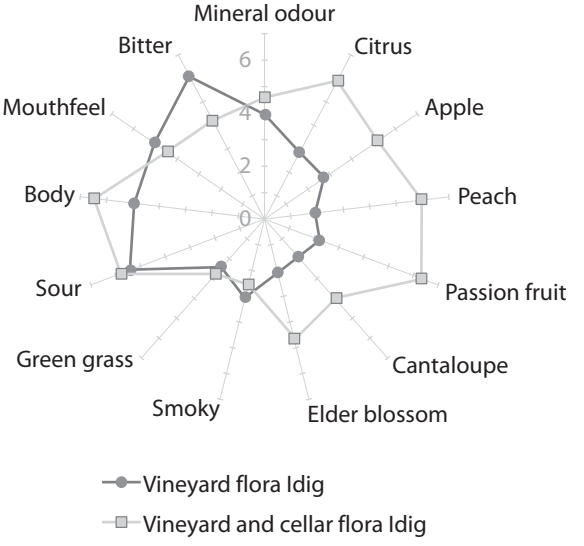


Figure 9. Sensory profiles of vineyard flora alone versus vineyard and cellar flora fermenting a 2004 Köngisbacher Idig Riesling (n = 15 judges x 2 repetitions)



According to the sensory profiles displayed in Figure 10, the sensory modulation due to the yeast flora coming from different vineyard sites was limited. The strongest differences were observed for the attribute passion fruit, followed by peach, cantaloupe and elder blossom. Among the taste attributes, bitterness was altered the most, followed by sourness, mouthfeel and body. However, intensities were changed or enhanced up to 50% or even 70% and the most pronounced modulation was observed in the same sensory attributes, which were responsible for the differences between spontaneous fermentations conducted by the vineyard flora alone and those in combination with the cellar flora in Figure 8 and Figure 9. Thus, it seems to be not the cellar flora alone that is responsible for the desired sensory properties, but the vineyard flora as well, maybe to a lesser degree.

Comparing the sensory diversity due to two active dry yeasts in Figure 11 with that created by the yeast flora from different vineyards in Figure 10, a similar range of sensory variation can be achieved by utilizing various commercial yeasts. This can be interpreted as an argument against such superficial comments as the use of ac-

Figure 10. Pasteurized 2004 Riesling must fermented by vineyard flora transferred from spontaneous fermentations of sterile picked grapes from three different sites (n = 15 judges x 2 repetitions)

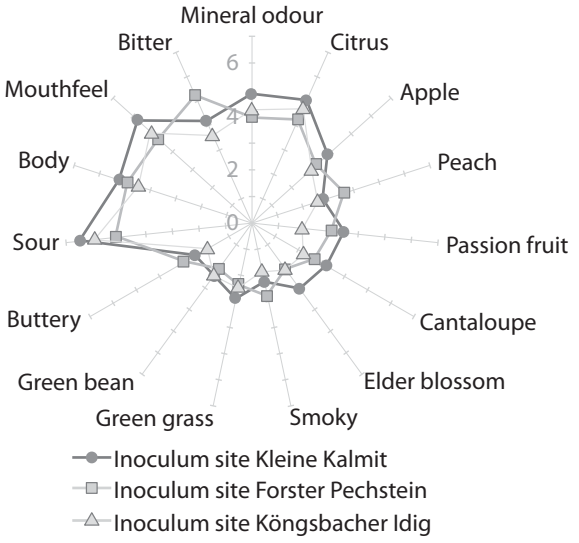


Figure 11. Pasteurized 2004 Riesling must fermented by active dry yeast (n = 15 judges x 2 repetitions)

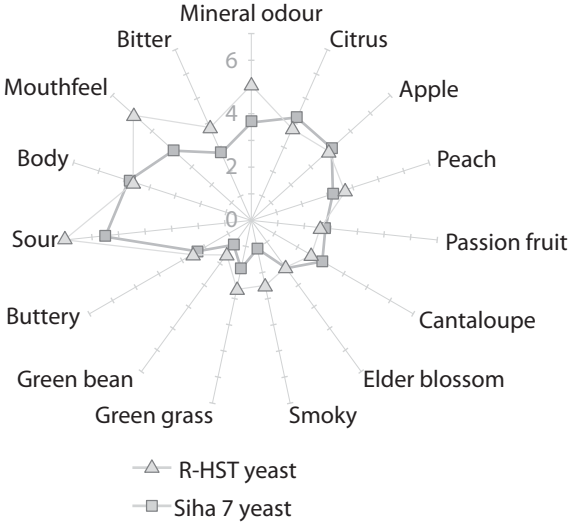


Figure 12. Principal component analysis based on sensory properties of experimental Riesling wines: Loadings of sensory attributes (n = 15 judges x 2 repetitions)

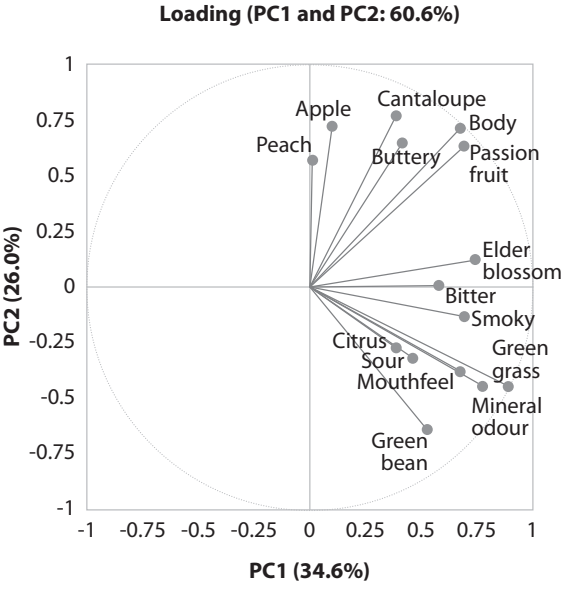
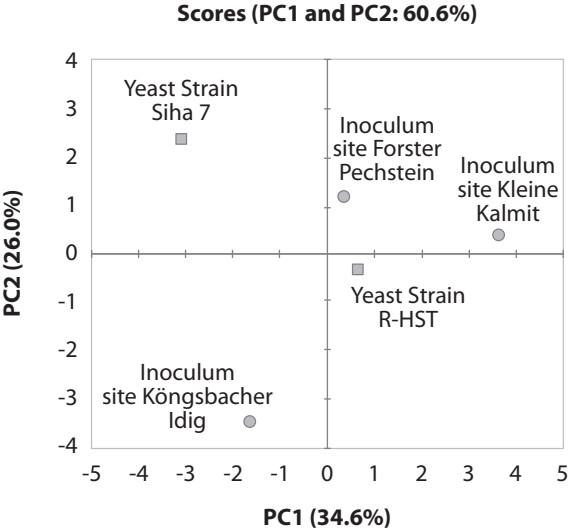


Figure 13. Principal component analysis of pasteurized 2004 Riesling musts inoculated by either 3 vineyard flora transferred from spontaneous fermentations of sterile picked grapes or by two yeasts: Scores of experimental wines



tive dry yeast will favour uniform wine styles. Processing the information given in Figure 10 and Figure 11 in a different way, by employing principal component analysis (PCA), the results displayed in Figure 13 reveal similar distances in the sensory plane between the two active dry yeasts on the one hand and the three inoculums obtained from the vineyard flora of three different sites on the other. While defining the sensory plane in Figure 12, it becomes obvious that wines located on the right side are more flavourful than those displayed on the left side. Further on, the upper half is dominated by such fruity notes as peach, apple, cantaloupe and passion fruit, while the lower half shows predominantly loadings of green and smoky attributes, as well as higher acidity and bitterness.

It is remarkable that neither of the two active dry yeasts could reproduce the sensory profile or position in the PCA of the most powerful vineyard-derived yeast flora from the Kleine Kalmit site. In conclusion, there is a rationale and a demand for further yeast selection work in order to find even better yeast cultures. This seems to be a worthwhile and challenging project, because the diversity of current Riesling styles has tremendously improved and enlarged in Germany, partially due to the production of higher quality grapes, but also due to the search for more individual wines in line with a pronounced focus on *terroir*.

Conclusion regarding yeast diversity

Comparing the sensory properties of spontaneous and induced fermentations of equal musts, both types of fermentation were able to exhibit superior sensory intensities, depending on the time course of fermentation. Examining the impact of site-dependent yeast flora on the sensory properties in wine, Riesling grapes were either processed and fermented under customary conditions in the wine estates or under sterile conditions in the pilot plant, excluding any yeasts coming from the cellar flora. According to much greater sensory intensities found in spontaneous fermentations taking place in the wine estates, the yeast flora originating from the individual cellars appeared to predominate over the vineyard flora in terms of sensory composition. At 3%/vol. alcohol, the vineyard flora was partially removed from the fermenting vessels and transferred into the same sterile must. Sensory evaluation of the finished wines demonstrated a similar variation among the different vineyard flora versus two commercial yeast strains. In conclusion, it is hypothesized that the yeast flora from individual wine cellars contribute predominantly to the sensory properties of spontaneous fermentation, as does the vineyard-specific yeast flora.

Liberation of aroma precursors by yeast

The results in the previous section have clearly demonstrated the crucial role of yeast for flavour formation and subsequent sensory diversity, regardless of whether it is predominantly non-*Saccharomyces* or solely *Saccharomyces* yeast. However, yeasts do not generate flavours *de novo*; in most cases they require the presence of precursors, such as amino acids, fusel alcohols, short-chain fatty acids or bound aroma compounds (Swiegers et al. 2005). Thus, two avenues lead to more flavourful wines: enrichment of aroma precursors in the grape must and improvement of yeast strains regarding their ability to liberate aroma compounds from the precursors. To enhance the aroma precursors, several authors have demonstrated the importance of reduced yield, more sun exposure for grape clusters and extended hang time on the vine, as well as skin maceration, to facilitate a better transfer of aroma precursors from berry skins into the must (Marais et al. 1991, Marais et al. 1992, Reynolds et al. 1996, Zoecklein et al. 1998, Bureau et al. 2000 and Fischer 2007). Literature characterizing the liberation of aroma compounds has mainly focused on the ß-glycosidase activity of yeast to generate free monoterpenes, and on the cystein-lyase activity of yeast that facilitates the release of thiols (Winterhalter et al. 1997, Swiegers et al. 2005, Tominaga et al. 2001 and Fischer 2007). As for the secondary fermentation – the key process in the production of sparkling wines – the role of aroma precursors had not yet been investigated and was therefore the objective of the following study.

Materials and methods

A 2004 Riesling base wine from the Pfalz region was fermented with two active dry yeasts: Fermicru VB 1 (Keller, Mannheim, Germany) and IOC 18-2007 (Institut Oenologique de Champagne, Epernay, France) after 24 g/L of sucrose was added. In order to mimic the physical changes during secondary fermentation, the base wine was either supplemented by 1.5%/vol. of ethanol alone (RSLG base wine + EtOH) or with ethanol and sufficient CO_2 to achieve a pressure of 6 bars (RSLG base wine + EtOH + CO_2). To observe the enzymatically induced changes of secondary fermentation without the impact of CO_2, the base wine was fermented after sugar supplementation in a glass carboy, allowing the escape of the CO_2.

For the second experiment, flavour precursors were separated from 2005 Riesling and Chardonnay base wines by the following protocol: after diluting the base wines 1:1 with water, they were poured through a preparative XAD-2 column. Polar compounds were eluted by washing with water. Methanol released the non-polar compounds from the column, and free aroma compounds were removed by

liquid-liquid extraction with diethyl ether. The methanol extracts were freeze-dried and further separated by applying high-speed, counter-current chromatography, which yielded a fraction of glycosidically bound aroma compounds for each variety. This fraction was added to the non-treated original Riesling or Chardonnay base wine in order to double the native content of aroma precursors. Both the non-treated and precursor-supplemented base wines received 24 g/L of sucrose and a yeast inoculum (IOC 182007). Secondary fermentation took place in six 0.75 L sparkling wine bottles, and the bottles were disgorged after six weeks of fermentation. After a further six weeks, sensory evaluation started.

For the descriptive analysis, 18 trained judges were asked to rate colour, odour (11 attributes) and taste (five attributes) of all the experimental wines and sparkling wines. Samples were presented at 10°C in randomized triplicates.

Sensory changes due to secondary fermentation

The sensory plane defined by PC1 and PC2 in Figure 14 describes the left side of the graph by higher intensities of orally perceived fruitiness and alcoholic perception. The right side of the plane is defined by sourness, fruit attributes, such as fruit drops or cantaloupe, as well as elder flower. By adding ethanol alone to the base wine, the alcoholic perception increases, as does the fruity taste, which could be explained by the sweet character of ethanol. Further carbonization shifted the score towards the right side, as CO_2 contributed to the sourness, decreased the mouthfeel due to its irritative properties, and facilitated a better volatilization of some aroma compounds, which in turn enhanced floral and fruity notes (Figure 15). Fermenting the base wine, regardless of the yeast strain, shifted the wine from the upper part of the sensory plane, which is defined by green aroma compounds, such as green bean, green grass and green banana, to the lower half of the graph due to enhanced fruitiness (apple, peach, juicy), yeasty character and bitterness. In general, secondary fermentation decreased the unripe, green notes of the base wine and amplified the fruity attributes. According to the scores in Figure 15, the yeast strain VB1 produced a Riesling sparkling wine as fruity and floral as the IOC strain. The stronger elder flower attribute, especially, which is typical for Sauvignon blanc wines, could be explained by the expression of a cysteine lyase activity of the VB1 strain, while this flavour release feature is yet not know for the IOC 18-2007 strain.

Figure 14. Principal component analysis based on sensory properties of experimental Riesling wines: Loadings of sensory attributes (n=18 judges x 3 repetitions)

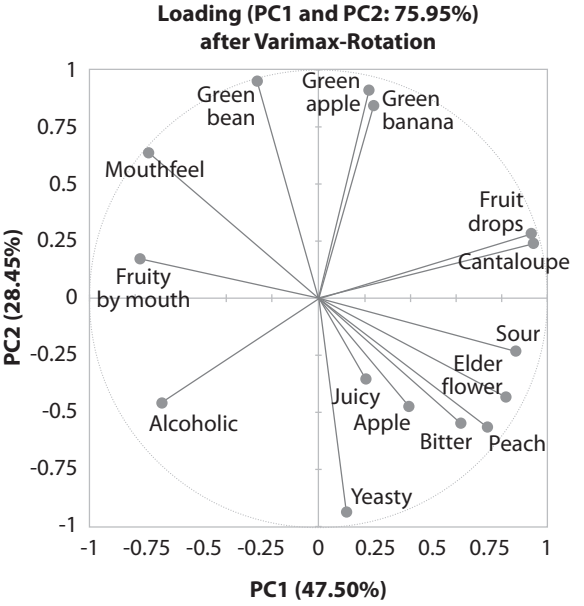
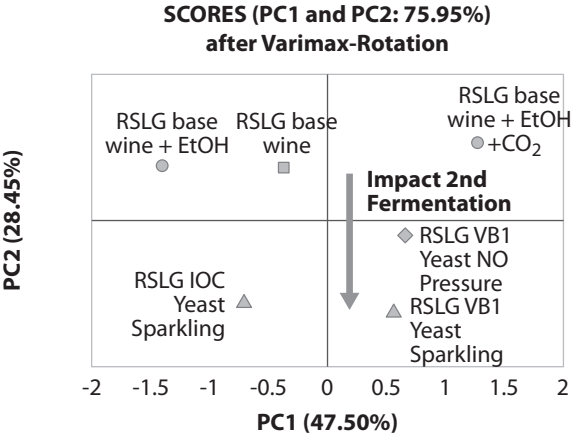


Figure 15. Principal component analysis of experimental Riesling wines before and after secondary fermentation: Scores of experimental wines



Sensory impact of flavour precursors

Doubling the concentration of aroma precursors in Riesling and Chardonnay base wines prior to secondary fermentation yielded more flavour-intense sparkling wines. The observed effect was stronger in the Riesling wine, where the attributes fruit drops, peach, cantaloupe and fruity by mouth increased, as well as such green and floral notes as elder blossom, green banana and green bean. Analysis of flavour compounds, which are not reported here (Ganß et al. 2005), revealed significantly higher concentrations of linalool, ß-damascenon and (Z)-hex-3-en-1-ol, which may have contributed to the flavour enhancement. For the Chardonnay, the attributes fruit drops and

Figure 16. Sensory impact of the addition of flavour precursors to a 2005 Riesling base wine. Evaluation of both sparkling wines in March 2007 (n = 14 judges x 2 repetitions)

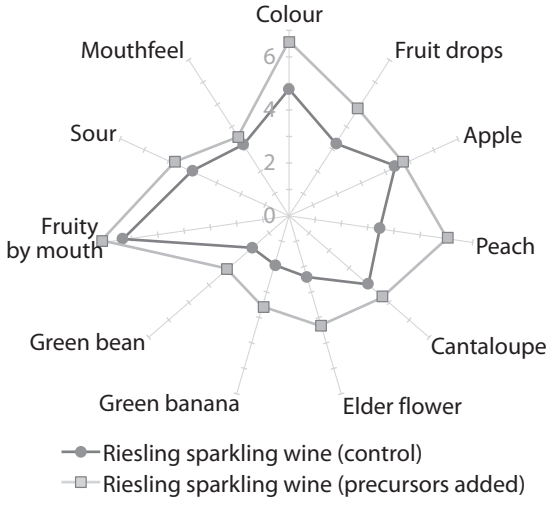
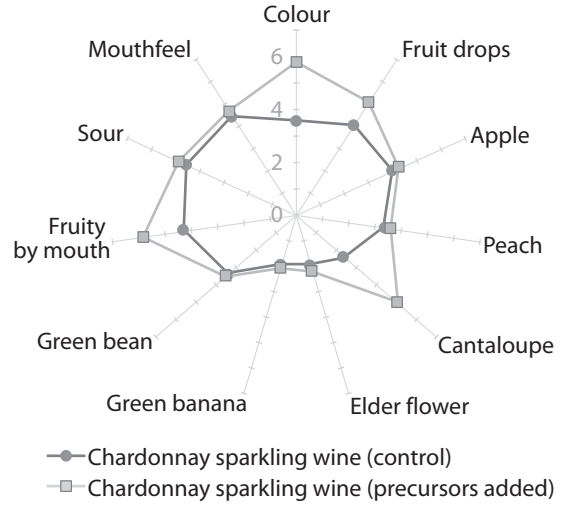


Figure 17. Sensory impact of the addition of flavour precursors to a 2005 Chardonnay base wine. Evaluation of both sparkling wines in March 2007 (n = 14 judges x 2 repetitions)



fruity by mouth were intensified and the odour of cantaloupe even doubled compared to the sparkling wine obtained from the original base wine. Amplification of the colour in both varieties could be a hint that the added fractions were not completely free of any coloured substances.

Conclusion: liberation of aroma precursors by yeast

The results of this experiment proved the as yet underestimated importance of aroma precursors in base wines for sparkling wine production, as they are a valuable source for further flavour release. At the same time, yeast strains that were selected specifically for sparkling wine production were able to liberate a wide range of aroma compounds, such as monoterpenes, C_{13}-norisoprenoids, C_6-alcohols and aromatic alcohols from their glycosidically bound precursors. Greater knowledge about the content of aroma precursors and the use of specific yeast strains or enzyme activities could improve the liberation of aroma compounds during secondary fermentation. In order to reach that objective, the development and implementation of analytical techniques to measure aroma precursors would be very helpful, as they provide objective figures regarding these intrinsic quality parameters when purchasing base wines and blending individual cuvées.

References

Bauer, A. 2008. Terroirausprägung bei der Rebsorte Riesling: Korrelation sensorischer, chemischer, bodenkundlicher und klimatischer Parameter. Dissertation, Technische Universität Carolo-Wilhelmina, Braunschweig.

Bauer, A., and U. Fischer. 2008. Impact of Terroir, vintage and winemaking on the sensory properties of German Riesling. In *Proceedings of Sensory Science Symposium: From the Vineyard to Consumer Preference*, 59th Annual ASEV Meeting, Portland, Oregon, June 6, 2008.

Beltran, G., M. J. Torija, M. Novo, N. Ferrer, M. Poblet, J. M. Guillamon, N. Rozes, and A. Mas. 2002. Analysis of yeast populations during alcoholic fermentation: a six year follow-up study. *Systematic and Applied Microbiology* 25(2):287-293.

Bureau, S. M., R. L. Baumes, and A. J. Razungles. 2000. Effect of vine or bunch shading on the glycosylated flavor precursors in grapes of *Vitis vinifera L.* cv. Syrah. *J. Agric. Food Chem*. 48(4):1290-1297.

Cavazza, A., and C. Zini. 1996. Changes in grape must microbial flora as affected by winemaking operations before yeast inoculation: An investigation in 12 wineries in Trentino (north Italy). *Wein-Wissenschaft* 51(3-4):180-186.

Constanti, M., M. Poblet, L. Arola, A. Mas, and J. M. Guillamon. 1997. Analysis of yeast populations during alcoholic fermentation in a newly established winery. *Am. J. Enol. Vitic.* 48(3):339-344.

Dittrich, H. H., and M. Großmann. 2005. *Mikrobiologie des Weines*. 3. Auflage ed.; Verlag Eugen Ulmer: Stuttgart, Germany, 240.

Douglas, D., M. A. Cliff, and A. G. Reynolds. 2001. Canadian *terroir*: characterization of Riesling wines from the Niagara Peninsula. *Food Res. Int.* 34(7):559-563.

Egli, C. M., W. D. Edinger, C. M. Mitrakul, and T. Henick-Kling. 1998. Dynamics of indigenous and inoculated yeast populations and their effect on the sensory character of Riesling and Chardonnay wines. *J. Appl. Microbiol.* 85(5):779-789.

Fischer, U. 2007. Wine aroma. In R. G. Berger (ed.). Flavour and Fragrances. *Chemistry, Bioprocessing, and Sustainability*. Springer Verlag, S. 241-268.

Fischer, U., and A. C. Noble. 1994. The Effect of Ethanol, Catechin Concentration, and pH on Sourness and Bitterness of Wine. *Am. J. Enol. Vitic.* 45(1):6-10.

Fischer, U., D. Roth, and M. Christmann. 1999. The impact of geographic origin, vintage and wine estate on sensory properties of *Vitis vinifera* cv. Riesling wines. *Food Qual. Pref.* 10:281-288.

Ganß, S., H.-G. Schmarr, T. Potouridis, and U. Fischer. 2005. Analytical and Sensory Investigations on Flavour Changes during Sparkling Wine Production. *Euro Food Chem XIII*, Hamburg, Germany (2):402-405.

Köhler, H., E. Schindler, R. Miltenberger, M. Gessner, and K. Curschmann. 1995. Trocken-Hefen-Vergleich. *Deutsches Weinmagazin* 28:15-20.

Mendes Ferreira, A., M. C. Climaco, and A. Mendes Faia. 2001. The role of non-*Saccharomyces* species in releasing glycosidic bound fraction of grape aroma components – a preliminary study. *J. Appl. Microbiol.* 91(1):67-71.

Marais, J. C., C. J. van Wyk, and A. Rapp. 1991. Carotenoid levels in maturing grapes as affected by climatic regions, sunlight and shade. *S. Afr. J. Enol. Vitic.* 12(2):64-69.

Marais, J. C., G. Versini, G., C. J. van Wyk, and A. Rapp. 1992. Effect of region on free and bound monoterpene and C13-norisoprenoid concentrations in Weisser Riesling wines. *S. Afr. J. Enol. Vitic.* 13(2):71-77.

Marais, J. C. 2002. The significance of 1,1,6-trimethyl-1,2-dihydronaphthalene in the production of high quality Riesling wines. *ACS Symposium Series* 802:273-284.

Martini, A., M. Ciani, and G. Scorzetti. 1996. Direct enumeration and isolation of wine yeasts from grape surfaces. *Am. J. Enol. Vitic.* 47(4):435-440.

Pretorius, I. S., T. J. v. d. Westhuizen, and O. P. H. Augustyn. 1999. Yeast biodiversity in vineyards and wineries and its importance to the South African wine industry. *S. Afr. J. Enol. Vitic.* 20:61-74.

Reynolds, A. G., D. A. Wardle, and M. Dever. 1996. Vine Performance, Fruit Composition, and Wine Sensory Attributes of Gewürztraminer in Response to Vineyard Location and Canopy Manipulation. *Am. J. Enol. Vitic.* 47(1):77-96.

Soden, A., I. L. Francis, H. Oakey, and P. A. Henschke. 2000. Effects of co-fermentation with *Candida stellata* and *Saccharomyces cerevisiae* on the aroma and composition of Chardonnay wine. *Aust. J. Grape Wine Res*. 6:21-30.

Swiegers, J. H., E. J. Bartowsky, and P. A. Henschke. 2005. Yeast and bacterial modulation of wine aroma and flavour. *Austr. J. Grape Wine Res.* 11(2):139-173.

Sturm, J., D. Rauhut, M. Grossmann, C. Dorn, and B. Berkelmann-Loehnertz. 2002. Studies on indigenous yeast populations of grape berry surfaces (*Vitis vinifera* L.) *Proceedings of the 22nd International specialised Symposium on Yeasts*, South Africa, Pilanesberg Game Park, March 25-28, 2002, 54.

Tominagea, T., L. Masneuf, and D. Dubourdieu. 2001. Powerful aromatic volatile thiols in wines made from several *Vitis vinifera* grape varieties and their releasing mechanism. *Symposium on Nutraceutical Beverages: Chemistry and Flavour Characteristics*; ACS national meeting in Chicago, IL, USA.

Winterhalter, P. 1991. 1,1,6-Trimethyl-1,2-dihydronaphthalene (TDN) formation in wine. 1. Studies on the hydrolysis of 2,6,10,10-tetramethyl-1-oxaspiro [4.5] dec-6-ene-2,8-diol rationalizing the origin of TDN and related C_{13} norisoprenoids in Riesling wine. *J. Agric. Food Chem*. 39:1825-1829.

Winterhalter, P., and G. Skouroumounis. 1997. Glycoconjugated aroma compounds: Occurrence, role and biotechnological transformation. In R. G. Berger (ed.): *Advances in Biochemical Engineering/Biotechnology. Volume 55: Biotechnology of Aroma Compounds*, Springer Verlag, Heidelberg 73-105.

Winterhalter, P., and M. Sefton. 1990. Volatile C_{13}-norisoprenoid compounds in Riesling wine are generated from multiple precursors. *Am. J. Enol. Vitic.* 41:277-283.

Zoecklein, B. W., T. K. Wolf, J. E. Marcy, and Y. Jasinski. 1998. Effect of Fruit Zone Leaf Thinning on Total Glycosides and Selected Aglycone Concentrations of Riesling (*Vitis vinifera* L.) Grapes. *Am. J. Enol. Vitic.* 49(1):35-43.

Acknowledgments

The authors wish to thank the Ministry for Economy, Transport, Agriculture and Viticulture Rheinland-Pfalz and the Forschungsring des Deutschen Weinbaus for kindly funding the *Terroir* project. Grapes, wines and information provided by the cooperating wine estates in Rheinland-Pfalz were important contributions, as was the support through the Department of Geology and Mining Rheinland-Pfalz in Mainz.

The research concerning release of aroma precursors during secondary fermentation was funded through the federal ministry for Economy and Technology via AiF and FEI e.V. Project AIF-FV 13932 N. The support by the association of German Sparkling Wine producers and the member companies is highly appreciated.

SENSORY SPACE OF TYPICAL CHARDONNAY WINES AND OTHER WINES, AND ITS RELATION TO VOLATILE COMPOSITION

Yves LE FUR[1], Julien JAFFRE[1, 2] and Dominique VALENTIN[2]

[1] Unité Mixte de Recherche Flaveur, Vision et Comportement du consommateur (FLAVIC)
INRA-AgroSup Dijon-Université de Bourgogne
17 rue Sully, BP 86510, 21065 Dijon CEDEX, France

[2] Unité Mixte de Recherche, Centre des Sciences du Goût (CSG)
CNRS-Université de Bourgogne-INRA
rue Hugues Picardet, 21000 Dijon, France

Introduction

Our goal is to demonstrate that the wines produced from the Chardonnay grape varietal can be organized into a single category (called the product space), which, despite the diversity of objects that compose it, is distinguished in sensory terms from other categories of white wines produced by other varietals. This lends credence to the existence of a sensory space specific to Chardonnay wines that near-perfectly overlaps the product space studied. Establishing the existence of its own sensory space has important repercussions: it justifies, for example, any ulterior approach of a physicochemical nature aimed at characterizing the circumscribed sensory category. In this regards, we were interested in the aromatic component of Chardonnay wines, and we attempted to confirm that the wines produced by the Chardonnay varietal possess distinctive aroma characteristics based on a combination of volatile compounds present in given concentrations.

Olfactory Image and the Notion of Specific Sensory Space

Having defined what we mean by product space and sensory space, we conducted two experiments, one in 2001 and the other in 2006. They relied on the same sensory methodology aimed at revealing the existence of a sensory space specific to Chardonnay wines, independent of the vintages, and the evaluation procedures utilized. We also considered that the tasters may or may not have shared representations of the wines produced from the Chardonnay varietal, which raises the notion of consensus (or convergence) among the tasters.

The product space can be defined as a preset wine category, based on varietal, technological or territorial criteria. In our case, it is necessary to study the product space in regards to the wines produced from the Chardonnay varietal. From this perspective, a wine may or may not belong to this product space. Therefore, the product space follows the rule of all or nothing.

On the other hand, the sensory space corresponds to a category of wines based on organoleptic criteria. Such a space extends from the wines that are the most representative to those that are the least, and is organized along a continuum, called the representivity gradient. Furthermore, the sensory space has indistinct limits, which are not necessarily those of the product space. In the hypothesis where the wines the most representative of the sensory space – considered the most typical wines or the best examples – belong to the product space studied, we can assert that the wine has its own sensory space. That legitimizes all following investigations, notably research conducted on the aroma component (or olfactory image) of the sensory space.

The sensory space can be studied in relation to the product space in terms of variable perimeters, depending on the criteria considered in order to set the boundaries: the nature of the varietal(s), the winemaking technique, the production zone, and the *Appellation d'Origine Contrôlée* (A.O.C.), etc. Indeed, it is possible to demonstrate its specific sensory space by bringing together the wines that belong to different but adjoining product spaces, selected according to the criteria chosen. In our case, we brought together a sampling composed not only of Chardonnay wines (C), but wines from other varietals as well: Pinot Blanc (P), Marsanne (M), Chenin (H), Sylvaner (Y), Melon de Bourgogne (B), Aligoté (A) and Sauvignon Blanc (S).

Our sensory methodology was utilized for two successive experiments. The first, carried out in 2001, focused on wines from the 1999 and 2000 vintages and included 48 wines – 29 Chardonnay wines and 19 wines from other varietals. The selection criteria for the wines were as follows: French wines, made from a single varietal, non-woody, award-winning, dry and young (aged one or two years, as the study was carried out in 2001) wines. Two types of sensory evaluations were applied independently: an initial orthonasal (nose only) evaluation, and an overall evaluation (nose and mouth). The wines were served in black glasses, coded and presented in a monadic way with a presentation order specific to each subject and to each evaluation procedure. The test was given to 28 tasters, all of whom are active in the winegrowing and winemaking industry.

The second experiment, carried out in 2006, focused on wines produced in 2003, 2004 (the majority) and 2005. The experiment included 46 wines – 23 Chardonnay wines and 23 wines produced from other varietals. The same selection criteria were adopted. The wines were aged from one to three years, as the study was conducted in 2006. Only the orthonasal method was utilized. This test was given to 22 tasters, all part of the winegrowing and winemaking industry. The same serving conditions were applied.

What was asked of the tasters was simple, but projected the taster into a given situation: *Imagine that you have to explain to friends what characterizes a wine produced by the Chardonnay varietal. To explain to them, you can have them taste a wine*. For each wine presented, we asked the tasters to answer the following question: *Do you consider this wine to be a good example or a bad example to explain to your friends what a wine from the Chardonnay varietal is like?* The taster received further information about the nature of the wines to be tasted: the vintage and other selection criteria (Chardonnay or non-Chardonnay, without indicating which are which). Wine by wine, the subjects put their answer on a non-structured continuum, where the left was a bad example and the right was a good example. Each of the subjects placed a mark that, according to each of them, positioned the wine on this typicity scale. For each wine we obtained as many different answers as there were tasters. Each response was then converted to a score from 0 to 10 that corresponds to the level of typicity.

In such a sensory analysis, the taster must have a particular status. It is clear that not every taster is able to answer such a question. For this reason, we opted to work with people in the industry, considered members of the human reference group, who have evaluation expertise as winegrowers, oenologists or technicians, etc. Over the Chardonnay wine-tasting period, given the diversity of the wines, these subjects gradually developed an image, a sensory representation, of what a Chardonnay wine is. Indeed, they all had participated in the "Chardonnay du Monde" competition. Consequently, they have memorized knowledge of the levels of sensory expression that Chardonnay wines can present. Nevertheless, it is reasonable to wonder about their capacity to have mutual reference points and to generate a consensus.

The first results presented concern only the first experiment: the correlation between the average typicity scores obtained in the orthonasal evaluation and the overall evaluation (Figure 1). We can see the continuum, with, at the bottom on the left, the wines considered to be bad examples and, at the top on the right, the wines considered to be good examples. Note that the tasters reason more easily in terms of the negative than the positive (they find it easier to exclude from the sensory space than to

Figure 1. Illustration of the continuum – Correlation between average orthonasal and overall typicity scores. First experiment: 1999 and 2000 vintages

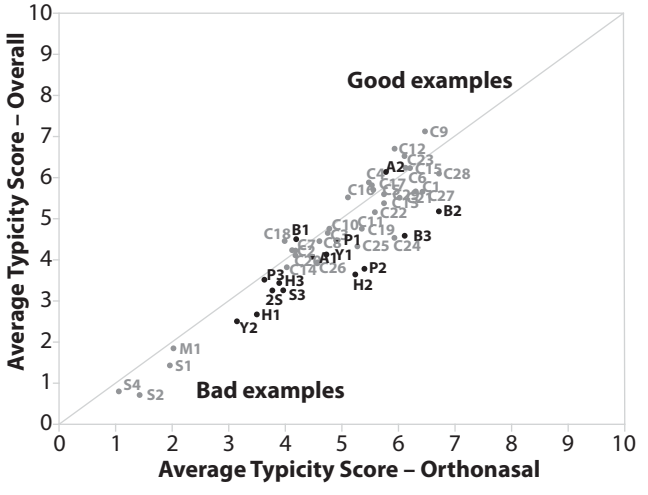


Figure 2. Description of the continuum from the first experiment with the 1999 and 2000 Chardonnay and other varietals

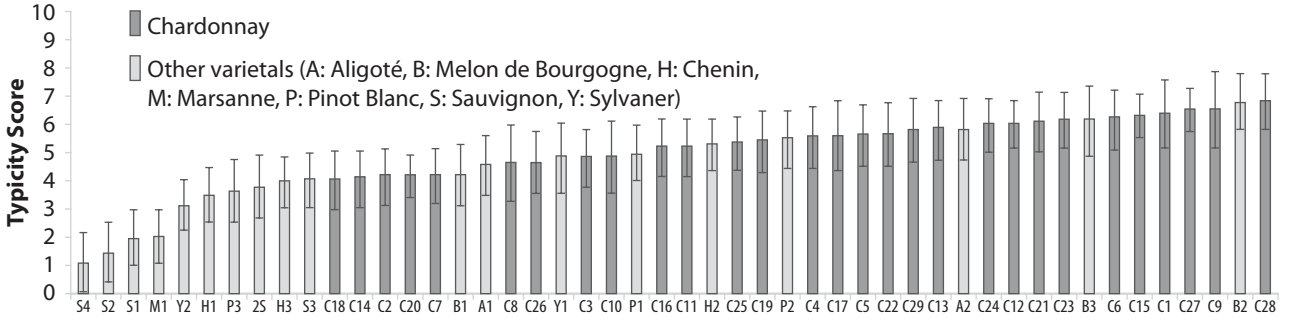
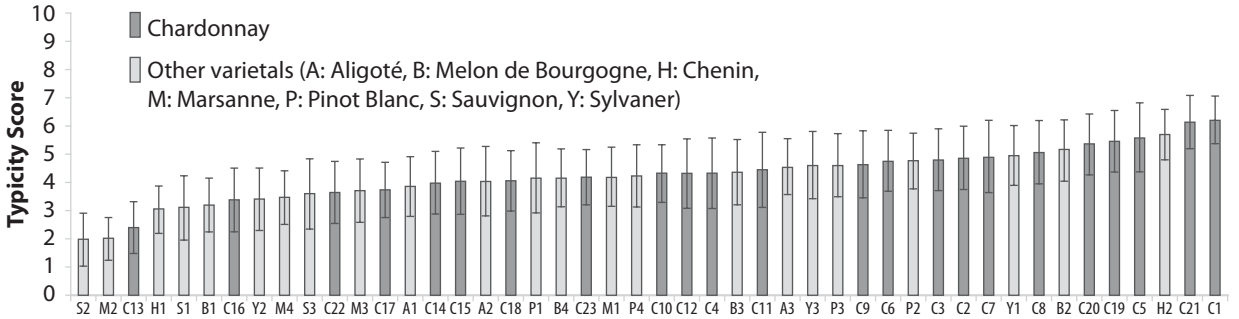


Figure 3. Description of the continuum from the second experiment with the 2003, 2004 and 2005 Chardonnay and other varietals



include), and, as a whole, they are reserved in regards to the good examples (on average, there were few very good scores, although individually certain subjects utilized the extreme right of the scoring scale). These results also testify to the correlation between the average scores produced by the two evaluation procedures. During the second experiment, we will not use the overall evaluation, as, in the case of white wines, it appears that by itself the orthonasal evaluation contains the main sensory information.

The following data (Figures 2 and 3) show the distribution of the average orthonasal scores obtained during both experiments. Again, the continuum is clearly apparent – even more so for the first experiment than for the second. In such situations, how can one demonstrate, despite the superimposition of the product spaces, the existence of a specific sensory space? Let us take the example of the orthonasal evaluation carried out during the first experiment. Thanks to the analysis of variance (ANOVA) and the least significant difference (LSD) methods, it is possible to significantly differentiate between two opposite wine populations by removing the intermediate population, made up of the wines that are neither good nor bad examples. This population illustrates the collective indecision of the tasters and should not hold our attention (see Figure 4). The bipolar pattern reveals the wines whose olfactory characteristics allow the subjects to categorize

Figure 4. Illustration of the wine samples removed from the continuum to separate the bad examples from the good examples of wines, in order to obtain a proper sensory space for Chardonnay wines

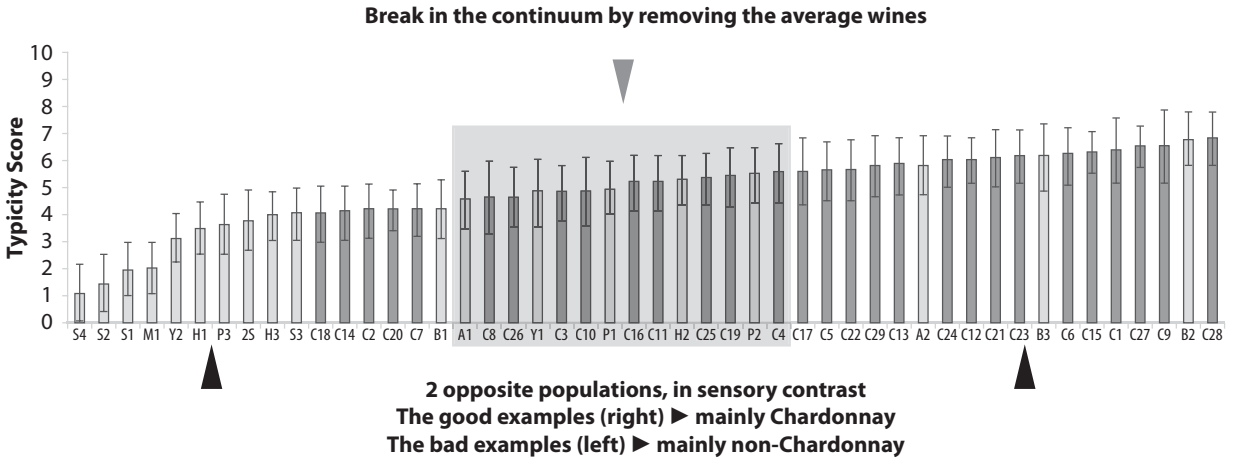
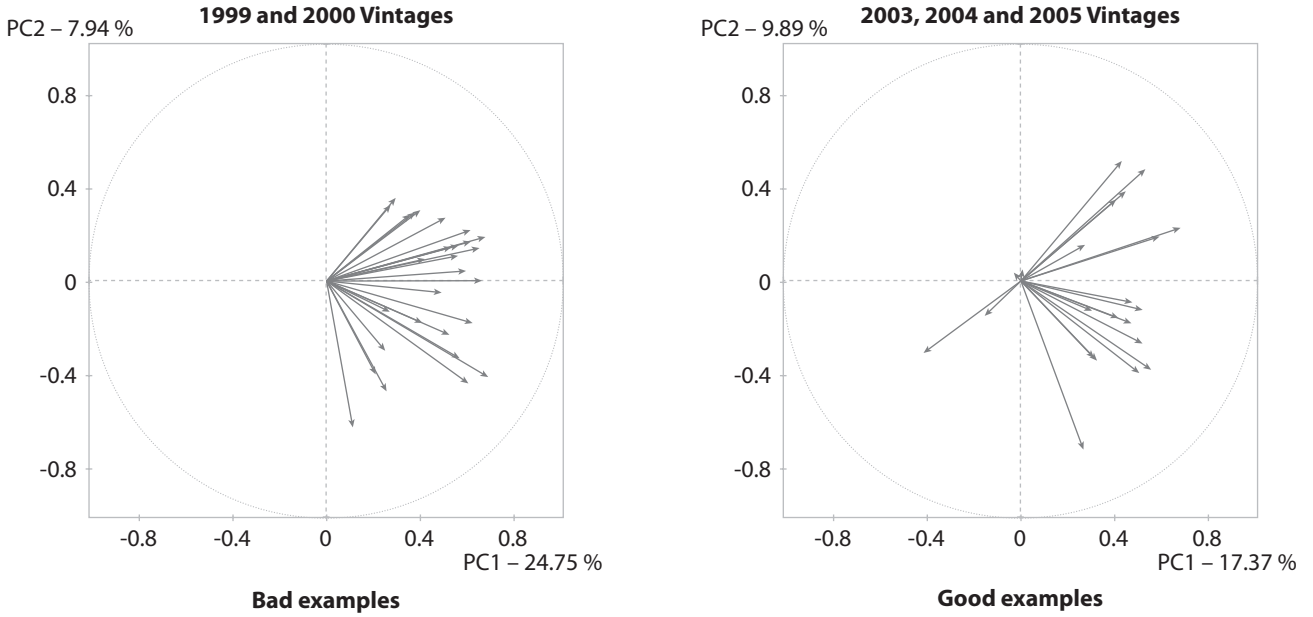


Figure 5. Principal component analyses of the individual scores given by each taster for each wine



them unanimously, either in the population of good examples of Chardonnay wines, or in the population of bad examples. Therefore, we will now focus on these two populations, which contrast from a sensory point of view, and are significantly different from each other. In the case of the orthonasal evaluation in the first experiment, it appears that the great majority of good examples is made up of wines produced by the Chardonnay varietal (15 wines out of 18) and that the majority of bad examples is constituted of non-Chardonnay wines (11 out of 16). The same distribution appears for the evaluations, both orthonasal and overall, conducted respectively during the first and second experiments. Thanks to these results, it is possible to conclude that, for non-woody young wines (aged one to three years), the sensory space specific to Chardonnay wines exists, independent of the evaluation procedure, the vintage and, not shown here, the provenance.

Now let us turn to the consensus among tasters. For that, we conducted two principal component analyses (PCA) on the individual scores of the two orthonasal evaluations from the first and second experiments (see Figure 5). In this case, the wines are considered to be individuals, and the subjects as variables. Whatever the experiment, the vectors related to each of the subjects form a more or less convergent direction arrayed around the horizontal axis (the typicity axis) on the good example side. Despite an inter-individual variability that is understandable in regards to such a concept, it is nevertheless possible to infer that the subjects possess a collective representation of good and bad examples of Chardonnay wines. The typicity evaluation of the wines must be entrusted to the members of the human reference group, sufficiently numerous (20 to 30 tasters) for this convergence effect to emerge.

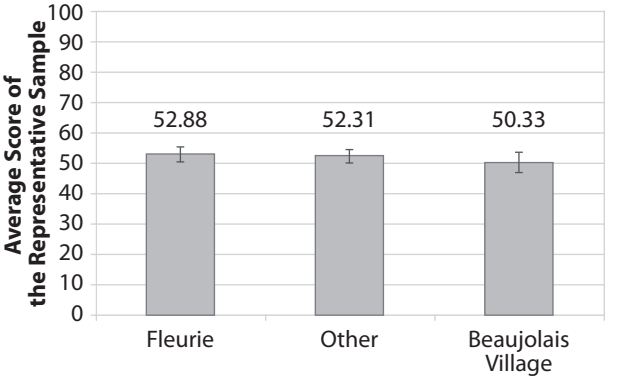
The expert tasters agree on the sensory definition on a specific category.

Within the framework of the study carried out on the Cru Fleurie (Beaujolais) wines (reds), a convergence among tasters appears, while the product spaces studied (Fleurie versus non Fleurie) are superimposed. Thus, a sensory space specific to Fleurie wines does not exist (see Figure 6).

Links between Sensory Data and Aromatic Components

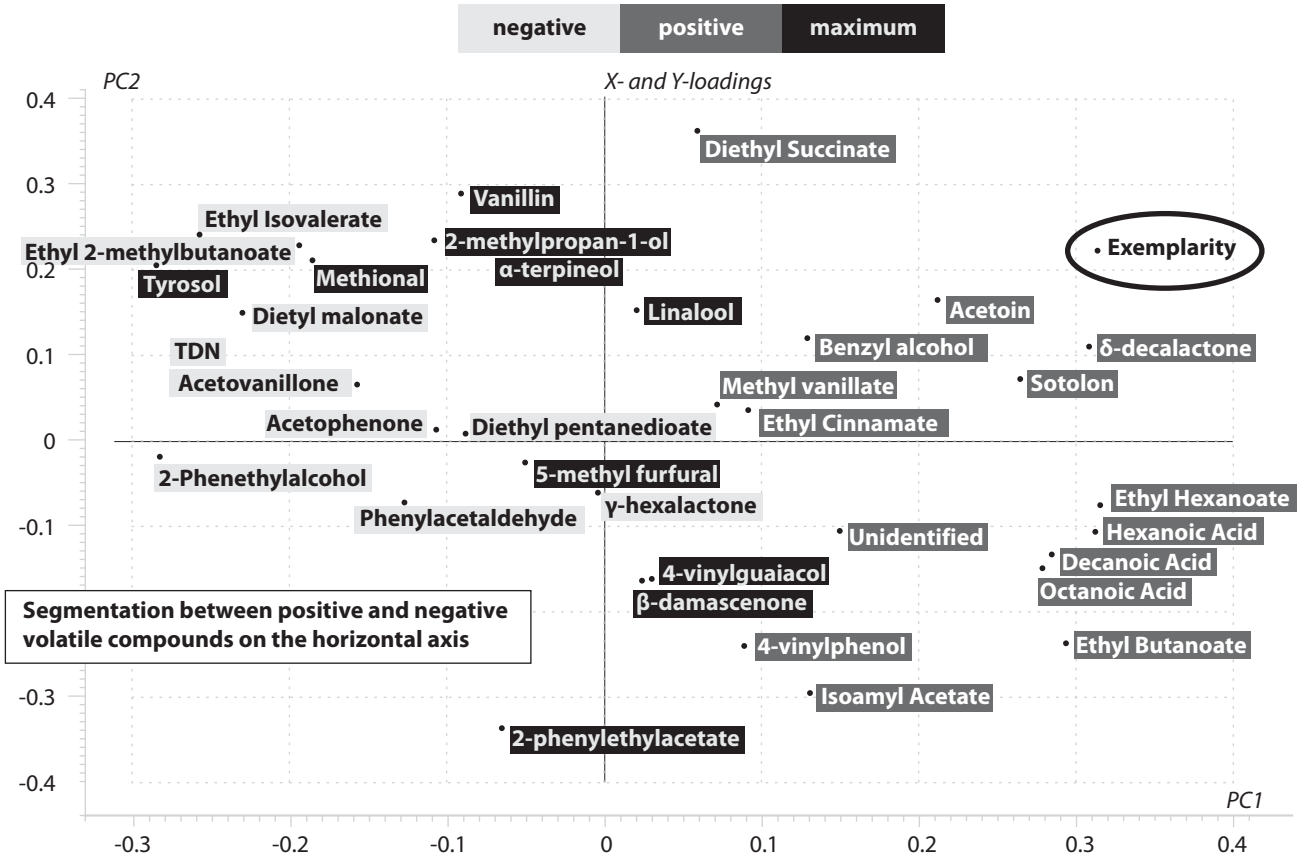
At the end of the first experiment, nine wines considered to be good examples and nine considered to be bad ex-

Figure 6. Evaluation of the product space of Cru Fleurie against Beaujolais Village wines as well as other Crus



(Student test: Fleurie vs. non Fleurie: non significant)

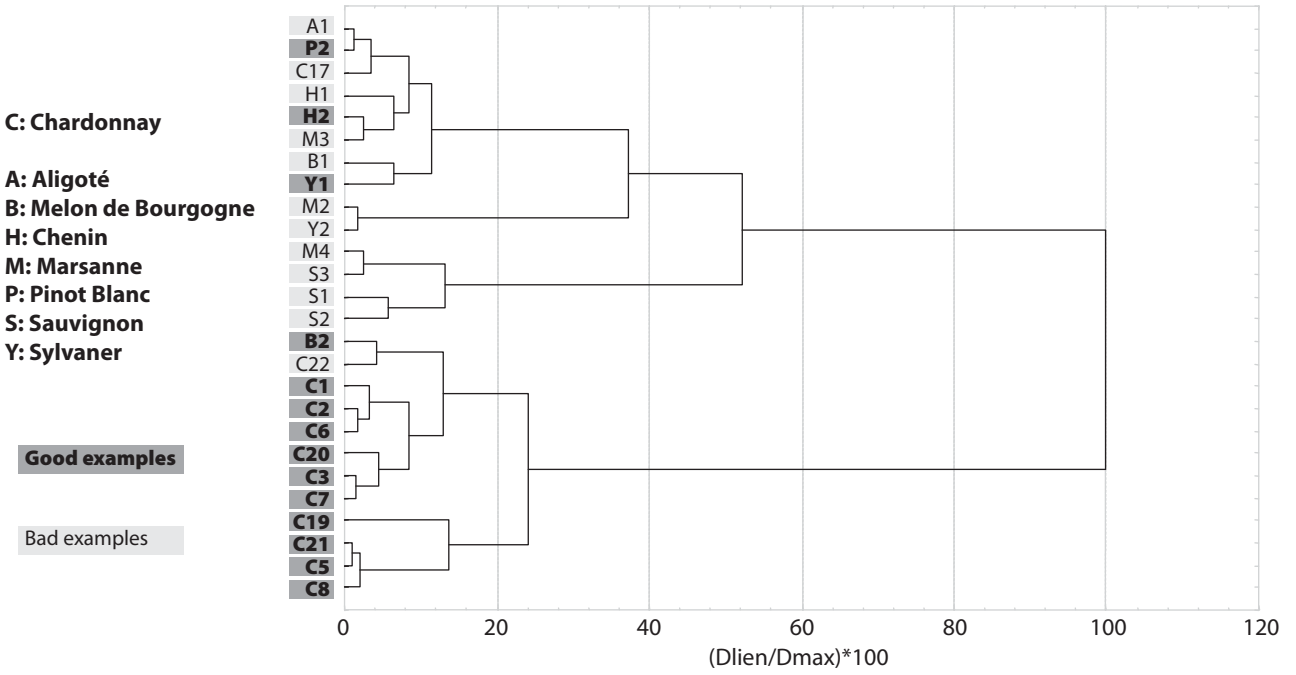
Figure 7. PLS results of the 35 compounds having an impact on Chardonnay wines



amples were analyzed by gas chromatography-olfactometry (GC-O). These wines were selected to illustrate the four cases in the following figures: Chardonnay, good example; Chardonnay, bad example; non-Chardonnay, good example; and non-Chardonnay, bad example. Thus, 76 odorant zones, common or specific to the 18 wines, were revealed, and 72 volatile compounds were identified by gas chromatography–mass spectrometry (GC–MS) then dosed by gas chromatography–mass spectrometry–selective ion monitoring (GC–MS–SIM). At the end of the second experiment, 28 sensory contrasting wines (14 good examples and 14 bad examples) were analyzed, but the olfactometric and identification steps in the analysis were considered to have been obtained. Only the dosage was recalibrated. In both cases, the semi-quantitative analysis determined the relative concentration (compared to an internal standard – ethyl heptanoate) of each of the compounds in the samples. Thus, we had two databases: the sensory data (typicity scores) and the volatile compound composition (relative concentrations). Subsequent to the first experiment, we tried to link both databases utilizing ANOVA–LSD, carried out on the semi-quantitative data of each of the compounds. After the second experiment, the ANOVA was completed by the partial least squares (PLS) analysis where all the sensory and quantitative data were taken into consideration. Only these final results are presented. Different trend groups are revealed. These trend groups will then constitute our working hypotheses: 1) the typicity score increases when the relative concentration of the compound increases (positive effect); 2) the typicity score increases when the relative concentration of the compound decreases (negative effect); 3) the typicity score increases when the concentration of the compound reaches an optimal level (optimum effect). In addition to these three trend groups, there is the case where the typicity score is not influenced by the concentration of the compound. This is the trend most often seen and the compounds that correspond to it can be considered responsible for the white wine environment with no real impact on the character of Chardonnay wine.

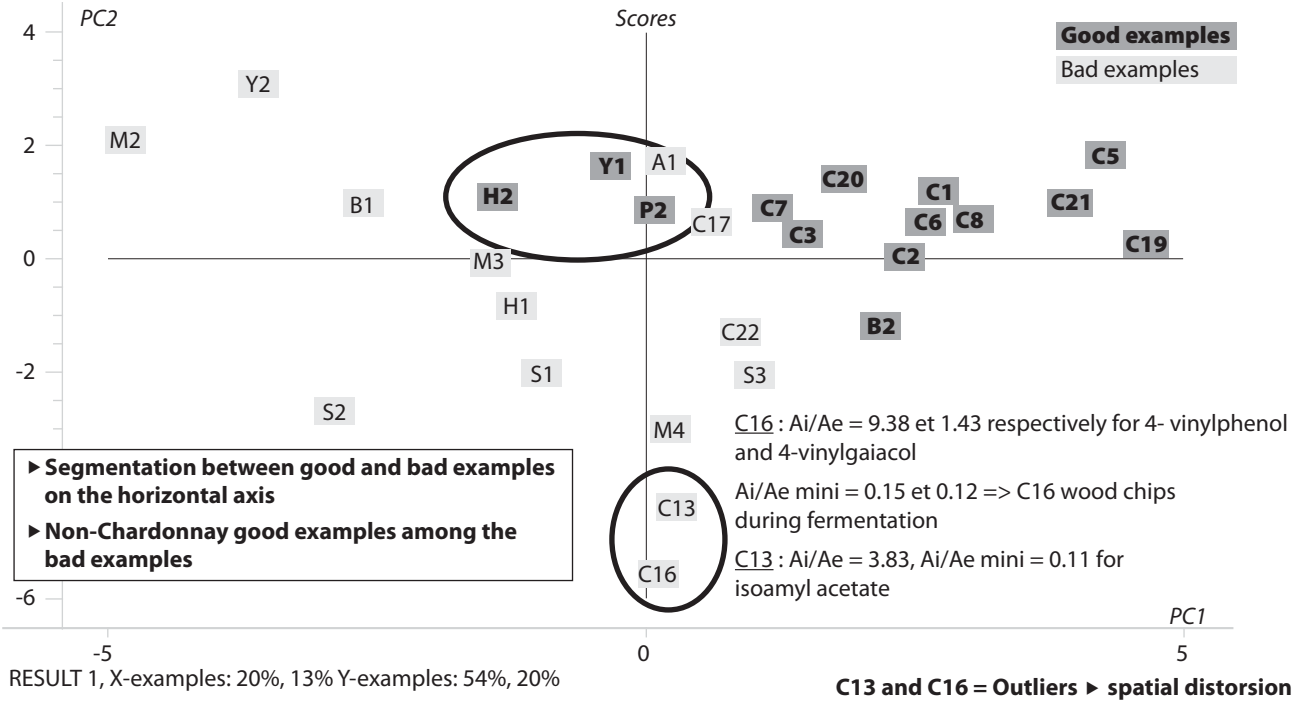
The PLS results show that out of the 35 compounds having an impact (first identified through ANOVA–LSD), 15 seem to present a positive effect and 10 a negative effect (see Figure 7). The segmentation between these two trend groups is clear-cut. On the other hand, the optimum effect seems more difficult to modelize for the last 10 compounds that could be distributed between the two main

Figure 8. New LSD (nwine = 26) followed by an ascending hierarchical ranking from the wine results given by LSD



trend groups: positive and negative. The mapping of the 28 wines shows a partial segmentation between good and bad examples, based on the aromatic component (see Figure 8). The non-Chardonnay wines considered to good examples (P2, H2 and Y1) are indeed in a singular position: they are more closely related to the bad examples. Two Chardonnay wines (C13 and C16), considered to be bad examples, have a distinctive aromatic composition. They were discarded from the rest before carrying out a second LSD analysis followed by an ascending hierarchical ranking (based on the LSD coordinates) (Figure 9). Very clearly, under our conditions for analysis, through the prism of

Figure 9. PLS results of the good and bad examples of Chardonnay wines with the position of the two discarded wines (C13 and C16)



C: Chardonnay
A: Aligoté, B: Melon de Bourgogne, H: Chenin, M: Marsanne, P: Pinot Blanc, S: Sauvignon, Y: Sylvaner

35 compounds having an impact, the Chardonnay wines recognized as good examples stand out from the bad examples (from a physicochemical perspective), with which the three wines previously identified (P2, H2 and Y1) were associated.

Conclusions

In the case of young, non-woody wines, the sensory space specific to wines produced from the Chardonnay varietal clearly exists. This illustrates the example of a typicity that is not related to the *terroir*. One can also see that the tasters share a collective representation of what Chardonnay wines are, compared to other white wines produced from other varietals.

Although very complex, there appears to be link between the typicity score and the aromatic component. Although the great majority of compounds do not present any influence on the Chardonnay character, they do constitute the environment in which the high-impact compounds are expressed, and about 30 of them present either positive or negative effects. This remains to be validated through further experiments subjected to sensory analysis.

MICROBIOLOGICAL ACETALDEHYDE KINETICS: CURRENT RESULTS AND MANAGEMENT IN THE CONTEXT OF SIMULTANEOUS ALCOHOLIC AND MALOLACTIC FERMENTATIONS

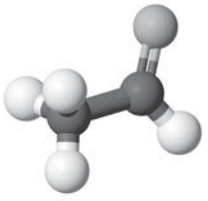
Sandra CHRISTEN, Nick JACKOWETZ and Ramón MIRA DE ORDUÑA

Department of Food Science & Technology, NYSAES, Cornell University, Geneva, NY 14456-0462

Introduction

Acetaldehyde is an important volatile carbonyl compound in wines. It is a small (see Figure 1) and chemically reactive molecule with an aroma that has been described as green, grassy, apple and oxidized / Sherry-like – an attribute that stems from the higher concentrations of acetaldehyde usually found in wines that have been subjected to oxidative conditions and in wines produced in Jerez (the Sherry region of Spain). While it is correct that Sherries tend to have higher acetaldehyde concentrations than most table wines, the production of Fino or Manzanilla Sherry wines is a reductive process and hence the term "aldehydic" would appear more suitable to describe wines with high acetaldehyde concentrations instead of "oxidized."

Figure 1. Acetaldehyde and basic chemical parameters



Melting point:	-123.5°C
Boiling point:	21°C
Flash point:	-38°C
MW:	44 g mol^{-1}
CAS:	75-07-0
EINECS:	200-836-8

Values provided in the literature for the odour threshold of acetaldehyde vary considerably according to the method and the study. An orthonasal odour threshold of 41 µg/L was measured in air by Rychlik et al. 1996, who also determined values of 25 µg/L (orthonasal) and 10 µg/L (retronasal) when dissolved in water (reviewed in Buettner and Schieberle 2001). In wine mimicking hydroalcoholic solutions, the odour threshold of acetaldehyde was found to be significantly higher, with reports of 0.5 mg/L (Guth 1997; Francis and Newton 2005) and 10 mg/L (Zea et al. 2008).

In beer, the flavour threshold ranges from 5 to 50 mg/L according to beer style (Meilgaard 1974; MacDonald et al. 1984), while levels of 100 to 125 mg/L are reported for wine (Zoecklein et al. 1995).

A recent study concluded that acetaldehyde present in alcoholic beverages could lead to saliva acetaldehyde concentrations, "…which are above levels previously regarded as potentially carcinogenic" (Lachenmeier and Sohnius 2008). The same authors presented a risk assessment study suggesting that acetaldehyde ingested from alcoholic beverages "…greatly exceeded the usual limits for cancer risks … resulting in a magnitude of risks requiring intervention" (Lachenmeier et al. 2009).

Homann et al. (2000) have studied the role of acetaldehyde in alcohol-associated carcinogenesis (Seitz et al. 2001) and presented data that supports the existence of a link between alcohol consumption and acetaldehyde-mediated oral (Homann et al. 2000, Homann et al. 2001), upper gastrointestinal (Homann 2001) and colon cancers (Homann et al. 2000b). At the same time, it remains unclear whether acetaldehyde has epidemiological relevance beyond subjects with poor dental status, heavy drinkers or those displaying genetic polymorphisms of enzymes involved in the metabolism of ethanol to acetaldehyde (Anonymous 1985), and no thorough study is available to evaluate the chronic toxicity of acetaldehyde.

The last opinion of the International Agency for Research on Cancer (IRC, a WHO agency) from 1985 concluded that there was inadequate evidence to suggest acetaldehyde carcinogenicity to humans while sufficient evidence was found to suggest carcinogenicity to animals from acetaldehyde inhalation (Anonymous 1985). The Scientific Committee on Cosmetic Products and Non-Food Products Intended for Consumers (European Union committee) adopted an opinion at the 28th plenary meeting May 25, 2004, rating acetaldehyde as having a low acute and subchronic toxicity (Anonymous 2004).

In fact, acetaldehyde is currently being used as a food additive, and is naturally present in many types of fruit, where acetaldehyde accumulation is caused by anaerobic fruit metabolism, e.g. during modified atmosphere storage conditions generally applied to delay ripening in fruit (Pesis 2005).

In the United States, acetaldehyde has GRAS status (Generally Regarded As Safe), according to 21 CFR 182.60; 27 CFR 24.246 permits the use of acetaldehyde for colour stabilization of [grape] juice prior to concentration as long as the added amount does not exceed 300 ppm and the finished concentrate contains no residues.

Significant amounts of acetaldehyde may be found in some fruit and fruit juices. In orange juice, it is essential for the typical and desirable aroma (Hinterholzer and Schieberle 1998, Perez-Cacho and Rouseff 2008). Concentrations of 6 to 8 mg/kg (Shaw 1991; Buettner and Schieberle 2001) to 90 to173 mg/L (Lund et al. 1981) have been measured in freshly pressed and heat-treated orange and grapefruit juices.

While the assessment of the human toxicity of the acetaldehyde found in alcoholic beverages requires further work, the main reason for reducing acetaldehyde in table wines lies in its ability to strongly bind SO_2, an essential wine preservative that has been associated with negative health effects in sensitive consumers (Yang and Purchase 1985, Snelten and Schaafsma 1992, Papzian 1996). Since acetaldehyde reduces the availability of free and, hence, molecular SO_2, its presence requires further additions of SO_2, roughly 1.5 times the concentration of acetaldehyde.

The reduction of acetaldehyde levels is also important in the production of base wines for distillates where high acetaldehyde levels are undesirable because they reduce production yields (Geroyiannaki et al. 2007), and may be limited by legislation, such as in the EU, where a limit of 5 mg of acetaldehyde per litre of absolute alcohol is applicable for distilled spirits (Anonymous 2008).

This work provides a brief summary of recent research results obtained in our laboratory concerning the acetaldehyde levels commonly found in local cool-climate wines, factors that affect acetaldehyde production by yeast and its degradation by oenological lactic acid bacteria. Differences in the course of acetaldehyde levels during simultaneous alcoholic fermentation (AF) / malolactic fermentation (MLF) will also be mentioned.

Results and Discussion

Acetaldehyde Levels

Since acetaldehyde is mostly bound to SO_2 in bottled wines or those intended for bottling, in most studies investigating wine aroma compounds by gas chromatography (GC) applying headspace methods either omits data collected on acetaldehyde, or presents headspace data, which is not likely to be representative of the total acetaldehyde levels of the wines analyzed, such as the 2 mg/L reported by Guth (1997) in Scheurebe and Gewürztraminer wines.

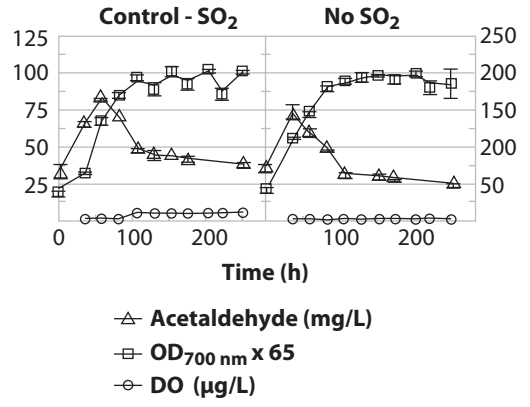
Initial studies carried out in our laboratory aimed at gaining an overview of total acetaldehyde concentrations across wines produced in the province of Ontario, Canada (n=92). The results showed that red wines had an average of 20 mg/L of acetaldehyde, while whites wines had mean acetaldehyde levels of 40 mg/L. No statistically significant correlations could be found among the acetaldehyde levels and vintage, variety (within whites and reds) or wine quality as expressed by legal definitions. Besides wine colour, significant differences were found among wines produced by different wineries, indicating large variations with regards to wine handling and, specifically, to protection from oxidation in post-alcoholic fermentation stages.

Acetaldehyde Production and Reuptake by Commercial *Saccharomyces cerevisiae* Yeast

It is well known that acetaldehyde may form from the oxidation of ethanol mediated by phenolic compounds and transition metals, such as copper and iron (Danilewicz 2007). However, acetaldehyde can also form under anaerobic conditions, namely by yeast during alcoholic fermentation.

In order to study acetaldehyde formation and degradation kinetics by commercially available yeast and considering the oenological parameters, two active dried yeasts (ADY) – DV10 and EC1118 – were used in the vinification of two varietals (Gewürztraminer and Sauvignon Blanc) and at various conditions for temperature (12° and 20°C), initial SO_2 addition (none and 30 mg/L), nutrient addition (none and 25 g/hL Fermaid K) and pH (native, i.e., 3.1 and 3.2;

Figure 2. Vinification with DV10 yeast in Gewürztraminer



Variable: Starting SO_2 concentration.
Addition of SO_2 creates an increase in the maximum and final concentration of acetaldehyde.

and increased to 3.5). Overall, 40 individual vinifications were carried out. Figure 2 shows an example comparing vinification with DV10 in Gewürztraminer with and without added SO_2. The data shows that during the initial phase of the AF, acetaldehyde levels rise to reach a maximum after which yeasts take up a certain amount of acetaldehyde to reach a final level, which depends on the residual yeast viability after AF.

Statistical analysis of the datasets revealed that among all parameters tested, lower temperatures led to increased acetaldehyde concentrations in some vinifications (Sauvignon Blanc) while SO_2 additions led to a statistically significant increase of maximum and final acetaldehyde levels across all treatments.

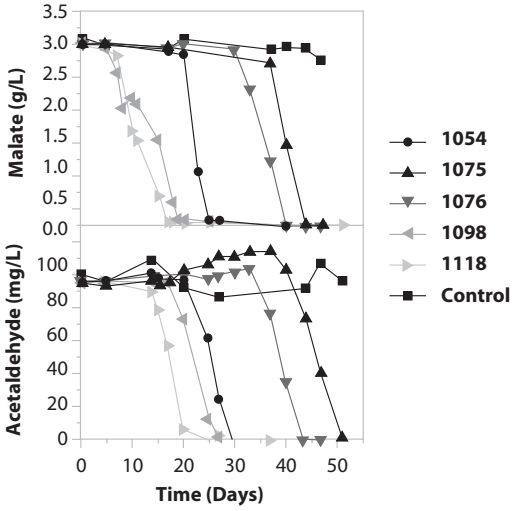
The Course of Acetaldehyde during Malolactic Fermentation

The experiment shown below is representative of a large number of incubations carried out with various strains of *Oenococcus oeni* and *Lactobacillus* species in the vinification of different varietals since 2000. For the work shown, the degradation of acetaldehyde and malic acid was followed during the entire course of MLF by *Oenococcus oeni* in Riesling. While the duration of MLF was strain dependent, all strains led to successful MLF. Acetaldehyde was also degraded by all strains and was complete within two to 10 days after the depletion of malic acid. (see Figure 3).

Overview of the Possible Acetaldehyde Course during Vinification

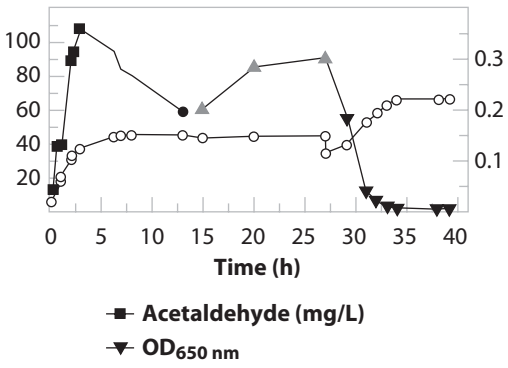
Figure 4 shows the possible course of acetaldehyde concentrations during vinification. The initial acetaldehyde concentration in the must depends mainly on the quality of the fruit. Significant levels should be expected when

Figure 3. Degradation of malic acid and acetaldehyde by five strains of *Oenococcus oeni* in Riesling



fruit is affected by microbial spoilage. Acetaldehyde formation by yeast may be affected by several factors, including, to some degree, the availability of nutrients in the must, Brix level, strain differences and fermentation temperature, but the most important factor is the addition of SO_2 to the must. The reuptake by yeast will be mediated by the viability of the biomass and the countering effect of wine oxidation. If wine oxidation is significant, a second significant increase may occur. It should be noted that oenological lactic acid bacteria have the ability to completely reduce the acetaldehyde in wine during MLF. For complete degradation, it is advisable to wait several days after malic acid degradation.

Figure 4. Possible course of acetaldehyde concentrations during vinification. Initial biological formation and reuptake by yeast during AF; second increase due to oxidative formation of acetaldehyde during storage or transfer; degradation of acetaldehyde by wine lactic acid bacteria during MLF.



Acetaldehyde Levels during Simultaneous Fermentation

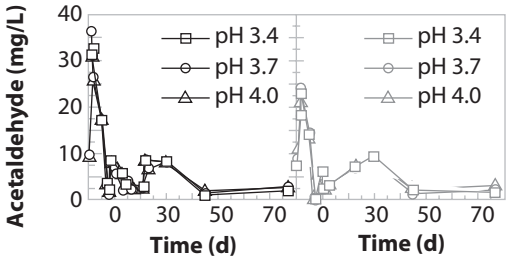
Yeast-bacteria co-inoculations can be an interesting alternative in the vinification of wine, especially white wines (Pan et al. 2007). Significant advantages include the duration of the overall vinification as MLF benefits from lower ethanol and higher nutrient concentrations when carried out simultaneously with AF.

However, malolactic bacteria have been shown to degrade acetaldehyde (see above), and a premature depletion of acetaldehyde, which is important for red colour development, could negatively influence red wine colour. Hence, a study was carried out to determine what the effect of co-inoculation, as compared to traditional consecutive fermentation, would be on the course of acetaldehyde levels, and if potential differences would affect the colour quality of the resulting wines. A weakly coloured variety – a cool-climate Pinot Noir – was used for this study and fermented at three different pH values, including the native pH 3.4, as well as pH 3.7 and 4.0 with *S. cerevisiae* ICV D254 and *O. oeni* ALPHA.

General results: The overall rate of sugar degradation was homogeneous across the different initial wine pH values and inoculation methods, and no stuck or sluggish AF occurred. The rate of alcohol production was similar in all treatments and independent of the pH as well. Malic acid degradation was complete in all treatments and at all pH values, but the rate of degradation was always faster after co-inoculation. The final acetic acid levels were slightly higher in simultaneous fermentations, but the differences were not significant from a sensory or legal perspective.

Acetaldehyde levels peaked during early fermentation phases regardless of the fermentation technique, but were significantly different. The maximum levels reached during simultaneous fermentations were approximately one third lower than in consecutive treatments. Except for the first few days, the courses of the two treatments were similar. Simultaneous treatments resulted in slightly higher final acetaldehyde levels (see Figure 5).

Figure 5. Course of acetaldehyde concentrations during consecutive (left) and simultaneous (right) fermentations of Pinot Noir at pH 3.4, 3.7 or 4.0.



Colour measurements: No significant differences could be observed between simultaneous and consecutive treatments using the CIELab method and by eye.

Overall, simultaneous AF/MLF was successful in the production of Pinot Noir, with a significant advantage in terms of vinification duration (two weeks) favouring co-inoculation. While the peak values measured for acetaldehyde were lower during co-inoculations, no effect on red wine colour could be measured, indicating that aerations/pumping over reduce the effect of acetaldehyde degradation by malolactic bacteria.

Conclusions

Acetaldehyde plays an essential role in wine colour, aroma and microbiological stability, the latter because of its ability to bind SO_2. Acetaldehyde is produced by yeast during alcoholic fermentation and may be produced by acetic acid bacteria during red wine skin maceration. After the depletion of sugar, yeast may take up a certain amount of acetaldehyde, but the further course of the acetaldehyde concentration depends on the rate of yeast uptake and the rate of acetaldehyde formation from the oxidation of ethanol in wines that are insufficiently protected from oxygen. Besides its uptake by yeast, acetaldehyde will also be significantly reduced during malolactic fermentation. A certain amount of acetaldehyde will be also used up in chemical reactions, including polymerization reactions of wine phenolics.

It should be noted that acetaldehyde may already be present in musts obtained from damaged grapes, e.g., through the activity of acetic acid bacteria (Drysdale and Fleet 1988) or by the oxidation of ethanol produced by yeast in damaged grapes. It has also been shown that acetic acid bacteria may be involved in the post-bottling production of acetaldehyde (Bartowsky and Henschke 2008). Table 1 summarizes the main sources and sinks of acetaldehyde.

Among the most important factors to be considered for the reduction of acetaldehyde in wines are grape quality, SO_2 utilization in the must stage and during active alcoholic fermentations, as well as protecting wines against oxidation. Malolactic fermentation plays an important role in the degradation of acetaldehyde. Chemical methods to reduce the levels of acetaldehyde and other carbonyls have been studied but are not yet commercially available (Blasi et al. 2007).

Table 1. Major acetaldehyde sources and sinks

Phase	Sources	Sinks
Vineyard	Damaged grapes: acetic acid bacteria, oxidation of ethanol produced by fermentative yeast	
Alcoholic fermentation	Yeast	Chemical reactions (e.g., phenolic polymerization)
Late alcoholic fermentation and post-AF	Acetic acid bacteria, oxidation of ethanol (lack of protection)	Yeast, chemical reactions
Malolactic fermentation	Acetic acid bacteria, oxidation of ethanol (lack of protection during slow MLF)	Malolactic bacteria, chemical reactions
Post-fermentative operations	Oxidation of ethanol (lack of protection during storage), mixing, transfer, cold stabilization, filtration, bottling	Chemical reactions
Post-bottling	Acetic acid bacteria	

References

Anonymous. 1985. Acetaldehyde. In IARC Monographs on the Evaluation of the Carcinogenic Risk of Chemicals to Humans. *Allyl Components, Aldehydes, Epoxides and Peroxides*, ed. Anonymous. Lyon, France: International Agency for Research on Cancer. 101-132.

Anonymous. 2004. *Evaluation and opinion on acetaldehyde*. The Scientific Committee on Cosmetic Products and Non-Food Products Intended for Consumers, SCCNFP/0821/04. 1-17.

Anonymous. 13 Feb. 2008. Regulation (EC) No 110/2008 of the European Parliament and of the Council of 15 January 2008 on the definition, description, presentation, labelling and the protection of geographical indications of spirit drinks and repealing Council Regulation (EEC) No 1576/89. *Official Journal of the European Union* L39:16-54.

Bartowsky, E. J., and P. A. Henschke. 2008. Acetic acid bacteria spoilage of bottled red wine – A review. *Int J Food Microbiol* 125:60-70.

Blasi, M., J. C. Barbe, B. Maillard, D. Dubourdieu, and H. Deleuze. 2007. New methodology for removing carbonyl compounds from sweet wines. *J Agric Food Chem* 55:10382-10387.

Buettner, A., and P. Schieberle. 2001. Evaluation of aroma differences between hand-squeezed juices from Valencia late and Navel oranges by quantitation of key odorants and flavor reconstitution experiments. *J Agric Food Chem* 49:2387-2394.

Danilewicz, J. C. 2007. Interaction of sulfur dioxide, polyphenols, and oxygen in a wine-model system: central role of iron and copper. *Am J Enol Vitic* 58:53-60.

Drysdale, G. S., and G. H. Fleet. 1988. Acetic acid bacteria in winemaking: a review. *Am J Enol Vitic* 39:143-154.

Francis, I. L., and J. L. Newton. 2005. Determining wine aroma from compositional data. *Austr J Grape Wine Res* 11:114-126.

Geroyiannaki, M., M. E. Komaitis, D. E. Stavrakas, M. Polysiou, P. E. Athanasopoulos, and M. Spanos. 2007. Evaluation of acetaldehyde and methanol in Greek traditional alcoholic beverages from varietal fermented grape pomaces (*Vitis vinifera* L.). *Food Control* 18:988-995.

Guth, H. 1997. Quantitation and sensory studies of character impact odorants of different white wine varieties. *J Agric Food Chem* 45:3027-3032.

Hinterholzer, A., and P. Schieberle. 1998. Identification of the most odour-active volatiles in fresh, hand-extracted juice of Valencia late oranges by odour dilution techniques. *Flavour and Fragrance Journal* 13:49-55.

Homann, N. 2001. Alcohol and upper gastrointestinal tract cancer: the role of local acetaldehyde production. *Addiction Biology* 6:309-323.

Homann, N., J. Tillonen, J. H. Meurman, H. Rintamaki, C. Lindqvist, M. Rautio, H. Jousimies-Somer, and M. Salaspuro. 2000. Increased salivary acetaldehyde levels in heavy drinkers and smokers: a microbiological approach to oral cavity cancer. *Carcinogen* 21:663-668.

Homann, N., J. Tillonen, H. Rintamaki, M. Salaspuro, C. Lindqvist, and J. H. Meurman. 2001. Poor dental status increases acetaldehyde production from ethanol in saliva: a possible link to increased oral cancer risk among heavy drinkers. *Oral Oncol* 37:153-158.

Homann, N., J. Tillonen, and M. Salaspuro. 2000b. Microbially produced acetaldehyde from ethanol may increase the risk of colon cancer via folate deficiency. *International Journal of Cancer* 86:169-173.

Lachenmeier, D. W., F. Kanteres, and J. Rehm. 2009. Carcinogenicity of acetaldehyde in alcoholic beverages: risk assessment outside ethanol metabolism. *Addiction* 104:533-550.

Lachenmeier, D. W., and E. M. Sohnius. 2008. The role of acetaldehyde outside ethanol metabolism in the carcinogenicity of alcoholic beverages: Evidence from a large chemical survey. *Food and Chemical Toxicology* 46:2903-2911.

Lund, E. D., C. L. Kirkland, and P. E. Shaw. 1981. Methanol, ethanol, and acetaldehyde contents of citrus products. *J Agric Food Chem* 29:361-366.

MacDonald, J., P. T. V. Reeve, J. D. Ruddlesden, and F. H. White. 1984. Current approaches to brewery fermentations. *Progress in Industrial Microbiology* 19:47-198.

Meilgaard, M. 1974. Flavor and threshold of beer volatiles. *Technical Quarterly, Master Brewers Association of America* 11:87-89.

Pan, W., D. Jussier, and R. Mira de Orduña. 2007. Controlling malolactic fermentation in a changing climate. The *19es Entretiens Scientifiques Lallemand – International Technical Meeting on Global Warming: Oenological Challenges*. Margaux, France: Lallemand Inc.

Papzian, R. December 1996. Sulfites: safe for most, dangerous for some. *FDA Consumer Magazine*.

Perez-Cacho, P.R., and R. L. Rouseff. 2008. Fresh squeezed orange juice odor: *A review. Critical Reviews in Food Science and Nutrition* 48:681-695.

Pesis, E. 2005. The role of the anaerobic metabolites, acetaldehyde and ethanol, in fruit ripening, enhancement of fruit quality and fruit deterioration. *Postharvest Biology and Technology* 37:1-19.

Rychlik, M., P. Schieberle, and W. Grosch. 1996. *Compilation of Odor Thresholds, Odor Qualities and Retention Indices of Key Food Odorants*. Deutsche Forschungsanstalt für Lebensmittelchemie, Garching, Germany.

Seitz, H. K., S. Matsuzaki, A. Yokoyama, N. Homann, S. Vakevainen, and X. D. Wang. 2001. Alcohol and cancer. *Alcohol Clin Exp Res* 25:137S-143S.

Shaw, P. E. 1991. Fruits II. In *Volatile Compounds in Fruits and Beverages*, ed. H. Maarse. New York: Marcel Dekker. 305-327.

Snelten, H. J., and G. Schaafsma. 1992. Health aspects of oral sulphite, and sulphite in wine. *Voeding* 53:88-90.

Yang, W. H., and E. C. R. Purchase. 1985. Adverse reactions to sulfites. *Can Med Assoc J* 133, 865-867.

Zea, L., L. Moyano, and M. Medina. 2008. Odorant active compounds in Amontillado wines obtained by combination of two consecutive ageing processes. *European Food Research and Technology* 227:1687-1692.

Zoecklein, B. W., K. C. Fugelsang, B. H. Gump, and F. S. Nury. 1995. *Wine Analysis and Production*. Kluwer Academic/Aspen Publishers, New York, USA.

EVOLUTION OF AROMA COMPOUNDS DURING THE MALOLACTIC FERMENTATION OF THE COOL-CLIMATE VARIETIES WELSH RIESLING AND SAUVIGNON BLANC

Tatjana VRŠČAJ VODOŠEK, Irena KRALJ CIGIĆ, Matija STRLIČ and Tatjana KOŠMERL

University of Ljubljana, Biotechnical Faculty, Department for food science and technology
Jamnikarjeva 101, Sl-1001 Ljubljana, Slovenia

Abstract

Malolactic fermentation (MLF) is a secondary fermentation in winemaking conducted by lactic acid bacteria (LAB) that is crucial for wine quality. *Oenococcus oeni* is the most desired LAB. As the main purpose of MLF is the reduction of acidity, it is desirable in wines from cooler winegrowing regions. And as MLF includes numerous and heterogeneous chemical reactions, which include many wine compounds, it is clearly not just a way to reduce acidity. Induced MLF fermentation and finish are much more predictable and controlled than spontaneous MLF. For this study, MLF trials were conducted on white Slovenian varieties of 2004 and 2005 vintages. We decided to include two varietals from the cooler winegrowing regions of Podravje – Welsh Riesling and Sauvignon Blanc. During the MLF trial period, chemical, microbiological and sensorial parameters were periodically analyzed. Microvinifications were carried out in 28 L stainless steel tanks and in 500 mL glass fermentors. Vinification procedures included the co-inoculation of grape musts and the inoculation of young wines after the completion of alcoholic fermentation (AF) with two different commercial LAB starters of the *Oenococcus oeni* species. Vinifications with induced MLF were carried out and compared to control vinifications, both with and without inoculation with yeast starters of the *Saccharomyces bayanus* species. During the trial in 28 L fermentors, the course of MLF was monitored by analyses of the organic acids, sugars, volatile acids, pH values, free amino acids, yeast and LAB population. The released CO_2 was monitored during the trial of the 2004 vintage in 500 mL fermentation flasks. After the completion of fermentation, several chemical parameters in the young wines were analyzed again after two months of aging. The results of individual parameters confirmed some hypotheses and new findings were made. The different chemical compositions of the musts of both vintages had an impact on the course of MLF. The concentrations of malic acid in musts of different grape cultivars and vintages influenced its level of degradation and also had an impact on the concentration of individual chemical parameters (e.g., volatile acids, citric acid, fructose, total extract, total phenols, diacetyl, ethyl lactate and acetoin). The course of both induced and spontaneous MLF was more rapid when the pH was higher and the concentration of malic acid lower in the grape must. During both induced and spontaneous MLF, a degradation of succinic acid was observed. Differences in MLF kinetics were observed among the LAB starters utilized, especially at the beginning and during MLF. The utilization of different LAB starters not only had an impact on the course of MLF in young wines and older ones, but on their chemical composition as well. We also established the influence of the timing of inoculation with LAB starters on the growth of LAB. The concentrations of higher alcohols and volatile compounds were more affected by the varietal and vintage than by the timing of inoculation and the LAB starters utilized. The impact of induced MLF on amino acid composition was shown. The varietal had the greatest impact on the amount of CO_2 released. It was also confirmed that MLF is faster at a higher temperature and, contrary to our predictions, more rapid MLF was observed in a smaller fermentation volume. For all our wines, induced MLF has proven to be the recommended method for improving

wine quality, although the contribution of MLF was higher in white wines from the cooler winegrowing region.

Introduction

Wine production is the result of numerous biochemical processes that are guided, above all, by both yeasts and bacteria. Clearly, alcoholic fermentation (AF) is the most important process in wine production. Guided fermentation is most often conducted by yeasts of the *Saccharomyces cerevisiae* species, while lactic acid bacteria (LAB) guide malolactic fermentation (MLF), a secondary but non-essential fermentation. Among LAB species, *Oenococcus oeni* is the most desirable, although *Lactobacillus hilgardii* is also used. The basic purpose of MLF is to reduce acidity, particularly in wines from cooler climates. MLF is complete when the malic acid content is below 0.2 g/L. Nowadays induced MLF is utilized more often as a tool for improving aromatic characteristics and microbiological stability, and is also utilized in wines from warmer climates. Reducing acidity in warm-climate wines can be hazardous due to the high pH value and lower acidity. In this case, the negative consequences of MLF occur much more often and winemakers must be aware of the high possibility of wine spoilage. The positive impact of MLF can include a reduction in vegetative notes and fruit the improvement of odour and flavour, as well as the microbial stability of the final product.

The basic action of MLF – the conversion of malic acid into lactic acid and CO_2 – is accompanied by numerous and heterogeneous chemical reactions among many wine compounds, including organic acids, sugars, aldehydes, ketones, glycosides, phenolic acids, esters, amino acids and amines. If the conditions are suitable, MLF usually occurs spontaneously after the completion of alcoholic fermentation. Oenologists are often afraid to use selected LAB in grape must, believing they could dominate over the selected wine yeasts and interrupt AF (i.e., cause stuck or sluggish fermentation), which could lead to unacceptable sensory quality because of the excess acetic acid, the synthesis of glucans, biogenic amines and precursors of ethyl carbamate. Indigenous populations of LAB are present on grapes, and the population in the must could quickly grow during vinification. However, spontaneous MLF is not recommended because it can lead to undesirable aroma development and a greater risk of spoilage. Controlled MLF can be induced through the simultaneous inoculation (co-inoculation) of yeast and LAB into the grape must. LAB inoculation can be realized during partial alcoholic fermentation or, as is more often the case, when alcoholic fermentation has finished, usually in young wines.

From the point of view of aroma characteristics, MLF develops and stabilizes certain aromatic and textural nuances, making the wine more complete with better overall quality. The wine bouquet is intensified, the varietal character is stronger and the taste is improved considerably, as long as the lactic notes are not excessive. MLF occurs in a variety of conditions, which make proving its existence difficult. If it takes place during or immediately following alcoholic fermentation, it can be completed without being noticed, but it can also occur several weeks or months after alcoholic fermentation. The duration of MLF is also influenced by the yeast used for AF (i.e., the yeast-bacteria interaction) and the maceration time, which raises the pH, increases grape polysaccharides and the acetaldehyde concentration.

Many different physical, biological and chemical factors affect the course of MLF and the development of LAB. The most important factors are a temperature between 20° and 25°C, a pH value above 3.20, an ethanol content of up to 13%/vol, and the SO_2 content below 20 mg/L for free SO_2 and below 50 mg/L for the bonded form. Furthermore, legislation regarding minimum total acidity and tartaric acid content in wine must be considered when planning MLF (Swiegers et al. 2005, Bartowsky 2005, Bauer and Dicks 2004, Alexandre et al. 2004, Liu 2002, Fleet 2002, Jackson, 2000, Ribéreau-Gayon et al. 2000, Lonvaud-Funel 1999, Versari et al. 1999, Zoecklein et al. 1999, and Boulton et al. 1996).

A deficiency of nitrogen compounds during vinification leads to unsuitable wine quality. Nitrogen has great importance in both AF and MLF. Many factors impact on nitrogen compound content, such as the cultivar, viticulture technology, vintage, vineyard location and vinification method. Many different types of nitrogen compounds are present in musts and wine. Amino acids represent the majority of nitrogen compounds in wine, particularly proline and arginine. During AF and MLF, free amino acids represent assimilable nitrogen for yeasts and bacteria, while proline cannot be used in anaerobic conditions. The lack of free amino acids is, in most cases, the main reason for stuck AF and/or MLF. Through yeast autolysis, amino acids are released back into the wine, especially during aging on lees. Amino acids are not only intermediates of heterogeneous aromatic compounds (e.g., higher alcohols and esters), they are intermediates of undesirable biogenic amines and ethyl carbamate (Arias-Gil et al. 2007, Cañas et al. 2007, Ferreira et al. 2002, Fleet 2002, Liu 2002, Jackson 2000, and Ribéreau-Gayon et al. 2000).

Amino acids are not only the source of assimilable nitrogen for LAB, but also of carbon and sulphur. Although precisely how LAB metabolize amino acids has not yet been fully studied, it is clear that many factors affect the metabolism of amino acids by LAB, including the citric acid content (Saguir and de Nadra 2002) and the proteolytic activity of LAB protease (Remize et al. 2005, and de Nadra et al. 1997).

In our study, we focused on MLF trials conducted by two different commercial strains of LAB starter culture (*Oenococcus oeni*) added to the grape must before the end of AF or to young wine after AF. We were interested in significant differences in free amino acid utilization during MLF, the evolution of aroma compounds, and the overall quality of trial wines compared to the control sample, which was vinified without the addition of LAB. Our hypothesis was that different vinification protocols would result in different amounts of aroma compounds produced and amino acids utilized during fermentation and released back into the wine after the completion of fermentation. The main purpose of our research was to improve the sensorial quality of the white wines Welsh Riesling and Sauvignon Blanc from cooler climates, through the utilization of MLF. Wines from both varietals are traditionally vinified through classic winemaking procedures, and often result in wines that contain less total dry extract, are untypical or have inexpressive fruitiness, unbalanced acidity and a lack of overall harmony. The utilization of different winemaking procedures would allow the winemaker to maintain and improve the varietal properties of wines, and to produce greater harmony in the acidity and freshness, which all contribute positively to the overall quality of Welsh Riesling and Sauvignon Blanc wines and to meeting the expectations of customers today.

Materials and Methods

Vinifications

Grapes of the cool-climate varietals Welsh Riesling and Sauvignon Blanc from 2004 and 2005 vintages were produced through an integrated system of grape production in the vineyards of the wine cellar Ptujska klet, which are in the winegrowing zone B. The harvest dates of cultivars in particular vintages are shown in Table 1. Welsh Riesling is the most represented (31.93%) white cultivar in the Haloze winegrowing district, while Sauvignon Blanc represents only 10.94% of the grapes grown there.

Trials with five different vinifications were conducted in each year. In the first part of the experiment, assigned as co-inoculation (CIN), the two different commercial LAB starters of the *Oenococcus oeni* species were added to the grape must at the same time as selected wine yeast starters of the *Saccharomyces bayanus* species. In the second part of the experiment, assigned as inoculation (IN), the LAB were added to the young wine after the completion of AF. Only wine yeasts without the addition of LAB fermented the control sample (CON), representing the classic vinification technique for Welsh Riesling and Sauvignon Blanc. The different vinifications were carried out in 28 L stainless steel tanks and in 500 mL glass fermentors. The commercial yeast starter (30 g/hL of *Saccharomyces bayanus* EC 1118), LAB (1 g/hL of *Oenococcus oeni* Uvaferm Alpha in LAB1) and Uvaferm Beta (1 g/hL in LAB2), are all produced by Lallemand.

Table 1. Harvest dates of cultivars

Vintage/Varietal	Welsh Riesling	Sauvignon Blanc
2004	November 2, 2004	October 14, 2004
2005	October 9, 2005	October 3, 2005

Analytical Methods

The grape must, the young wines after 42 days and the wines after two months of maturation were analyzed. The course of MLF during the trial was observed by the kinetics of individual organic acids (malic, lactic, citric and succinic acids), sugars (glucose, fructose, sucrose), glycerol, pH value and volatile acidity. During the MLF, the kinetics of 21 free amino acids (aspartic acid, glutamic acid, asparagine, serine, glutamine, histidine, glycine, threonine, arginine, alanine, tyrosine, cysteine, valine, methionine, tryptophan, phenyalanine, isoleucine, leucine, lysine, hydroxyproline, proline) were determined in three individual phases. In addition, the following basic chemical parameters were determined in the wines produced: the pH value, total acidity, buffer capacity, reducing sugars, total dry extract, alcohol and volatile compounds. Five different wines were sensory evaluated by the Buxbaum 20-point system after two months of maturation at 6°C.

The analysis of the organic acids (malic-MA, lactic-LA, citric-CA and succinic-SA), sugars (glucose, fructose and sucrose) and glycerol was carried out with a modified HPLC analytical technique employing the Bio-Rad Aminex HPX-87H column (300 mm x 7.8 mm) (Klein and Leubolt 1993). For the analysis of the organic acids, the UV-VIS detector and 0.0125 M H_2SO_4 as mobile phase were used. The RI detector and 0.0025 M H_2SO_4 as mobile phase were used for the detection of sugars and glycerol.

To determine the amino acid composition, reversed-phase HPLC-DAD with pre-column derivatization and gradient elution was used. The separation was carried out using the Zorbax Eclipse AAA column (4.6 mm x 150 mm, 5 µm) with a pre-column. The primary amino acids were derivatized with the reagent o-phthaldialdehyde (OPA) and the

secondary amino acids (Hyp, Pro) with 9-fluorenilmethyl-chloroformic acid (FMOC) (Henderson et al. 2000).

Determinations of higher alcohols (isoamyl alcohol, 1-propanol, isobutanol, 2-phenylethanol) and other volatile compounds (acetaldehyde, methanol, ethyl lactate, ethyl acetate, isoamyl acetate, diacetyl and acetoin) were performed by a modified GC-FID analytical technique using the HP FFAP column (50 m x 0.2 mm x 0.3 mm) in distilled wine samples (Košmerl and Kordiš Krapež 1996).

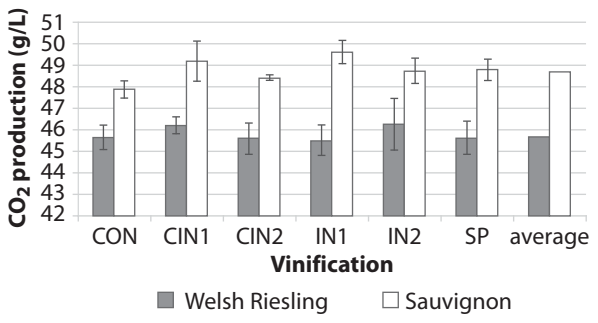
Total acidity (g of tartaric acid/L) and buffer capacity (mmol/L/pH) were determined by potentiometric titration. The contents of volatile acids (g of acetic acid/L) were analyzed in distilled samples, also with potentiometric titration. Sugar content (°Oe) in fresh grape must was measured by digital refractometry. The concentrations of reducing sugars (g/L) in wine samples were determined by the Rebelein titration method. Indirectly in wine distillate, the alcohol and total dry extract contents were determined (Košmerl and Kač 2004).

The concentrations of total phenols (mg of gallic acid/L) were detected with the Folin-Ciocalteu reagent at wavelength 765 nm (Ough and Amerine 1988). The levels of free amino nitrogen (FAN) (mg N/L) were analyzed spectrophotometrically, after reacting with ninhydrin and utilizating the threonine moderate curve and absorbance spectre from wavelengths of 450 to 700 nm (Nicolini et al. 2004).

Results and Discussion

The total CO_2 released during fermentation trials in 500 mL glass fermentors of the 2004 vintage is shown in Figure 1. Significantly higher CO_2 production was observed in the Sauvignon Blanc compared to the Welsh Riesling. In addition, MLF with LAB1 produced more CO_2 than LAB2 (only in Sauvignon Blanc), while we could not come to a conclusion about higher CO_2 production in regards to the timing of the LAB addition.

Figure 1. Total CO_2 production (g/L) during MLF trials in the 2004 vintage



For the released CO_2, the impact of the cultivar was more noticeable compared to the impact of timing and the LAB starters utilized. The kinetics of released CO_2 during vinification of the same cultivar were very similar. Contrary to our hypothesis, MLF was faster when in a smaller fermentation volume.

The results of the basic chemical parameters of the grape must (Table 2) show significant differences in the composition of the varietals investigated, as a consequence of vintage. The concentrations of total acidity, tartaric and malic acid had higher than normal average values. In the grape musts of the 2004 and 2005 vintages, important differences in the concentrations of malic acid, the main substrate of LAB, were noticed. The concentrations of malic acid in grape musts of the 2004 vintage were almost twice as high as the 2005 grape musts. The different chemical compositions of the grape musts had an impact on the course of MLF and the chemical composition of wines of both vintages. In terms of the concentrations of shikimic acid and amino acids in the grape musts, the similarities of grape musts from the same winegrowing region were revealed. The concentration of malic acid in grape musts of different vintages and cultivars influenced the onset, course and duration of MLF. In the trial of the 2004 vintage, MLF took longer than in the trial of the 2005 vintage.

In the trial of the 2004 vintage, MLF took an average of seven days more than the 2005 vintage trial. The longer duration of MLF was expected due to the higher concentrations of malic acid. Typical or untypical differences were established in the parameters analyzed on the basis of malic acid degradation. Total and volatile acidity, pH, organic acids (malic, lactic, citric and tartaric acids), glucose, fructose, total extracts, acetaldehyde, ethyl lactate and diacetyl were the most important parameters for wine quality. Chemical results were confirmed by sensory analysis; the only exceptions were spontaneous fermentations. We proved that MLF did not impact the concentration of shikimic acid and sucrose. Between the two LAB starters utilized, differences in MLF kinetics were observed, especially at the beginning and during MLF. Vintage also had a major impact. Because conditions for MLF were suitable, a quicker onset in both induced and spontaneous MLF was observed in the trial of the 2005 vintage, compared to the 2004 vintage. Induced MLF had no impact on the concentrations of tartaric acid and glycerol, confirming the absence of spoiling LAB.

During the vinification of Welsh Riesling grapes of the 2005 vintage, which had a high concentration of malic acid (2.23 g/L) and low pH (3.13), no significant impact was noticed between co-inoculation with LAB and inocu-

Table 2. Basic physical and chemical analyses of grape musts

Parameter (unit)	2004 Vintage		2005 Vintage	
	Welsh Riesling	Sauvignon	Welsh Riesling	Sauvignon
pH	3.18	3.14	3.13	3.22
Total acidity (g/L)	9.77	9.12	7.28	6.77
Buffer capacity (mmol/L/pH)	58.86	48.90	32.39	38.99
Volatile acidity (g/L)	0.36	0.11	0.20	0.14
Tartaric acid (g/L)	1.93	2.01	1.19	1.10
Malic acid (g/L)	5.89	5.75	2.23	2.29
Lactic acid (g/L)	0.15	0.65	0.24	0.31
Citric acid (mg/L)	635	445	368	457
Succinic acid (mg/L)	511	354	175	301
Shikimic acid (mg/L)	20	25	12	25
Sugar content (°Oe)	84	85	84	77
Glucose (g/L)	92.92	95.17	91.46	85.25
Fructose (g/L)	98.68	99.63	95.47	88.72
Sucrose (g/L)	0.81	0.39	0.77	0.46
Total phenols (mg/L)	368	250	144	219
FAN (mg N/L)	265	240	94	193
A_{420}	0.106	0.047	0.211	0.289

Table 3A. Content of higher alcohols and other volatile compounds in Welsh Riesling wines of the 2004 vintage after 30 days of vinification in 500 mL fermentation volume (average values of trials in two repetitions)

Compound (mg/L)	CON	CIN1	CIN2	IN1	IN2	CON+nutrients
Isoamyl alcohol	218.3±4.0	194.9±3.6	187.8±3.9	176.9±3.3	184.8±4.0	210.9±3.6
1-Propanol	42.2±2.5	35.7±2.3	38.1±2.5	33.4±2.0	37.9±2.6	42.9±2.3
Isobutanol	49.1±2.8	44.3±2.6	45.8±2.9	39.9±2.4	40.4±2.7	54.0±2.3
2-Phenyl ethanol	21.3±2.1	18.1±2.2	16.3±1.8	17.5±1.9	15.3±2.0	22.0±2.2
Methanol	44.2±2.2	40.9±2.4	41.0±2.3	39.2±2.5	41.2±2.2	45.1±2.6
Ethyl lactate	4.7±1.0	19.2±1.4	20.6±1.5	17.2±1.3	16.5±1.5	4.1±0.8
Methyl lactate	0.0	0.0	0.0	0.0	0.0	0.0
Isoamyl acetate	2.6±0.4	1.5±0.5	1.3±0.3	1.4±0.3	1.5±0.4	4.0±0.6
Ethyl acetate	29.5±1.2	38.7±1.6	37.4±1.4	36.7±1.6	35.3±1.5	33.9±1.4
Acetaldehyde	24.8±2.4	22.4±2.2	23.8±1.9	24.2±2.3	23.7±2.2	20.7±2.5
2-Phenyl ethylacetate	0.0	0.0	0.0	0.0	0.0	0.0
Diacetyl	0.6±0.1	2.7±0.3	2.4±0.3	1.9±0.2	1.7±0.2	0.4±0.2
Acetoin	4.3±0.3	7.3±0.4	6.8±0.3	6.4±0.3	6.2±0.2	3.2±0.1

lation. The LAB starter cultures were not inhibited by the fermentation activity of the wine yeasts. As we expected, the AF and MLF were completed almost at the same time for both the co-inoculation and the inoculation trials. Due to the more suitable MLF conditions, a quicker onset of both the induced and the spontaneous MLF was noticed for the trial of the 2005 vintage compared to the 2004. Interruptions in the onset and course of induced MLF were noticed during the vinification of the Sauvignon Blanc, 2004 vintage, and the Welsh Riesling, 2005 vintage. Microbiological analyses revealed small differences in LAB growth between the co-inoculation with LAB and inoculation. For the wines co-inoculated with LAB, slower LAB growth was observed at the beginning of MLF, but then

Table 3B. Content of higher alcohols and other volatile compounds in Sauvignon Blanc wines of the 2004 vintage after 30 days of vinification in 500 mL fermentation volume (average values of trials in two repetitions)

Compound (mg/L)	CON	CIN1	CIN2	IN1	IN2	CON+nutrients
Isoamyl alcohol	223.6±3.8	209.1±4.0	191.5±4.2	202.4±3.8	184.0±3.6	242.2±3.2
1-Propanol	36.0±2.3	33.9±2.5	31.5±2.1	34.5±2.6	30.7±2.3	31.0±2.4
Isobutanol	27.2±2.9	34.0±2.4	26.3±2.6	29.1±2.3	25.2±2.4	31.6±2.7
2-Phenyl ethanol	16.8±2.0	15.6±1.8	15.5±2.0	14.3±1.7	15.8±1.8	22.7±2.1
Methanol	34.3±2.5	34.0±2.1	33.0±2.3	33.5±2.3	32.0±2.0	33.2±2.4
Ethyl lactate	3.2±0.7	23.3±1.5	19.5±1.3	19.3±1.5	16.3±1.6	2.8±0.5
Methyl lactate	0.0	4.6±0.2	0.0	0.0	0.0	0.0
Isoamyl acetate	2.3±0.6	1.7±0.4	2.0±0.6	2.1±0.5	1.2±0.4	3.5±0.4
Ethyl acetate	27.5±1.4	23.3±1.2	22.5±1.5	26.9±1.6	29.7±1.6	32.7±1.8
Acetaldehyde	24.9±1.8	19.8±2.0	20.0±2.1	21.6±1.8	23.1±2.0	19.2±2.2
2-Phenyl ethylacetate	3.3±0.6	0.0	0.0	0.0	0.0	3.9±0.4
Diacetyl	0.4±0.1	3.7±0.3	3.5±0.3	1.9±0.1	1.8±0.2	0.3±0.1
Acetoin	3.4±0.2	7.8±0.4	7.0±0.3	4.4±0.2	4.3±0.1	2.4±0.2

Table 4A. Results of physical and chemical analyses of Welsh Riesling wines of the 2005 vintage after completion of MLF and further maturation (trials in 28 L fermentation volume)

Parameter (unit)	After completion of MLF							After further 2 months of maturation						
	CON	CIN1	CIN2	IN1	IN2	SP	Significance	CON	CIN1	CIN2	IN1	IN2	SP	Legend
pH	3.25 d	3.41 a	3.38 b	3.42 a	3.36 c	3.34 c	***	3.19 e	3.38 b	3.37 b	3.40 a	3.33 c	3.28 d	***
Total acidity (g/L)	7.47 a	5.97 c. d	5.94 d	5.72 e	6.10 c	7.25 b	***	6.95 a	5.28 d	5.24 d	5.18 d	5.60 c	6.62 b	***
Volatile acidity (g/L)	0.39 d	0.51 c	0.56 b. c	0.54 c	0.60 b	0.77 a	***	0.42 d	0.54 c	0.58 c	0.56 c	0.64 b	0.84 a	***
Tartaric acid (g/L)	0.77 c	0.67 d	0.86 a	0.87 a	0.83 b	0.76 c	***	0.71 a	0.72 a	0.73 a	0.71 a	0.73 a	0.70 a	ns
Malic acid (g/L)	1.98 a	0.42 e	0.45 d	0.37 f	0.88 c	1.87 b	***	1.78 a	0.31 c. d	0.24 e	0.29 d	0.33 c	1.13 b	***
Lactic acid (g/L)	0.49 f	2.66 c	2.81 b	2.95 a	2.17 d	0.63 e	***	1.20 e	2.89 c	2.99 b	3.02 a	2.28 d	0.99 f	***
Citric acid (mg/L)	354 a	245 e	267 d	178 f	275 c	334 b	***	338 a	227 c	245 b	139 f	212 d	201 e	***
Succinic acid (mg/L)	146 a	0 b	0 b	0 b	0 b	0 b	***	104 a	0 b	0 b	0 b	0 b	0 b	***
Shikimic acid (mg/L)	20 b	21 a	20 b	20 b	19 c	19 c	**	20 a	20 a	19 a	19 a	19 a	20 a	ns
Reducing sugar (g/L)	1.10 b	1.00 b	0.95 b	1.15 b	0.95 b	2.50 a	**	1.10 a	0.64 b	0.55 b	0.52 b	0.45 b	1.35 a	***
Total dry extract (g/L)	22.9 a	21.1 c	20.9 c. d	20.8 c. d	20.6 d	21.9 b	***	20.1 a	18.9 b	18.5 c	18.2 c. d	18.1 d	20.3 a	***
Alcohol (vol.%)	11.31 d	11.48 b. c	11.53 b	11.49 b. c	11.44 c	11.72 a	***	11.29 b	11.50 a	11.51 a	11.51 a	11.53 a	11.16 c	***
Glucose (g/L)	0.98 b	0.63 d	0.69 c	0.47 e	0.69 c	1.36 a	***	0.55 a	0.52 a. b	0.49 b. c	0.49 b. c	0.46 c	0.54 a	***
Fructose (g/L)	1.49 b	0.32 e	0.39 d	0.23 f	0.73 c	1.70 a	***	1.33 a	0.14 d	0.12 d	0.38 c	0.42 b	1.31 a	***
Sucrose (g/L)	0.54 a	0.55 a	0.52 a	0.51 a	0.54 a	0.53 a	ns	0.30 a	0.29 a	0.30 a	0.29 a	0.29 a	0.32 a	ns
Total phenols (mg/L)	102 a	89 c	92 b	80 d	77 e	71 f	***	87 a	78 b	79 b	67 c	63 d	53 e	***
FAN (mg N/L)	5 c	7 b	6 b. c	7 b	7 b. c	20 a	***	5 b	6 b	6 b	7 b	6 b	16 a	***
A_{420}	0.113 f	1.125 a	0.534 d	0.677 c	0.719 b	0.119 e	***	0.084 d	0.096 b	0.098 b	0.102 a	0.091 c	0.076 e	***

Legend: *** P≤0.001; ** P≤0.01; * P≤0.05; ns P>0.05

a higher LAB population was detected. Antagonism from *Saccharomyces bayanus* yeast species was the most probable reason. During the observation of LAB growth, it was established that the timing of inoculation had a greater impact than the LAB starter species utilized. In cases of spontaneous MLF, comparable populations of indigenous LAB instead of LAB starters were established, but the growth of indigenous LAB was slower, as expected.

Table 4B. Results of physical and chemical analyses of Sauvignon Blanc wines of the 2005 vintage after completion of MLF and further maturation (trials in 28 L fermentation volume)

Parameter (unit)	After completion of MLF							After further 2-months of maturation						
	CON	CIN1	CIN2	IN1	IN2	SP	Meaning	CON	CIN1	CIN2	IN1	IN2	SP	Significance
pH	3.30 c	3.51 a	3.51 a	3.50 a	3.51 a	3.44 b	***	3.32 c	3.53 b	3.53 b	3.53 b	3.54 a. b	3.55 a	***
Total acidity (g/L)	7.40 a	5.58 c	5.50 c	5.34 d	5.46 c. d	6.50 b	***	6.97 a	5.18 c	5.03 d	5.06 c. d	4.99 d	5.88 b	***
Volatile acidity (g/L)	0.35 e	0.41 d	0.47 c	0.46 c. d	0.53 b	0.65 a	***	0.37 d	0.44 c	0.48 c	0.48 c	0.55 b	0.73 a	***
Tartaric acid (g/L)	0.84 a	0.86 a	0.81 b	0.86 a	0.76 c	0.67 d	***	0.69 b	0.60 d	0.63 c	0.73 a	0.66 b	0.63 c	***
Malic acid (g/L)	2.01 b	0.00 c	0.00 c	0.00 c	0.00 c	2.04 a	***	1.57 a	0.00 c	0.00 c	0.00 c	0.00 c	1.36 b	***
Lactic acid (g/L)	0.95 f	3.70 b	3.87 a	3.46 c	3.23 d	1.26 e	***	2.64 d	3.86 b. c	3.97 a	3.84 c	3.88 b	1.96 e	***
Citric acid (mg/L)	450 a	167 d	56 f	111 e	212 c	401 b	***	390 a	123 d	0 e	0 e	156 c	246 b	***
Succinic acid (mg/L)	249 a	0 c	0 c	0 c	0 c	47 b	***	0 a	0 a	0 a	0 a	0 a	0 a	
Shikimic acid (mg/L)	31 a	30 b. c	30b. c	30 b. c	29 c	31 a	***	32 a	32 a	32 a	32 a	32 a	30 b	**
Reducing sugar (g/L)	1.25 a	0.82 b	0.91 b	0.70 b	0.75 b	1.35 a	***	1.10 a	0.74 c	0.80 b. c	0.63 c	0.68 c	1.02 a. b	*
Total dry extract (g/L)	25.3 a	24.2 b	23.5 c	22.9 d	22.6 d	23.3 c	***	23.9 a	21.2 b	21.1 b. c	20.8 c. d	20.6 d. e	20.3 e	***
Alcohol (vol.%)	10.23 c	10.37 a	10.37 a	10.33 a. b	10.31 b	10.07 a	***	10.17 c	10.31 a. b	10.33 a	10.30 a. b	10.27 b	10.21 a	***
Glucose (g/L)	1.04 b	0.65 d	0.63 d	0.65 d	0.69 c	1.94 a	***	0.49 c. d	0.46 d	0.46 d	0.54 a	0.50 b. c	0.53 a. b	***
Fructose (g/L)	0.87 b	0.35 d	0.36 d	0.40 c	0.42 c	3.14 a	***	0.85 b	0.21 c	0.21 c	0.19 c	0.18 c	1.38 a	***
Sucrose (g/L)	0.34 a	0.33 a. b	0.33 a. b	0.30 b. c	0.29 b. c	0.27 c	*	0.25 a	0.26 a	0.24 a	0.26 a	0.26 a	0.25 a	ns
Total phenols (mg/L)	147 a	131 b	128 c	117 d	115 d	109 e	***	124 a	114 b	110 c	98 e	101 d	89 f	***
FAN (mg N/L)	12 c	19 b	18 b	19 b	20 b	51 a	***	10 d	17 b. c	15 c	17 b. c	19 b	42 a	***
A_{420}	0.140 f	0.275 d	0.198 e	0.317 c	0.730 b	0.752 a	***	0.119 d	0.139 b	0.125 c	0.126 c	0.169 a	0.106 e	***

Legend: *** P≤0.001; ** P≤0.01; * P≤0.05; ns P>0.05

Table 5A. Content of higher alcohols and other volatile compounds in Welsh Riesling wines of the 2005 vintage after completion of MLF and further maturation (trials in 28 L fermentation volume)

Compound (mg/L)	After completion of MLF							After further 2-months of maturation						
	CON	CIN1	CIN2	IN1	IN2	SP	Meaning	CON	CIN1	CIN2	IN1	IN2	SP	Significance
Isoamyl alcohol	121.2 c	117.5 d	127.2 b	131.0 a	116.4 e	111.4 f	***	127.8 b	118.8 e	123.6 c	133.3 a	118.7 e	120.8 d	***
1-Propanol	38.9 c	39.9 b	40.1 b	40.7 a	40.3 a. b	33.3 d	***	41.5 c	40.3 c	38.9 b	41.4 a	41.3 a	37.1 d	***
Isobutanol	19.2 d	20.2 c	22.5 b	23.7 a	20.5 c	15.9 e	***	20.1 c	20.4 c	21.8 b	23.9 a	20.5 c	17.6 d	***
2-Phenyl ethanol	9.4 a	6.6 c	9.0 a. b	7.9 b	6.6 c	6.3 c	***	9.1 a	8.0 a	8.5 a	9.3 a	8.8 a	8.7 a	ns
Methanol	38.1 d	37.0 f	38.9 b	38.5 c	37.7 e	39.4 a	***	39.0 c	38.2 d	37.9 d	40.2 b	38.9 c	41.5 a	***
Ethyl lactate	4.9 a	2.6 d	2.0 e	4.5 b	3.5 c	2.0 e	***	1.8 c	4.3 b	4.1 b	4.8 a	4.0 b	2.0 c	***
Methyl lactate	0.0 b	4.3 a	0.0 b	4.1 a	0.0 b	0.0 b	***	0.0 b	0.0 b	0.0 b	4.4 a	0.0 b	0.0 b	***
Isoamyl acetate	2.9 c	3.7 b	3.7 b	4.0 b	3.8 b	4.8 a	***	3.2 c	3.4 b. c	3.3 b. c	3.6 b	3.6 b	4.8 a	***
Ethyl acetate	33.5 f	42.6 d	43.5 b	42.9 c	40.8 e	49.7 a	***	39.7 e	40.7 d	35.9 f	41.6 c	41.9 b	49.6 a	***
Acetaldehyde	37.2 a	31.4 d	31.6 d	27.5 e	34.1 c	35.7 b	***	36.0 a	28.8 c	27.5 d	25.8 e	31.2 b	36.7 a	***
2-Phenyl ethylacetate	0.0 a	0.0 a	0.0 a	0.0 a	0.0 a	0.0 a	ns	0.0 a	0.0 a	0.0 a	0.0 a	0.0 a	0.0 a	***
Diacetyl	2.5 c	3.1 a. b	3.1 a. b	3.4 a	2.8 b. c	0.6 d	***	0.5 d	2.4 b	2.4 b	3.2 a	2.9 a. b	1.4 c	***
Acetoin	6.3 b	7.5 a	6.2 b	8.3 a	6.4 b	6.5 b	*	6.4 b	6.7 b	6.2 b	8.6 a	8.4 a	6.9 b	***

Legend: *** P≤0.001; ** P≤0.01; * P≤0.05; ns P>0.05

Table 5B. Content of higher alcohols and other volatile compounds in Sauvignon Blanc wines of the 2005 vintage after completion of MLF and further maturation (trials in 28 L fermentation volume)

Compound (mg/L)	After MLF completion							After further 2-months of maturation						
	CON	CIN1	CIN2	IN1	IN2	SP	Meaning	CON	CIN1	CIN2	IN1	IN2	SP	Significance
Isoamyl alcohol	146.2 e	175.8 b	159.1 c	177.5 a	148.5 d	105.4 f	***	141.2 d	184.7 a	115.4 f	174.8 b	160.5 c	125.6 e	***
1-Propanol	24.7 c	25.8 b	24.8 c	40.2 a	40.1 a	18.2 d	***	42.6 b	43.2 a	39.7 c	39.7 c	42.6 b	24.6 d	***
Isobutanol	15.6 e	18.7 c	17.1 d	31.2 a	23.3 b	16.9 d	***	20.5 c	30.0 a	20.3 c	30.7 a	24.9 b	20.2 c	***
2-Phenyl ethanol	18.1 c	24.9 a	23.8 a. b	23.1 b	17.0 c	11.1 d	***	16.0 c	22.2 a	8.5 e	18.7 b	16.1 c	12.8 d	***
Methanol	39.3 e	44.7 c	42.6 d	46.0 a	45.2 b	45.4 b	***	45.4 d	49.8 a	37.5 e	45.9 c	48.9 b	48.8 b	***
Ethyl lactate	9.7 c	5.9 d	9.7 c	10.9 a	10.4 b	6.0 d	***	2.6 e	3.4 d	4.1 c	6.4 a	6.1 a	5.7 b	***
Methyl lactate	0.0 c	0.0 c	0.0 c	3.8 b	3.5 b	4.1 a	***	4.4 a	0.0 b	0.0 b	0.0 b	0.0 b	0.0 b	***
Isoamyl acetate	2.9 a	3.0 a	2.8 a	1.6 c	3.1 a	2.2 b	***	3.2 b	2.2 c	3.6 a	1.3 d	2.6 c	2.3 c	***
Ethyl acetate	36.1 a	36.1 a	34.3 b	22.2 e	32.4 c	27.6 d	***	34.8 b	28.9 e	41.3 a	17.9 f	30.9 d	31.6 c	***
Acetaldehyde	16.7 b	7.7 d	7.4 d	11.3 c	20.3 a	19.4 a	***	25.4 b	18.3 e	29.9 a	19.9 d	21.5 c	25.1 b	***
2-Phenyl ethylacetate	0.0 a	0.0 a	0.0 a	0.0 a	0.0 a	0.0 a	ns	0.0 b	0.0 b	0.0 b	0.8 a	0.0 b	0.0 b	***
Diacetyl	0.5 b	0.7 b	0.4 b	0.6 b	1.3 a	0.6 b	*	0.3 d	3.6 a	2.5 b	0.5 d	1.8 c	3.2 a	***
Acetoin	6.1 b	6.2 b	7.0 b	6.2 b	8.5 a	6.4 b	***	6.3 c	6.2 c	6.3 c	10.3 a	6.8 b. c	7.5 b	***

Legend: *** P≤0.001; ** P≤0.01; * P≤0.05; ns P>0.05

The observation of MLF kinetics revealed the degradation of three organic acids in the following sequence: succinic, malic and citric acid. In contrast to our forecast, the degradation of succinic acid was noticed during both induced and spontaneous MLF. Complete degradation of succinic acid was confirmed in cases of complete MLF. Citric acid was completely degraded by LAB during the vinification of the 2004 vintage, whereas it was not confirmed during vinifications of the 2005 vintage.

Both varieties (Welsh Riesling and Sauvignon Blanc) are well known as wines with higher acidity because of a higher proportion of malic acid (especially in 2004), which often results in less harmonious wines. During the vinification of the Sauvignon Blanc 2005 vintage, which had lower concentrations of malic acid and a higher pH, a quicker onset and shorter duration for MLF were characteristic of LAB co-inoculation, as was seen in the other varietal. Due to suitable conditions for MLF (specifically, higher pH), a quicker onset of spontaneous MLF in the control sample was observed. Interruptions in the onset and course of induced MLF were not seen in any of the vinifications, despite lower FAN content in both grape musts of the 2005 vintage, especially the Welsh Riesling. LAB starter culture additions did not inhibit the fermentation activity of wine yeasts.

As expected, AF and MLF were completed almost at the same time in both the grape musts that were co-inoculated or received only inoculation. One strain of LAB utilized in the grape must performed significantly better in sensory terms, compared to the other strain and when added to the wine after AF. The total malic acid conversion in all four wines that underwent MLF was accompanied by citric acid conversion to diacetyl and acetoin. The reduction of citric acid in young wines was greater in the cases of co-inoculation, and therefore the levels of diacetyl and acetoin were higher as well. In these samples (Welsh Riesling inoculated with LAB1), higher concentrations of isoamyl alcohol, 1-propanol and isobutanol were observed. During the two months of aging, the citric acid level was further reduced to different extents, while the succinic acid level remained stable.

Differences in the LAB starters utilized are expressed in the levels of most of the higher alcohols analyzed (isoamyl alcohol, 1-propanol and 2-phenylethanol), in the volatile MLF products (ethyl lactate, ethyl acetate, acetaldehyde and volatile acids) and lactic acid. On the other hand, the impact of the timing of LAB addition is expressed in the levels of citric acid, reducing sugars, total dry extract, glucose and total phenols. Analyses of matured wines showed the differences expected in the majority of parameters observed. The pH values over 3.50 in the wines were not optimal, and special care (a sulphur addition) was needed to prevent wine spoilage.

The Welsh Riesling and Sauvignon Blanc wines produced by MLF contained less total dry extract and reducing sugars in comparison to the control sample. In spite of this,

Table 6A. Levels of free amino acids (AA) in Welsh Riesling grape must and wines depending on the type of vinification, and in control samples (2005 vintage, trials in 28 L fermentation volume)

AA (mg/L) (by turns in must)	Grape must	CON			CIN1			CIN2			IN1			IN2			SP		
		1/3 AF	2/3 AF	End of MLF	1/3 AF	2/3 AF	End of MLF	1/3 AF	2/3 AF	End of MLF	1/3 AF	End of AF	End of MLF	1/3 AF	End of AF	End of MLF	1/3 AF	2/3 AF	End of MLF
Asp (12.)	16.5	/	/	2.6	/	/	4.7	/	/	7.1	/	/	8.9		1.2	8.7	5.0		7.2
Glu (10.)	34.0	12.5	4.4	12.6	3.8	5.3	21.3	3.3	5.3	19.3	2.8	7.4	22.6	4.3	6.8	20.0	29.3	10.6	21.6
Asn (14.)	11.4	6.0	11.4	14.3	2.3	7.3	11.6	1.8	7.9	12.1	1.6	8.6	13.4	2.3	5.0	8.5	11.9	2.6	12.0
Ser (5.)	47.3	0.8	0.6	2.2	0.7	0.6	4.3	0.6	0.6	3.7	0.5	1.2	4.5	0.2	1.3	3.8	43.6	1.1	4.0
Gln	31.4	4.3	5.5	3.1	3.3	6.2	5.1	1.5	5.6	4.2	2.0	5.0	4.7	1.4	3.6	3.7	41.0	47.8	20.8
His (8.)	35.2	/	/	3.3	/	/	6.3	/	8.1	5.6	/	/	7.4	6.2	/	8.1	37.3	/	5.9
Gly (15.)	7.5	3.5	/	2.6	0.6	0.4	5.6	/	0.2	4.5	0.2	0.5	2.7	0.6	2.3	4.1	6.9	6.8	8.6
Thr (4.)	48.5	/	/	/	/	/	1.7	/	/	1.0	/	/	/	/	/	2.7	44.3	/	0.9
Arg (1.)	828.1	19.8	9.5	14.3	17.1	12.3	30.5	10.7	9.7	21.8	9.2	11.3	26.3	18.0	13.5	26.4	792.1	331.5	166.5
Ala (2.)	131.5	4.1	8.9	14.4	2.0	9.2	19.5	1.6	10.1	18.6	1.1	11.6	21.2	2.1	9.3	17.5	123.0	27.0	30.3
Tyr (13.)	13.6	/	/	/	/	/	5.6	/	/	4.6	/	/	7.4	/	0.8	6.6	11.5	2.5	8.8
Val (3.)	72.7	37.0	25.2	30.5	41.4	27.1	26.9	36.5	29.7	34.4	44.2	29.3	37.3	45.5	29.2	36.8	84.3	60.7	41.8
Met		/	0.3	2.8	/	0.6	6.1	/	1.1	4.3	/	1.2	5.0	/	1.0	4.0	/	/	4.0
Trp (16.)	5.9	/	/	/	/	/	/	/	/	/	/	/	/	/	/	/	5.4	/	/
Phe (6.)	42.9	/	0.6	6.8	/	0.6	15.2	/	0.1	12.3	/	1.6	17.2	/	3.3	11.8	38.5	/	13.6
Ile (11.)	19.4	/	/	1.6	/	/	6.7	/	/	4.2	/	/	7.4	/	/	5.2	17.7	/	3.4
Leu (9.)	32.4	/	2.4	12.7	/	3.3	28.3	/	3.2	22.0	/	/	28.4	/	/	26.1	4.7	/	22.6
Lys (17.)	2.8	7.0	3.2	15.1	2.2	4.4	35.0	2.9	4.2	27.3	2.1	5.6	37.6	3.2	10.3	35.6	20.2	/	25.4
Pro (7.)	36.3	274.0	298.5	308.8	239.7	294.9	316.2	263.2	288.5	306.2	279.6	290.8	309.4	245.3	248.3	263.4	51.2	123.5	265.6
Σ	1417.5	369.0	370.5	447.7	313.3	372.3	550.7	322.2	374.2	513.1	343.4	374.2	561.3	329.2	335.8	492.9	1367.7	614.0	663.0
Σ AK-Pro	1381.2	95.0	72.0	138.9	73.5	77.3	234.5	59.0	85.7	206.8	63.8	83.4	251.9	83.9	87.5	229.5	1316.5	490.5	397.4
Asp. Glu. Ser. Arg. Thr. Ala	1105.9	37.2	23.5	46.0	23.7	27.3	82.0	16.3	25.7	71.5	13.6	31.5	83.4	24.7	32.1	79.1	1037.3	370.2	230.5

Table 6B. Levels of free amino acids (AA) in Sauvignon Blanc grape must and wines depending on the type of vinification, and in control samples (2005 vintage trials in 28 L fermentation volume)

AA (mg/L) (by turns in must)	Grape must	CON			CIN1			CIN2			IN1			IN2			SP		
		1/3 AF	2/3 AF	End of MLF	1/3 AF	2/3 AF	End of MLF	1/3 AF	2/3 AF	End of MLF	1/3 AF	End of AF	End of MLF	1/3 AF	End of AF	End of MLF	1/3 AF	2/3 AF	End of MLF
Asp	/	/	/	8.0	/	1.4	18.5	/	2.3	17.8	/	0.5	18.8	/	2.4	19.4	/	/	15.3
Glu (7.)	41.6	5.4	9.1	22.4	2.3	7.8	39.7	4.1	10.4	37.6	1.7	8.8	36.5	4.2	12.2	39.4	22.0	21.2	37.1
Asn (13.)	5.9	1.1	3.9	7.1	/	1.1	9.0	0.8	1.9	7.6	/	1.4	8.0	0.6	3.4	8.7	/	1.0	6.7
Ser (8.)	41.1	0.0	1.3	4.4	/	1.1	9.1	0.2	1.5	8.0	/	1.5	9.1	0.1	1.9	8.6	5.8	1.4	7.6
Gln (2.)	274.1	5.0	4.7	2.9	0.4	2.6	4.9	2.1	3.0	5.2	0.1	1.4	4.3	1.5	2.6	5.3	66.2	16.8	11.5
His	/	/	/	8.9	/	4.1	14.9	/	6.0	10.4	/	2.4	9.8	/	5.7	11.4	/	/	11.4
Gly (15.)	2.6	/	/	7.7	/	3.9	13.3	0.1	3.6	11.5	0.1	4.9	14.7	0.1	4.4	12.8	9.6	8.5	14.4
Thr (6.)	49.1	4.4	7.4	2.4	4.2	0.4	7.3	4.3	0.8	5.4	3.6	0.5	7.5	5.4	0.4	6.1	/	5.5	3.8
Arg (1.)	1098.2	/	20.9	31.4	4.6	11.8	37.6	9.9	17.8	38.9	2.3	11.3	34.4	9.5	17.8	43.8	966.9	331.9	274.3
Ala (3.)	175.6	7.4	11.0	15.7	5.7	10.8	29.5	7.5	10.9	25.7	3.4	11.4	30.3	7.1	12.0	28.0	102.8	20.3	36.5
Tyr (12.)	6.7	/	0.7	5.5	/	0.4	16.4	/	0.6	11.0	/	1.8	15.1	/	3.1	15.1	/	/	12.3
Val (4.)	82.0	27.2	31.0	31.2	23.4	24.4	47.7	30.6	31.4	25.7	24.6	27.9	44.9	31.8	31.6	45.4	67.1	42.5	16.3
Met	/	/	1.6	4.2	6.4	1.5	9.1	/	1.7	8.9	/	1.6	9.5	/	1.9	9.7	/	/	4.6
Trp (14.)	3.8	/	/	/	/	/	5.4	/	/	/	/	/	3.2	/	/	4.4	/	/	/
Phe (9.)	38.9	/	3.3	12.2	/	3.2	27.0	/	5.5	24.8	/	5.7	26.9	/	6.8	27.5	/	/	21.3
Ile (11.)	7.1	/	/	3.7	/	0.2	12.3	/	1.3	10.3	/	1.0	11.3	/	0.9	11.7	/	/	6.7
Leu (10.)	21.2	/	9.0	20.8	0.7	8.1	46.3	1.5	10.3	41.8	0.1	9.5	45.9	1.1	12.4	47.2	/	3.8	35.6
Lys	/	2.3	13.6	28.8	3.5	14.0	57.9	5.6	18.2	54.0	2.4	15.4	56.8	4.9	20.2	62.7	/	/	19.6
Pro (5.)	77.3	429.5	456.6	482.5	410.7	458.6	399.1	390.6	396.9	370.6	366.0	352.8	381.9	378.2	373.7	393.3	89.7	246.7	351.7
Σ	1925.3	482.2	574.1	699.5	461.8	555.4	805.0	457.3	524.0	715.3	404.5	459.7	769.0	444.6	513.2	800.3	1330.0	699.7	886.7
Σ AK-Pro	1848.0	52.7	117.5	217.0	51.1	96.8	405.9	66.7	127.1	344.7	38.5	106.9	387.1	66.4	139.5	407.0	1240.3	453.0	535.0
Asp Glu Ser. Arg Thr Ala	1405.7	17.2	49.7	84.2	16.8	33.3	141.6	26.0	43.6	133.4	11.1	34.0	136.7	26.4	46.6	145.3	1097.4	380.4	374.6

the concentrations of diacetyl and ethyl lactate were comparable in the induced vinifications of the same cultivar in both vintages. In wines of the 2004 vintage, analysis showed higher concentrations of acetaldehyde. There was less acetaldehyde in the wines with induced MLF than in the control vinifications. On other hand, the concentrations of higher alcohols and volatile compounds were more affected by cultivar and vintage than by the timing of inoculation and the LAB starters utilized. The impact of LAB starters was also revealed in the chemical composition of wine before and after two months of aging, not just during the course of MLF. We established that the influence of the timing of inoculation of LAB starters on yeasts and LAB population kinetics is due to antagonism. On the basis of the timing of LAB inoculation, the experiment showed differences in the population numbers for yeasts and LAB. Meanwhile, differences in the population numbers due to the species of LAB starters were negligible.

Different concentrations of individual amino acids in grape musts for the two varietals were expected (Tables 6A and 6B). Amino acid kinetics during MLF showed significant reductions in the majority of amino acids present in the grape must after one third of AF, but they increased later on. Higher concentrations of individual amino acids were determined in young wines and grape musts. Sauvignon Blanc grape must was an exception, because of the high concentration of arginine and lower concentration of proline. Among the 21 free amino acids, the presence of lysine was determined in young wines, with the exception of Welsh Riesling. In young wines, we proved the impact of MLF on amino acid composition. The vinifications were sampled (at 15° to 17°C) at the following benchmarks: one third of AF (five days), two thirds of AF (nine days), end of AF (15 days for inoculation trials), and the end of MLF (three weeks later, a total of 42 days).

Higher concentrations of individual amino acids were found in the young wines as in the grape must, which contained 1.9 g/L (Sauvignon Blanc) and 1.4 g/L (Welsh Riesling) of total free amino acids. The amino acids most represented in the grape musts (Asp, Glu, Ser, Arg, Thr and Ala) represent 73.0% and 78.1% of total free amino acids in the Sauvignon Blanc and the Welsh Riesling, respectively, while, as expected, their levels were lower in the wines produced and comparable to the levels in the MLF trials conducted (Figures 2A and 2B).

Winemakers systematically supplement grape musts with diammonium phosphate (DAP) to prevent nitrogen-related fermentation problems. The timing of the nitrogen additions influenced the biomass yield, the fermentation performance, the patterns of ammonium and amino

Figure 2A. Levels of all free amino acids (mg/L) in trial wines after the completion of MLF (2005 vintage)

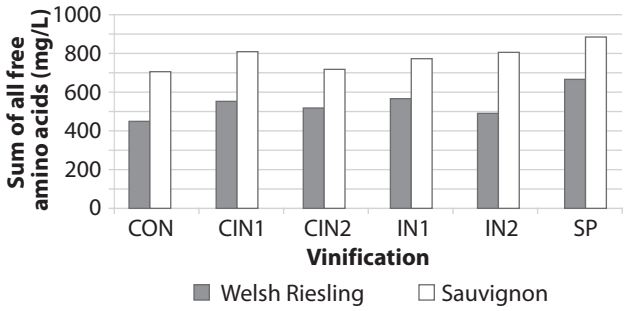
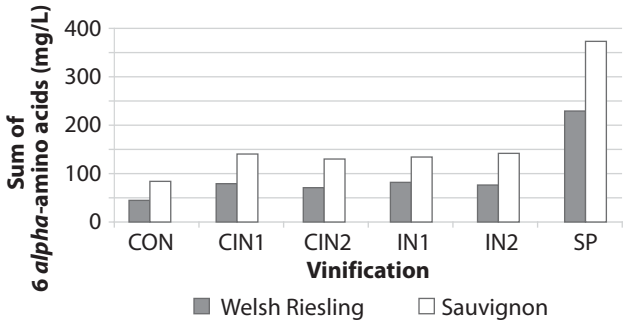


Figure 2B. Levels of six *alpha*-amino acids (mg/L) in trial wines after the completion of MLF (2005 vintage)



acid consumption, and the production of secondary metabolites. These nitrogen additions induced a nitrogen-repressed situation in the cells, and this situation determined which nitrogen sources were selected. Nitrogen assimilation also depends on fermentation temperature, which is an important factor determining the utilization of nitrogen sources during the fermentation of grape juice, and influences the quantity and the quality of the nitrogen requirement. Ammonium and glutamine, the preferred sources for biomass production, are consumed at a slower rate at low temperatures. Likewise, amino acids that are only taken up under de-repressed conditions (e.g., arginine, alanine, asparagine) are consumed at a faster rate at low temperatures.

The first free amino acid was arginine (0.8 mg/L), which represented 57.0% of total free amino acids in the Sauvignon Blanc and 58.4% in the Welsh Riesling. The second highest free amino acid in Sauvignon Blanc was glutamine (274 mg/L), but in Welsh Riesling the second highest was unexpectedly alanine (132 mg/L), which was noticeably consumed after one third of AF. Towards the end of AF, yeast cell lysis resulted in the release of nutrients that favour the growth of LAB and consequently its activity. It is known that a slow AF will inevitably lead to sluggish or stuck MLF. Therefore it is essential that all the nutrients needed by LAB are present in the grapes before crushing.

Table 7. Results of sensory analysis of wines by the 20-point Buxbaum system after the completion of MLF

Welsh Riesling	After 42 days of vinification					
	CON	CIN1	CIN2	IN1	IN2	SP
Appearance	2.0	1.9	1.8	1.8	1.9	1.9
Colour	1.6	1.9	1.8	1.8	1.8	1.9
Odour	2.9	3.4	3.3	3.2	3.1	3.5
Taste	4.4	4.9	5.1	5.0	4.8	5.0
Harmony	4.4	4.7	5.1	4.9	4.6	4.9
Total	15.2	16.8	17.1	16.8	16.2	17.1
Sauvignon	**After 42 days of vinification**					
	CON	CIN1	CIN2	IN1	IN2	SP
Appearance	1.7	1.9	1.8	1.9	1.9	1.8
Colour	1.8	1.9	1.8	1.9	1.8	1.9
Odour	3.2	3.6	3.2	3.5	3.3	3.4
Taste	4.6	4.7	4.9	5.0	5.0	4.8
Harmony	4.5	4.6	4.7	5.1	4.8	4.9
Total	16.0	16.9	16.5	17.3	16.8	16.9

Among 18 determined free amino acids in Welsh Riesling, a significantly higher level of lysine was determined in young wines (15 to 38 mg/L) compared to grape must (2.8 mg/L). After completion of MLF, wines contained from 10.3% (CON) to 34.8% (SP) free amino acids. The most represented amino acids in young wines were Asp, Glu, Ser, Arg, Thr and Ala. After completion of MLF, their content varied from 46 mg/L (CON) to 230.5 mg/L (SP). In the case of inoculation, their content was higher (27.0% to 16.0%) in comparison to the co-inoculation trials (14.9% to 13.9%). The starters utilized had a significant impact on the free amino acid composition of young wines.

Sensorially, the wines produced through MLF were considered to have more mouthfeel, and to be fresher and more harmonious than the control wines, where spontaneous MLF was also completed. The results of sensory analysis (Table 7) confirmed the results obtained through chemical analyses.

The production of diacetyl (2,3-butanedione) is known to impart buttery or nutty aromas. Comparative results of diacetyl content are shown in Figure 3. This compound has sensory thresholds of 0.2 mg/L in Chardonnay, 0.9 mg/L in Pinot Noir and 2.8 mg/L in Cabernet Sauvignon. When the concentration exceeds 5 mg/L, it is considered spoilage. Overproduction of particular esters could be responsible for a pleasant fruity nose. Comparative results for ethyl lactate, isoamyl alcohol and its acetate esters are presented in Figures 4A, 4B and 4C. LAB are also responsible for the liberation of monoterpenes, which are often present in grapes in non-volatile, flavourless forms, but the ß-glucosidase activity of *O. oeni* LAB can free the volatile free form, and *O. oeni* can also metabolize acetaldehyde, as well as other aldehydes, to produce ethanol and acetic

Figure 3. Comparative results of diacetyl content (mg/L) in wines after the completion of MLF (for both varietals and vintages)

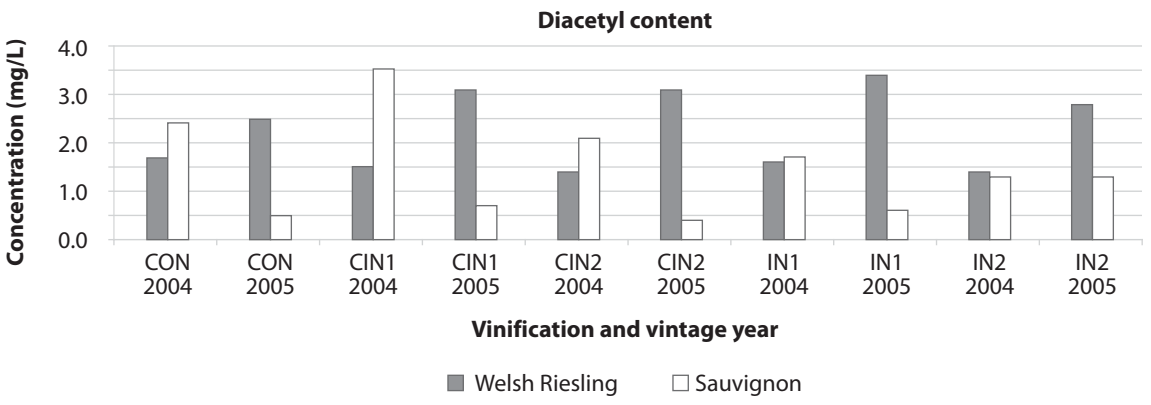


Figure 4A. Comparative results of ethyl lactate content (mg/L) in wines after the completion of MLF (for both varietals and vintages)

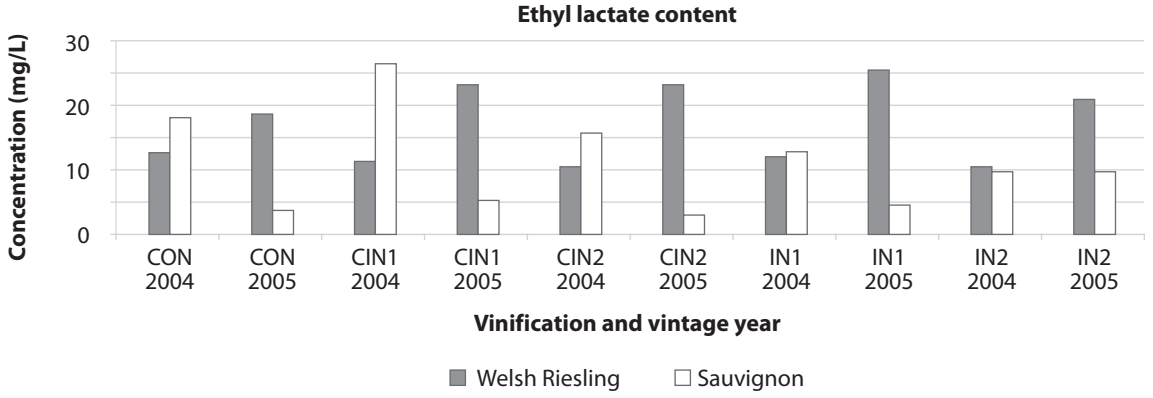


Figure 4B. Comparative results of isoamyl alcohol content (mg/L) in wines after the completion of MLF (for both varietals and vintages)

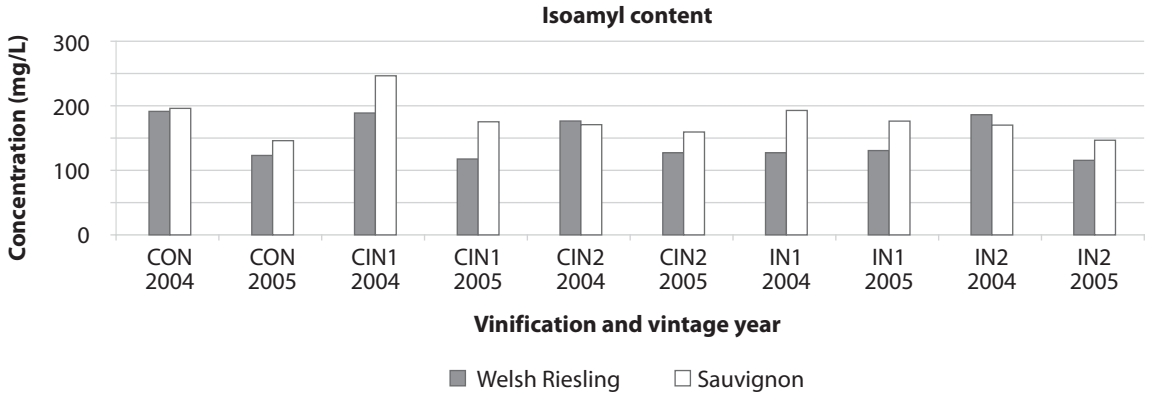
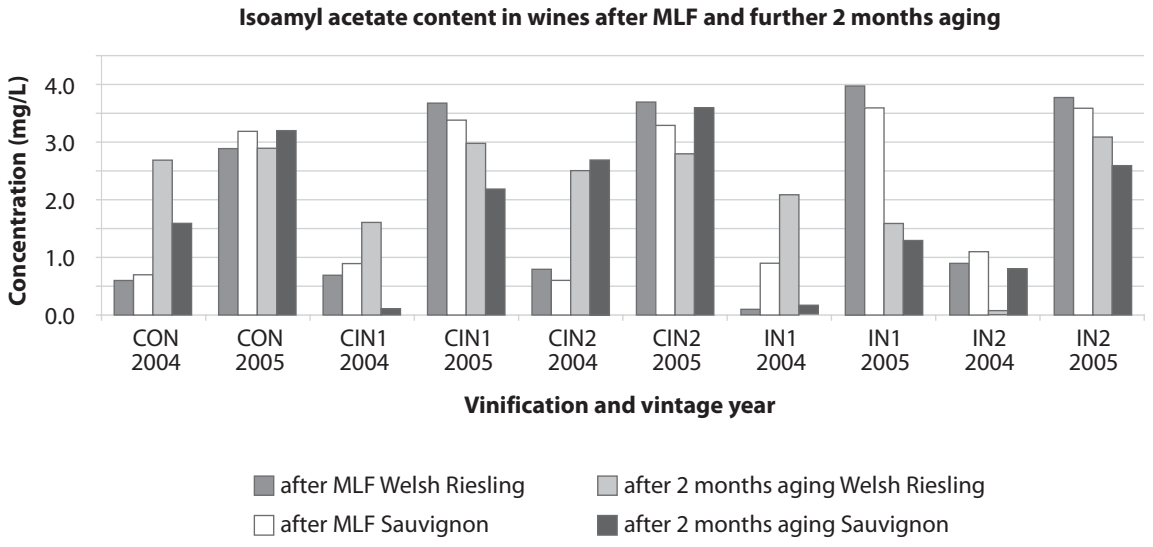


Figure 4C. Comparative results of isoamyl acetate content (mg/L) in wines after the completion of MLF and further wine maturation (for both varietals and vintages)



acid. Depending on the case and extent, this can be desirable (as acetaldehyde causes an off-aroma) or undesirable (as acetaldehyde also plays a role in colour development). MLF also increases body or mouthfeel, possibly due to the production of polyols. A comparison of the results obtained for acetaldehyde and glycerol content is shown in Figures 5 and 6, respectively.

Despite the fact that concentrations of diacetyl and ethyl lactate in wines of the same cultivar in both vintages after

Figure 5. Comparative results of acetaldehyde content (mg/L) in wines after the completion of MLF (for both varietals and vintages)

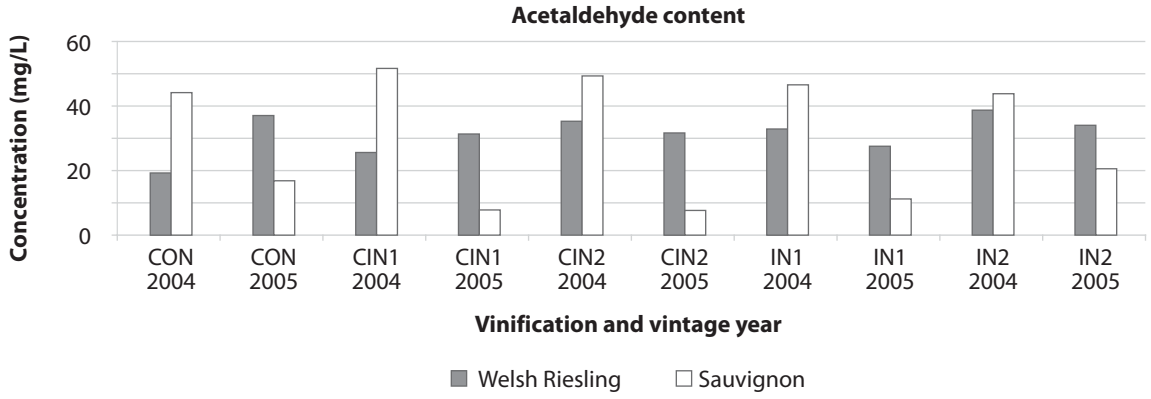
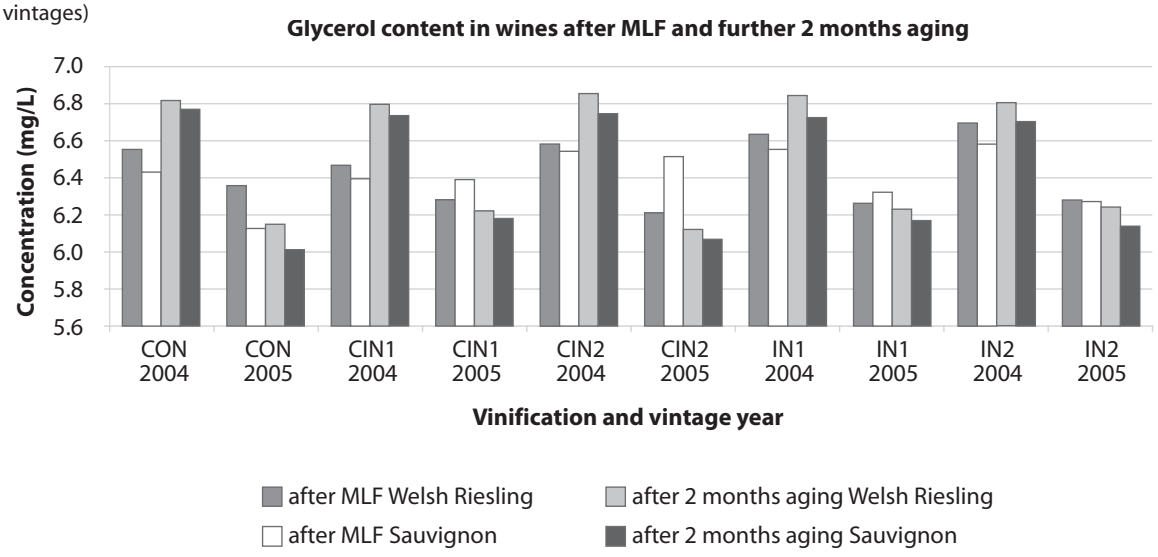


Figure 6. Comparative results of glycerol content (g/L) in wines after the completion of MLF and further wine maturation (both varieties and vintages)



induced MLF were comparable, the wines of the 2004 vintage had higher concentrations of acetaldehyde. Wines with induced MLF had less acetaldehyde than the control wines. On the other hand, the concentration of higher alcohols and volatile compounds was more affected by cultivar and vintage than by the timing of inoculation and the LAB starters utilized.

Conclusion

Results of our research proved that the malolactic fermentation of Welsh Riesling and Sauvignon Blanc significantly improved the chemical and sensorial parameters of the wines produced. For numerous chemical parameters, there was no significant difference between the two strains of LAB utilized, although one strain produced better sensorial characteristics (e.g., varietal character, fruitiness and overall harmony).

The vintage and variety also have an important influence on MLF. The differences in free amino acid utilization during MLF were significant. When the level of free amino acids and/or free amino nitrogen is low, we highly recommend the addition of nitrogen compounds to avoid stuck alcoholic and/or sluggish MLF. We can confirm that MLF is recommended in the production of both varietals, especially Welsh Riesling, which is the most representative of cool-climate white wines. The complexities of aroma, mouthfeel and roundness, as well as the acidity balance (all consequences of MLF) are the most important factors that defined the sensory quality of wines that underwent MLF.

References

Alexandre, H., P. J. Costello, F. Remize, J. Guzzo, and M. Guilloux-Benatier. 2004. *Saccharomyces cerevisiae - Oenococcus oeni* interactions in wine: Current knowledge and perspectives. *International Journal of Food Microbiology*. 93:141-154.

Arias-Gil, M., T. Garde-Cerdán, and C. Ancín-Azpilicueta. 2007. Influence of addition of ammonium and different amino acid concentrations on nitrogen metabolism in spontaneous must fermentation. *Food Chemistry*. 103:1312-1318.

Bartowsky, E. J. 2005. Oenococcus oeni and malolactic fermentation – moving into the molecular arena. *Australian Journal of Grape and Wine Research*. 11:174-187.

Bauer, R., and L. M. T. Dicks. 2004. Control of malolactic fermentation in wine. A review. *South African Journal of Enology and Viticulture*. 25:74-88.

Boulton, R. B., V. L. Singleton, L. F. Bisson, and R. E. Kunkee. 1996. *Principles and Practices of Winemaking*. Chapman&Hall, New York.

Cañas, P. M. I., E. G. Romero, S. G. Alonso, M. F. Gonzáles, and M. Ll. P. Herreros. 2007. Amino acids and biogenic amines during spontaneous malolactic fermentation in Tempranillo red wines. *Journal of Food Composition and Analysis*, doi:10.1016/j.jfca.2007.11.002.

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

Ferreira, R. B., M. A. Picarra-Pereira, S. Monteiro, V. B. Loureiro, and A. R. Teixeira. 2002. Wine proteins. *Trends in Food Science & Technology*. 12:230-239.

Fleet, G. H. 2002. *Wine microbiology and biotechnology*. Taylor & Francis, London.

Henderson, J. W., R. D. Ricker, B. A. Bidlingmeyer, and C. Woodward. 2000. *Rapid, Accurate, Sensitive, and Reproducible HPLC Analysis of Amino Acids. Amino Acid Analysis Using Zorbax Eclipse-AAA Columns and the Agilent 1100 HPLC*. Agilent Technologies, Palo Alto, CA.

Jackson, R. S. 2000. *Wine Science: Principles, Practice, Perception. Second edition*. Academic Press, San Diego, CA.

Klein, H., and R. Leubolt. 1993. Ion-exchange high-performance liquid chromatography in the brewing industry. *Journal of Chromatography* A. 650:259-270.

Košmerl, T., and M. Kordiš Krapež. 1996. *Aroma compounds in wine*. V: P. Raspor, D. Pitako, and I. Hočevar (Eds). 1. Slovenian food and nutrition congress, Bled, April 21-25, 1996. Technology, food, health: Book of abstracts. Ljubljana: Association of Food and Nutrition Specialists of Slovenia.

Košmerl, T., and M. Kač. 2004. *Basic chemical analyses of grape must and wine*. 2nd edition. Ljubljana, University of Ljubljana, Biotechnical Faculty, Department of Food Science and Technology.

Liu, S.-Q. 2002. Malolactic fermentation in wine – beyond deacidification. *Journal of Applied Microbiology*. 92(4):589-601.

Lonvaud-Funel, A. 1999. Lactic acid bacteria in the quality improvement and depreciation of wine. *Antonie van Leeuwenhoek international journal of general and molecular microbiology*. 76(1-4):317-331.

Nicolini, G., G. Versini, L. Corradin, R. Larcher, C. Beretta, A. Olivari, and E. Eccli. 2004. Misura dell'azoto prontamente assimilabile dal liecito nei mosti d'uva ed esempi di applicazione. *Rivista di Viticoltura e di Enologia*. 1-2:13-27.

Ough, C. S., and M. A. Amerine. 1988. *Methods for analysis of musts and wines*. John Wiley & Sons, New York.

Remize, F., Y. Augagneur, M. Guilloux-Benatier, and J. Guzzo. 2005. Effect of nitrogen limitation and nature of the feed upon *Oenococcus oeni* metabolism and extracellular protein production. *Journal of Applied Microbiology*. 98:652-661.

Ribéreau-Gayon, P., D. Dubourdieu, B. Doneche, and A. Lonvaud. 2000. *Handbook of Enology, Volume 1, The Microbiology of Wine and Vinifications*. John Wiley & Sons, New York.

Saguir, F.M., and M. C. M. de Nadra. 2002. Effect of L-malic and citric acids metabolism on the essential amino acid requirements for *Oenococcus oeni* growth. *Journal of Applied Microbiology*. 93:295-301.

Swiegers, J. H., E. J. Bartowsky, P. A. Henschke, and I. S. Pretorius. 2005. Yeast and bacterial modulation of wine aroma and flavour. *Australian Journal of Grape and Wine Research*. 11(2):139-173.

Versari, A., G. P. Parpinello, and M. Cattaneo. 1999. *Leuconostoc oenos* and malolactic fermentation in wine: A review. *Journal of Industrial Microbiology and Biotechnology*. 23(6):447-455.

Zoecklein, B. W., K. C. Fugelsang, B. H. Gump, and F. S. Nury. 1999. *Wine Analysis and Production*. Chapman & Hall, New York.

Acknowledgements

We would like to express our sincere gratitude to the main oenologists of the Slovenian wine cellar (Mr. Bojan Kobal from the winery Ptujska klet vinarstvo d.o.o.), who provided the grape musts for this study; for the scholarship of Tatjana Vrščaj Vodošek's doctoral thesis, thanks to Slovenian Research Agency. Special thanks are earmarked for Lallemand for the starter cultures of yeast, lactic acid bacteria and nutrients; and to Gordana Veber (Jurana, Maribor, Slovenia), Nenad Maslek (Lallemand, Zagreb, Croatia) and to Dr. Sibylle Krieger (Lallemand Danstar Ferment AG, Renningen, Germany) for all their useful information, discussion and help.

Cover Design: Bruno Loste – Layout and Printing: MODULI INC.

LALLEMAND S.A.S. – 19, rue des Briquetiers - B.P. 59 - 31702 Blagnac CEDEX – Tel.: +33 (0)5 62 74 55 55 – Fax: +33 (0)5 62 74 55 00
www.lallemandwine.com

Cover design: Bruno Loste

SENSORY DEVELOPMENT OF COOL-CLIMATE VARIETALSDURING WINE FERMENTATION

GEISENHEIM INSTITUTE
GERMANY, APRIL 24, 2009

LALLEMAND