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SULPHUR COMPOUNDS

PRODUCTION AND SENSORY IMPACT ON WINE

PROCEEDINGS
OF

LES XX^{es} ENTRETIENS SCIENTIFIQUES LALLEMAND

LALLEMAND

FOREWORD

This year, the *XXes Entretiens Scientifiques Lallemand* focused on one of the most important wine faults: sulphur-related compounds. The meeting gathered some of the top scientists in the field to learn about the sensory impact of sulphur-related compounds and their production during both alcoholic and malolactic fermentations, as well as their prevalence in the wine world.

The results of the fault clinic at London's International Wine Challenge (IWC), run by Sam Harrop, Master of Wine, were summarized by Dr. Jamie Goode (U.K.), based on the wines categorized as faulty during the past three years of this international event. An average of 5% of all the 10,000 wines tasted were considered faulty, in seven different categories of faults. The number of reduction defects (sulphur-compound off-aromas) was found to be increasing significantly.

Based on the research at the University of California, Davis (U.S.A.) in the laboratory of Dr. Linda Bisson, Dr. Angela Lee Linderholm explained the genetics surrounding H_2S formation in *Saccharomyces cerevisiae*. The choice of the proper yeast for fermentation, as well as the best must composition, are crucial in order to avoid this problem because yeast, when stressed, will be tend to produce H_2S. Proper fermentation management is just as important.

Although the production of sulphur compounds is often associated with sluggish or stuck alcoholic fermentations, little is known about the impact of malolactic fermentation on this issue, as shown by Dr. Doris Rauhut from Forschungsanstalt Geisenheim (Germany). Preliminary results show that not only is the production of different sulphur compounds dependent on the bacteria strain, it is also dependent on the pH value and the nutrient composition of the must.

Dr. Chris Curtin, from the Australian Wine Research Institute (AWRI), presented some results on the production of thiols found in such varietals as Sauvignon Blanc, and how wine drinkers respond to those aromas. There were also discussions on the topic of the proper nutrient addition to avoid producing sulphur-compound defects.

The conference ended with a presentation by Dr. Michaël Moisseeff, from the Association Asquali (France), who demonstrated, with various aromas, the evolution of certain compounds when reduced or oxidized.

The findings presented at the conference show the importance of continuing research on sulphur-related compounds and focusing our work on the proper selection of yeast and bacteria, as well as their proper nutrition, to understand their behaviours under different conditions – while keeping in mind the importance of their sensory impact.

CONTENTS

WINE FAULTS AND THEIR PREVALENCE: DATA FROM THE WORLD'S LARGEST BLIND TASTING

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Abstract: The International Wine Challenge, held in London, England, is the world's largest annual blind tasting, with some 10,000 wines submitted each year. These wines come from all around the world, and are assessed by panels made up of three to five experienced wine professionals. It represents an ideal opportunity to gather data on the real world prevalence of such wine faults as cork taint, reduction, *Brettanomyces* and oxidation. For the last three years, a concerted effort has been made to gather and validate such data through a faults clinic run by Sam Harrop, Master of Wine. If a wine is judged to be faulty by a tasting panel, it is labelled and sent through to the faults clinic, where the nature of the fault is verified by sensory analysis. In this presentation we discuss some of the results from the last three years, and examine trends. We also discuss the limitations of this sort of study, which is based solely on sensory analysis.

Wine fault data

Wine is a natural drink in the sense that it is made from the fermentation of crushed grapes, with the only additions allowed being sugar or acid, plus processing aids, such as fining materials, cultured natural yeasts and nutrients. It is also a traditional product, often made with simple equipment in old wineries by people with little technical knowledge or training. As a result, sensory defects (known more commonly as "wine faults") are relatively common. These include musty taint (usually from cork), oxidation, reduction (commonly referred to as "sulphides" or volatile sulphur compounds), *Brettanomyces* and volatile acidity. Awareness of faults has increased in recent decades. Improved winemaking has led to the dominance of clean, fruit-driven wines that expose the presence of faults much more clearly than old-fashioned styles, and the emergence of alternative closures to cork has highlighted problems associated with lower-priced natural corks and focused attention on post-bottling wine chemistry.

While much good science has been published about faults, what really matters to the trade is the incidence of faults in the real world, and we lack really good data on just how prevalent the various wine faults are. Such data are important for three reasons. First, it is important to know how frequently consumers will encounter each type of fault in order to prioritize research and education efforts aimed at eradicating these faults. Second, these data would enable us to see whether the various faults are a growing or decreasing problem, as well as seeing whether interventions targeted against specific faults are efficacious. Finally, a geographical breakdown of these data would identify countries whose wine industries are having particular struggles with various types of faults.

With this in mind, the results of the faults clinic from the International Wine Challenge (I.W.C.) are of particular interest. Held annually in London, England, this is the world's largest blind-tasting competition, with some 10,000 entries each year from around the wine-growing world. As each wine entered may be tasted a number of times during the judging process, the data from the most recent competition, 2008, involved 15,000 bottles. The faults clinic was initiated in 2006 when the I.W.C. came under new ownership, as part of a raft of measures seeking

to maintain the position of the I.W.C. as the world's premier blind-tasting competition.

The wines are judged by panels of three to five tasters, with each table being led by an experienced member of the trade. The panels are mixed, and might typically contain a journalist, a buyer, a retailer and a winemaker. In the first week of tasting, all wines are assessed with a view to deciding solely whether they should go through to the medal-judging round or not. All wines not passed by the panel are re-tasted by the co-chairs to check that good wines haven't been overlooked. During this tasting process, any wine deemed faulty by the panel is sent to the faults clinic with the suspected fault marked on it, and a second bottle is requested for tasting. In the following week, the panels judge the remaining wines with a view to deciding which medal, if any, to award them. The faults clinic is also in operation for this second round of tasting.

The faults clinic is run by Sam Harrop, M.W., one of the four co-chairs, who for the last couple of years have been joined by a fifth honorary co-chair from overseas. It is Harrop's job to re-taste wines flagged as faulty, to confirm or refute the panel's diagnosis. Harrop may enlist the help of his co-chairs if necessary, and is conservative: only if he is sure of the fault will he flag it and enter the wine as faulty in the database. Also, a wine may have more than one fault, but will only be scored faulty on one count. All this time the identity of the wine is kept secret, with the only identifier being the number on the bottle tag.

One of the strengths of the I.W.C. data is that they come from an extremely large sample with no geographic skewing. The wine market in the United Kingdom is extremely broad and has representation from all countries where wine is made. While a French competition, for example, would have extremely strong representation from France, the U.K. has a tiny, almost commercially insignificant wine industry and so no such skewing exists. The other strength is the extremely large sample, which means that the fault rates obtained in this way should more closely represent the actual rate across all wines.

However, the fact that these data are based solely on sensory analysis is a weakness. It is likely that in the absence of chemical analysis some faults will be misdiagnosed. There may be some false positives, and some faults may be missed entirely. Chemical analysis for a battery of fault compounds for each wine in the tasting would prove to be impossibly expensive. More feasible might be verification of diagnosed faults in a randomly chosen selection of faulty bottles by chemical analysis, and this is something that the I.W.C. may wish to pursue in future years should a suitable partner company be found. It is likely that the data are more robust with faults that the tasting panels will have more experience of (such as musty taint) than others (such as reduction or *Brettanomyces*). There is also the complicating factor that, in some wine contexts, the same level of "fault" compounds can be seen as positive while in others they are negative. *Brettanomyces* would be a good example of this, although many tasters regard any evidence of *Brettanomyces* to be negative in all contexts.

Results

What do the data show? Here we are able only to provide a brief summary of the results (Table 1), which at some stage will be published in fuller form by the owners of the I.W.C., William Reed Business Publishing. The faults were divided into seven categories: TCA/Musty Cork Taint; Volatile Phenols/*Brettanomyces*; Sulphides; SO_2; Volatile Acidity; Oxidation; and "Rot." The total percentage of faulty bottles fell from 7.1% in 2006 (the first year data were collected) to 5.9% in 2008. It will be interesting to see whether this is a continuing trend in future I.W.C.s.

Table 1. Incidence of wine faults at the I.W.C., 2006–2008.

	2006	2007	2008
Total faults %	**7.1**	**NA**	**5.88**
Cork taint (% of faults)	27.83	29.68	31.11
Brett	10.59	12.82	15.79
Oxidation	24.29	22.88	19.11
Sulphides	29.18	26.53	28.89

Cork taint

Musty taint, which is assumed to be a consequence of compounds such as 2,4,6-trichloroanisole from contaminated corks, is one of the most prevalent and easily recognized of all wine faults. The common assumption that musty taints are cork-derived does have some basis; while it is recognized that the winery environment can impart musty taints on wine, the incidence of such winery taints is low because in almost every case over the last few years, wines sealed with non-cork closures have not shown musty taints. While people differ in their perception thresholds for cork taint, it is likely that almost all the cork-tainted wines will be picked up by the panels, and so the sensory data on cork taint are likely to be accurate.

Of cork sealed bottles, 2.9% showed musty taint in 2006, rising slightly to 3.3% in 2007. This is slightly lower than some claims of the prevalence of cork taint that have been made. The data analysis for 2008 was not complete, but there is a suggestion that the rate may have fallen a little this year.

Brettanomyces

Brettanomyces (brett), a widely occurring spoilage yeast, is found almost exclusively in red wines. Its sensory impact tends to differ depending on the context of the wine and the degree to which the brett infection has progressed. Wines with low pH, high alcohol and high levels of phenolics are particularly vulnerable. Brett is quite a complex and controversial wine fault. At very high levels, wines thus affected can be quite unpleasant, with animal-like odours and strong medicinal notes, but at lower levels the more subtle earthy spiciness can be quite attractive in some wines. At what level Brett becomes a fault depends, to a degree, on the judgment of the taster, and some tasters are more tolerant of it than others. It can be expected that not all bretty wines found their way to the faults clinic, and that different groups of tasters might have different ideas about whether a certain wine is bretty to the point of being faulty. It is also possible that some tasting panels marked down a wine for being bretty without sending it to the faults panel, perhaps because some tasters aren't very good at identifying brett, even though they don't like its sensory impact.

Its incidence? The figures here are an underestimate, because no whites or sparkling wines have shown brett, and the baseline should be red wines, not all wines. But it is an increasing problem in terms of percentage of wine faults, accounting for 10.6% of faulty bottles in 2006, 12.8% in 2007 and 15.8% in 2008.

Reduction, sulphides and volatile sulphur compounds

Reduction is the term used in the trade to describe the sensory impact of a suite of volatile sulphur compounds, including sulphides, disulphides, thioesters and mercaptans (also known as thiols). This is a particularly intriguing and complicated topic which is currently the subject of a lot of attention. These sulphur compounds are produced by yeasts, and the level of their production depends on a variety of factors, such as yeast strain, yeast-available nitrogen (YAN) levels in the must and thermal stresses. Reduction defects in finished wines range from the eggy/drain character of hydrogen sulphide, through to the struck match/flint character of certain mercaptans, with a whole range of aromas in between depending on the nature of the sulphur compound involved and its concentration. Some winemakers believe that in the right style of wine, a little reduction can actually be a positive thing.

The reason for the recent interest in reduction has largely stemmed from the controversy around the use of tin-lined screwcaps as closures. These allow very little oxygen transmission and thus create a very low redox environment in the wine post-bottling. If there are sulphur compounds present at bottling, they can change their form such that a below-threshold level of disulphide can suddenly become an above-threshold level of mercaptans.

It is likely that tasters differ widely in their ability to pick up reduction issues, especially at low levels. There will probably be some false positives, but many incidences of low-level reduction go unreported. The data indicate that this is a real world problem for winemakers. In 2006, sulphides accounted for 29.2% of faults, in 2007 it was 26.5%, and in 2008 28.9%. And screwcap reduction? Harrop reports that, in 2007, 2.6% of screwcapped bottles showed reduction while in 2006 this figure was 2.2%.

The overall conclusions from a preliminary analysis of the I.W.C. faults data are that 1) winemaking faults are beginning to overtake closure-related faults for the first time; 2) the number of corks being used to seal bottles is reduced, and those that are used may be performing better; 3) winemakers are beginning to understand the vagaries of low ox-trans closures such as tin-lined screwcaps; and 4) sulphides, oxidation and brett are still big problems.

In summary, the faults data from the I.W.C. represent a valuable insight into the incidence of wine faults in the real world setting. They also have the potential for revealing the changing incidence of wine faults with time, and national trends with regards to wine faults. The I.W.C. is not the only large blind-tasting competition in the world, and it would be extremely valuable if other large competitions could also implement a similarly organized, rigorous data collection exercise on the incidence of the various faults. Properly collected, such data could be an extremely valuable resource.

THE GENETICS OF SULPHIDE TAINT PRODUCTION IN *SACCHAROMYCES*

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Abstract: Hydrogen sulphide and S-containing volatile compounds confer negative aroma attributes to wines. H_2S can arise during fermentation and the level formed is influenced by several environmental and genetic factors in wine yeast. Other sulphur-containing taints arise *sur lie*, during the aging of wine on the yeast lees. Copper treatment can be used to remove some sulphide compounds, but given the health issues concerning copper levels in the diet, elimination of the need to add this compound to wine is desirable. Toward that end, we have been investigating the genes responsible for sulphide formation in wine yeasts by isolating and examining naturally occurring low-sulphide producing strains and identifying the genes leading to the loss of sulphide formation. In a screen of nearly 200 yeasts, several were identified that produced low to undetectable levels of sulphide. In addition, one of those strains, UCD932, showed a white colony phenotype on selective media making genetic dissection of the trait feasible. In many crosses the loss of sulphide production showed simple Mendelian segregation suggesting a single recessive gene was responsible for the trait. In other crosses it appeared that the loss of sulphide production was under multigene control. Genetic and DNA sequence analyses led to the identification of the *MET10* gene as the genetic factor responsible for the lack of sulphide formation in UCD932. Exchanging of the native *MET10* allele in high sulphide-producing strains with the *MET10* allele from UCD932, $MET10^{UCD932}$, converted those recipient strains into low sulphide formers. This exchange can be accomplished either by using recombinant DNA methods or via classical genetic breeding as the white colony phenotype on BiGGY agar allows ready detection of the *MET10* allele in backcrosses. Further analysis of the $MET10^{UCD932}$ allele indicated that a single change of a threonine residue to a lysine residue is responsible for the loss of sulphide release.

The availability of low and high sulphide-producing strains isogenic for all genes other than the *MET10* allele has enabled testing of the role of sulphide formation during the primary fermentation in the appearance of S-taints during *sur lie* aging. Several taints were identified and varied by strain, but there was no correlation between the ability to form S-taints *sur lie* and H_2S production during the primary fermentation.

Sulphur taints in wine

Several sulphur compounds negatively impacting the aroma profile can be formed in wine (Thoukis and Stern 1962). These compounds are problematic because of the low thresholds of detection. Their chemical reactivity can, over time, lead to the formation of more deleterious compounds during further aging. With the exception of copper fining, these compounds are difficult to remove from wine and not easily masked in blends. The most common of these compounds is hydrogen sulphide, which confers a rotten-egg-like character. There are various mechanisms through which hydrogen sulphide may be produced by *Saccharamyces cerevisiae*. H_2S may be generated through the degradation of sulphur containing amino acids, the reduction of elemental sulphur, or the reduction of sulphite or sulphate (Acree et al. 1972, Karagiannis and Lanaridis 1999, Rankine 1963, Schutz and Kunkee 1977, Wainwright 1970). Hydrogen sulphide can be formed

during primary fermentation either early at the point of peak biomass production or late in the final stages of sugar consumption (Spiropoulos et al. 2000). Early formation of H_2S is thought to be due to sulphate reduction and the release of reduced sulphide prior to incorporation into the organic carbon acceptor, O-acetyl –L-homoserine, in the biosynthesis of methionine, cysteine and their derivatives. Inefficiency of incorporation of the reduced sulphur into the precursors of these amino acids coupled with release of the reduced sulphide from the active site of sulphite reductase has been proposed to result in leakage of sulphide from the pathway and the formation of H_2S (Eschenbruch et al. 1978, Jiranek et al. 1992, Rankine 1963, Spiropoulos et al. 2000). Late formation of sulphide may be due to turnover of S-containing components in the cell, such as methionine, cysteine or glutathione, or may be a consequence of the role of the sulphate reduction pathway in stress tolerance. Late in fermentation the production of sulphite may be crucial to reduce the toxicity of acetaldehyde (Aranda and Olmo 2004). Release of sulphide as H_2S may be necessary to free up the enzymatic pathway to result in more conversion of sulphate to sulphite for detoxification of acetaldehyde. Although appealing, there is not strong support for this hypothesis in the literature as other compounds may also lead to acetaldehyde tolerance. The sulphate reduction pathway may be induced under stress conditions for other reasons, such as the need to produce glutathione and S-adenosylmethionine to maintain cellular viability. The role of turnover of glutathione and S-adenosylmethionine in the generation of S-taints has not been adequately explored. When other preferred nitrogen sources are depleted, *Saccharomyces* can degrade sulphur-containing amino acids to utilize the nitrogen, resulting in the release of H_2S or other volatile sulphur compounds as by-products. However, the concentration of sulphur-containing amino acids in grape juice is generally not high enough to be responsible for the observed levels of H_2S. Extensive research has provided evidence that yeast strain, therefore genetic background, is an important variable in H_2S production, and that strains respond differently to physiological and environmental factors in the production of reduced sulphide (Acree et al. 1972, Giudici et al. 1993, Giudici and Kunkee 1994, Jiranek and Henschke 1991, Rankine 1963, Rauhut and Kerbel 1994, Schutz and Kunkee 1977, Spiropoulos and Bisson 2000, Spiropoulos et al. 2000, Stratford and Rose 1985, Takahashi et al. 1980, Thomas et al. 1993, Vos and Gray 1979). As a consequence of this strain variability, it has not been possible to devise fermentation management strategies guaranteed to reduce or eliminate the appearance of H_2S.

The quantity of H_2S remaining in the finished wine is more important than the total amount made. Several factors influence the retention of sulphide in wine, such as loss resulting from volatilization and entrainment due to carbon dioxide release, which is in turn affected by the temperature of fermentation and the timing of formation versus cessation of fermentative activity. Hydrogen sulphide is reactive and may form reaction products in wine depending on the redox status of the wine, pH and availability of co-reactants. Light exposure and temperature can also influence the reactions occurring in the wine. There is a complex interplay between grape and wine composition, physical parameters of fermentation and aging, and the genetic constitution of the yeast strain or strains present with respect to both the spectrum and levels of S-taints formed and their ultimate fates. Thus environmental factors in addition to production levels affect the final H_2S levels in wine. However, identification of yeast strains not producing H_2S would eliminate the need to ferment wine under conditions leading to its loss.

In addition to the formation of H_2S during primary fermentation, S-taint compounds may arise as wine is aged on the yeast lees. Aging wine in the presence of active yeast lees, termed biological aging, has been shown to have positive effects on wine quality, particularly for white wines. Such wines have been described as having more mature tannins, enhanced mouthfeel, a more prolonged finish and greater complexity, depending on the grape varietal (Ribereau-Gayon et al. 2000). However, one problem associated with aging on yeast lees is the development of sulphur-containing volatile compounds that have offensive odours (Palacios et al. 1997, Rapp et al. 1985, Rauhut 1993, Rauhut et al. 1996, Ribereau-Gayon 2000). A variety of S-containing compounds have been found to be produced *sur lie*. The common S-volatile off-odours include methyl and ethyl thiols, their corresponding acetates, dimethyl and diethyl sulphide, and mercaptans that confer characters of corn, asparagus, cabbage, clam, onion, garlic, rubber, burnt match, rotten meat and poultry taints (Bobet et al. 1990, Goniak and Noble 1987, Karagiannis and Lanaridis 1999, Pisarnitskii 2001, Rauhut 1993). Thioethers, cyclic and heterocyclic S-compounds have also been reported in wine to be associated with a raw potato or rancid vegetable character (Rauhut 1993). The appearance of these characters has been correlated with the presence of S-containing amino acids during fermentation (Moreira et al. 2002), but this effect was not observed in all musts tested.

The presence of oxidative or reductive conditions in wine can lead to the chemical interconversion of forms of S-containing compounds. Treatments to remove S-containing

compounds may instead simply convert them into a form that is below its threshold of detection. Chemical conversion back to the original or a different form with a lower threshold of detection results in the reappearance of the S-taint. Thiols can be oxidized to their corresponding disulphides, which have a higher threshold of detection, but can then be cleaved by sulphite under the anaerobic conditions created at bottling and reappear in the bottle (Bobet et al. 1990, Jones et al. 2004). Thioacetates can also undergo hydrolysis reactions producing thiols (Sea et al. 1999). Therefore, S-compounds may initially be produced in chemical forms below their sensory detection threshold, but, during the reductive aging conditions in the barrel or bottle, generate different chemical forms that exceed their threshold of detection. This chemical reactivity of S-containing compounds makes it challenging to discern the conditions under which they were initially synthesized versus when they were noticed by being converted to a volatile compound present above its detection limit. There currently are no effective methods for the removal of these compounds from wine pre-bottling, and the best strategy is to make sure they are not formed in the first place.

Identifying genetic factors impacting hydrogen sulphide formation

The goal of our study was to simultaneously screen the yeast deletion set to identify genes impacting sulphide formation, either increasing or decreasing it as compared to the parental strain (Linderholm et al. 2008), and to also screen a collection of native and commercial isolates to identify naturally arising low to no sulphide formation. Systematic screening of all of the over 4,800 mutants in the yeast deletion set will identify those pathways and biological functions that impact sulphide release most, and allow identification of the most likely sources of this compound during fermentation. Once the genes leading to an increase or decrease in sulphide are known, genetic crosses can be used to determine the identity of the genes altered in the native strains. In this way, mutations leading to loss of sulphide formation but not adversely impacting fermentation biology can be identified.

There are several potential candidates for genes impacting sulphide formation as control of the sulphate reduction pathway is multifaceted and responsive to numerous regulatory inputs (Breton and Surdin-Kerjan 1977, Cherest et al. 1973, Hansen and Johannensen 2000, Mountain et al. 1991, Ono et al. 1996, Paszewski and Ono 1992). As a consequence, mutational change of a wide array of genes may impact sulphide formation and release. Sulphite reductase is responsible for reducing sulphite into sulphide and is regulated by general amino acid control, as well as methionine (Mountain et al. 1991). Other research suggests that cysteine or its derivatives rather than methionine is the main end product regulating pathway activity (Hansen and Johannensen 2000, Ono et al. 1996). It is likely that the regulation of the pathway varies by media and growth conditions, with pathway activity controlled by the factor for which there is the greater cellular demand (Spiropoulos et al. 2000).

Sixteen yeast deletants were identified as leading to elevated levels of hydrogen sulphide in synthetic juice media and in actual juices (Linderholm et al. 2008). Gene function was taken from the *Saccharomyces* Genome database (Hong et al. 2006). Five of the positive H_2S-producing strains are defective in genes encoding for enzymes involved in sulphur-containing amino acid or precursor biosynthesis, and were associated with the sulphate assimilation pathway (*MET17, CYS4, HOM2, HOM6, SER33*), reinforcing the importance of this pathway in sulphide formation and release. Some of these genes are involved in precursor formation and others are involved in consumption of the reduced sulphide. Interestingly, not all genes of the sulphate reduction pathway impact sulphide formation or release when mutated. Seven strains are defective in genes involved in transport, secretion, or cell wall or membrane integrity or other functions (*CGR1, FCY22, GOS1, HHT2, IKI3, SIT4, TPO2*). Three of these genes impact the histone elongator complex involved in transcriptional elongation. However the elongator complex is comprised of multiple other genes that do not impact sulphide release. One strain contained a mutation affecting ATP synthesis (*ATP11*). The remaining strains had deletions in genes of unknown function (*RXT2, PSY4, YPL035C*). The four mutations leading to white colonies encoded catalytic or regulatory genes of sulphite reductase (*MET1, MET5, MET8, MET10*).

Of the nearly 200 native and commercial yeast strains tested, six were found that did not produce H_2S during the initial screen on synthetic media and in red juice (Syrah). One of these strains, UCD932, an Italian vineyard isolate, displayed a white colony colour on BiGGY agar that co-segregated with the loss of sulphide formation and allowed a convenient screen for genetic analysis. This strain did not produce sulphide in a variety of other media and juices, with one exception. In severely vitamin-limited conditions under which no other strain grew, UCD932 did grow and produce trace, but detectable, amounts of H_2S. The mutation in UCD932 causing white colony formation and loss of sulphide production lies in the *MET10* gene. The *MET10* gene was sequenced from UCD932 and found to contain several base pair changes leading

to differences in five amino acids. Four other genes of the sulphate reduction sequence were also found to carry mutations in UCD932 (*MET5, HOM6* and *CYS4*), and tests indicated that these variants were not responsible for the low-sulphide phenotype, at least in isolation from the other mutant alleles of the pathway (Linderholm et al. 2006, Spiropoulos and Bisson 2000). Sequence analysis of the *MET10* genes from various other strains indicated that only one of these changes, at amino acid 662, was unique to UCD932 (Figure 1). The amino acid at position 662 is a non-conservative change of a threonine to a lysine. Sequence prediction models place this residue in the sulphide reductase active site of the protein.

Figure 1. Comparison of MET10 sequences of UCD932 with other yeast strains at amino acid residue 662.

***MET10* Sequence Comparisons**									
S288C	AAT	AGA	CGT	GTT	ACG	CCT	GCT	GAT	TAT
UCD932	AAT	AGA	CGT	GTT	AAG	CCT	GCT	GAT	TAT
UCD950	AAT	AGA	CGT	GTT	ACG	CCT	GCT	GAT	TAT
Amino Acid	**N**	**R**	**R**	**V**	**T/K**	**P**	**A**	**D**	**Y**

This naturally arising mutation of *MET10* eliminated both the early and late peaks of sulphide formation. Early sulphide production can often be driven off by the carbon dioxide formed during active fermentation, although there is some concern that the sulphide can become chemically trapped in the medium and then released upon aging of the wine, depending on the redox conditions of the wine. Hydrogen sulphide and other sulphides can be removed by treatment of the wine with copper. However, copper has come under scrutiny for its potential role in the neural degenerative diseases of aging. While the amount of copper in wine represents only a minor dietary source of copper versus the water supply, reduction in its use would be prudent. Therefore we sought not only to identify the genes associated with low-sulphide formation but to determine the utility of transferring those genes to other genetic backgrounds to confer the low-sulphide production trait.

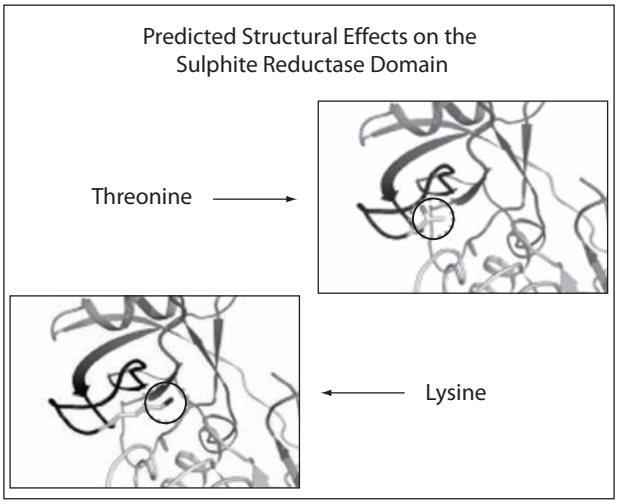
Genetic alteration of hydrogen sulphide producing ability: Use of the UCD923 *MET10* allele

The native *MET10* alleles of three high H_2S producers were replaced using allele swap technology with the $MET10^{UCD932}$ allele. The *MET10* alleles from UCD932 and UCD950 were cloned and each single base systematically converted difference to the base of the opposite allele using site-directed mutagenesis. The resulting alleles were identical to the parent allele, with the exception of the single swapped base change. The modified alleles were then inserted back into both strains and BiGGY agar was used as an indicator of a change in sulphite reduction and likely H_2S production. The single base change leading to a different amino acid at position 662 was identified by this screen as the mutation responsible for the change in colony colour. These strains were also examined for H_2S production during small-scale fermentations in synthetic wine juice. In all cases, sulphide production was eliminated in alleles with an altered amino acid at position 662. Further tests were run to determine if the change of the threonine at position 662 to lysine was sufficient to affect the loss of sulphide release. The unchanged UCD950 *MET10* allele and the UCD932 allele with the mutation to the UCD950 allele at position 1985 (932 *MET10* 1985 A-C) resulted in H_2S production, while the unchanged allele UCD932 *MET10* and the UCD950 allele with the change to UCD932 at position 1985 (950 *MET10* 1985C-A) resulted in no detectable H_2S production. These results indicate that the single base change at position 1985 (amino acid 662) is the key determinant of the difference in H_2S production of these alleles. These results were then strengthened by examining the production of H_2S when the single mutant alleles were place into two high H_2S-producing commercial strains, UCD522 and UCD940. Both of these strains produced H_2S with the 932 *MET10* 1985A-C allele, but no H_2S was detected with the 950 *MET10* 1985C-A allele. Thus the change of this single residue is indeed sufficient to block sulphide formation.

To determine if the *MET10* allele of UCD932 conferred a growth disadvantage, direct competition experiments were performed of unmodified UCD522 (native *MET10* allele) and modified UCD522 ($MET10^{UCD932}$). Equal numbers of cells of each type were mixed at the beginning of fermentation and the relative ratios of the two alleles in the population assessed using BiGGY agar, as there is a discernable colour difference of the colonies for each of the alleles. There was no statistically significant difference in the level of dominance of the two strains. The presence of the modified allele did not confer a growth or fermentation disadvantage.

Although more analyses are required to determine the nature of the mutation and its role in reducing sulphide release, analysis of sulphite reductase activity suggests that there is no significant difference in activity between strains carrying the $MET10^{UCD932}$ allele versus other wild alleles. Thus the lack of sulphide release is not due to a deficiency of enzyme activity. Instead, it is likely that the change of the threonine for a positively charged lysine stabilizes the reduced sulphide in the substrate pocket, not releasing it as readily as the wild-type protein (Figure 2).

Figure 2. Predicted location of the amino acid residue at position 662, indicated by the circle.



This would then allow release when the acceptor substrate molecule is present. This modification is consistent with a yeast strain that evolved in the vineyard under nutrient-limitation conditions. This would ensure that sulphate is more often incorporated than released. This explanation is consistent with the isolate's ability to grow under conditions of vitamin limitation preventing the growth of most other strains of *Saccharomyces*.

Analysis of the factors impacting S-volatile compound formation *sur lie*

Sur lie aging of wine is reported to confer several important organoleptic properties to wine, including enhanced complexity and mouthfeel. One of the problems associated with *sur lie* aging is the formation of S-containing volatile off-odours that can be difficult to remove. It is therefore desirable to identify strains that will yield the positive effects of *sur lie* aging, but not result in S-taints. There are several hypotheses and models explaining the formation of S-taints *sur lie*. These taints have been associated with the levels of sulphide produced during primary fermentation, with late formation of sulphide, with the levels of the S-containing amino acids, and with the specific yeast strain used. The conflicting nature of these analyses indicates the challenges of these studies and the multitude of factors that can impact S-taint formation. We are attempting a more systematic analysis of these factors with the goal of eliminating their formation during *sur lie* aging. Strain differences are also important here, as winemakers fond of *sur lie* aging have reported that UCD522 is an excellent strain *sur lie*, with the exception of the release of H_2S. The existence of paired sets of strains with differing *MET10* alleles that are high- and low-sulphide producers allows differentiation among these hypotheses. We are also examining the compositional factors that increase S-volatile formation.

An analysis of S-taints in Chardonnay juice was performed using two commercial strains, UCD522 (high H_2S producer, low S-taint producer *sur lie*), UCD713 (low H_2S producer, moderate sur lie S-taint producer) and UCD950 (high H_2S and S-taint producer) (Table 1). Curiously, all three strains produced 2-mercaptoethanol. This compound is highly reactive and not normally reported in wine. The levels formed, though below the threshold of detection, could be important given the reactivity of this compound. Furthermore, the presence of 2-mercaptoethanol at this stage suggests turnover of glutathione, which is being investigated further. Consistent with its reputation, UCD522 did not show evidence in this study of formation of other S-taints, while the other two strains did, particularly UCD950. This study confirms the role of yeast genetic factors in the formation of S-taints *sur lie*.

Table 1. The level of sulphur compounds detected *sur lie* in Chardonnay wines for UCD522, UCD713 and UCD950.

Sulphur Compounds (µ/L) in *Sur lie* Chardonnay Wines			
	522-Day 35	713-Day 35	950-Day 35
H_2S	–	3.0	7.0
Methanethiol	–	0.4	0.7
Ethanethiol	–	–	0.1
2-Mercapto-ethanol	13.0	4.0	14.0
Diethyl Sulphide	–	–	6.8
Carbon Disulphide	–	–	0.1

Conclusions

We have demonstrated that a single base pair change at position 1985 in the *MET10* allele dictates the production of hydrogen sulphide. The nucleotide difference at 1985 changes the encoded amino acid, thus any change in the surrounding nucleotide sequence that changes the sequence in the encoded amino acid will likely eliminate H_2S production. The serine present in the high-producing alleles may act as a regulatory point that changes the flux of the pathway. However, the role of this amino acid change in sulphide production remains to be determined. Genetic differences also have been shown to impact S-taint formation *sur lie*. The nature of those genetic differences is currently under investigation.

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FORMATION OF AROMA-ACTIVE S-COMPOUNDS BY *OENOCOCCUS OENI* DURING MALOLACTIC FERMENTATION IN WINE-LIKE MEDIA AND WINE

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ABSTRACT

During recent years, an increase of "reduced" or "reductive" sulphur (S-) off-flavours in wines during storage after treatment and bottling has become noticeable. Those off-flavours can be related to the release of unpleasant volatile compounds from non-volatile or volatile precursors.

The cause of an increase in reduced S-off-flavours after malolactic fermentation (MLF) and during storage can be attributed to the increase of the pH, which can trigger further chemical reactions. However, the addition of SO_2 or ascorbic acid can reduce disulphides to the more odour-active thiols. Furthermore, the metabolism of lactic acid bacteria (LAB) is discussed as a cause for the development of S-off-odours in wine. It is well known that LAB can metabolize S-containing amino acids like methionine, and that several S-aroma compounds can be formed by degradation of these amino acids. Therefore, research was focused on the influence of methionine, cysteine and glutathione (also in combination) on the growth of *Oenococcus oeni* and the duration of MLF.

Preliminary results showed that an addition of cysteine and glutathione after alcoholic fermentation to a fermented wine can promote the development of LAB and MLF. This effect appears to be influenced by the added concentration and by the nutrient composition of the wine, which is also affected by the nutrient requirement of the chosen yeast strain for alcoholic fermentation, and by the applied strain of *O. oeni*.

Furthermore it was confirmed that *O. oeni* is able to produce methanethiol, dimethyldisulphide, methional and methionol at wine-like conditions from methionine, whereas the addition of glutathione can lead to an increase of H_2S. The results indicate that the formation of volatile sulphur compounds depends on the concentration of methionine and glutathione, the pH, and the ability of the particular *O. oeni* strain.

Increased production of volatile S-compounds was observed only at concentrations of methionine and glutathione far over the normal levels in wines after the end of alcoholic fermentation. Therefore, off-flavours due to volatile S-compounds after MLF do not appear to be related to the metabolism of methionine and glutathione by *O. oeni*. It is assumed that the chemical or biochemical transformation of other volatile or non-volatile S-precursors in wine is the cause for an increase in reduced S-off-flavours after MLF.

Introduction

Malolactic fermentation (MLF) by *Oenococcus oeni* is well known for transforming harsh malic acid in wine

into smooth lactic acid and carbon dioxide. Recently, it has been shown that MLF improves organoleptic characteristics, impacts mouthfeel and structure, and provides biological stability in the final wine (Henick-Kling 1993, Krieger and Hammes 1988, Lonvaud-Funel et al. 1998). Moreover, "reduced" or "reductive" sulphur (S-) off-flavours can be detected in some wines after MLF.

Volatile S-containing compounds play a significant role in the flavour of wines. This is related to their high volatility, reactivity and impact at very low concentrations. Some of the S-substances are necessary for wine quality, while others are the cause of strong objectionable flavours (e.g., rotten eggs, cooked cabbage, cauliflower and burnt rubber, etc.), even at extremely low concentrations (e.g., H_2S, methanethiol [MeSH], and ethanethiol [EtSH]). Certain thiols contribute to the typical sensory impression of grape varieties like Sauvignon Blanc, Chenin Blanc, Scheurebe, etc. It is well known that the formation of volatile S-compounds is affected by organic and inorganic S-containing substances and pesticides in grapes and musts. Other factors are the nutrient level in grapes and musts, and the yeast metabolism during fermentation.

During recent years, a reoccurrence of off-flavours in wines during storage after treatment and bottling has become noticeable. These off-flavours can be related to the release of unpleasant volatile compounds from non-volatile or volatile precursors, such as the hydrolysis of thioacetic acid esters to thiols and acetic acid. This depends on the chemical equilibrium. Therefore, an off-flavour can increase or reoccur. In comparison to thioacetic acid esters, mercaptans have very low threshold values (> 40 µg/L and < 2 µg/L, respectively). Consequently, mere trace amounts of mercaptans are sufficient to induce a sulphur off-flavour.

The cause for an increase of reduced S-off-flavours after MLF or storage can be attributed to the increase of the pH, which can change the chemical equilibrium or trigger further chemical reactions. However, the addition of SO_2 or ascorbic acid can reduce disulphides to the more odour-active thiols. Furthermore, the metabolism of lactic acid bacteria (LAB) is discussed as cause for the development of reduced off-odours in wine. It is well known that LAB can metabolize S-containing amino acids like methionine, and that several S-aroma compounds, including methanethiol, dimethyl sulphide, dimethyldisulphide, methionol, methional and 3-(methylsulphanyl)propionic acid can be formed by the degradation of this amino acid (Dias and Weimer 1998, Bonnarme et al. 2000, Pripis-Nicolau et al. 2004). Most of these S-compounds are relevant for the variations in cheese flavours, as well.

Cysteine can be the precursor of S-containing heterocycles, such as certain thiazoles in wines and foods (Pripis-Nicolau et al. 2000). Therefore, in the first stage research was focused on the influence of methionine, cysteine and glutathione (also in combination) on the growth of *O. oeni*, the duration of MLF and the formation of volatile S-compounds (Schwarz 2003, Dörflinger 2006, Schäfer 2007) in different synthetic wine-like media with higher additions of these organic S-sources than normally expected in wines (Henschke and Jiranek 1993). An extract of preliminary results is presented in the following section.

Material and methods

The trials in synthetic wine-like media were carried out based on the research of Pripis-Nicolau et al. 2003. Trial I was conducted to optimize the synthetic media with the aim of studying the behaviour of different LAB strains in an improved wine-like synthetic medium. Therefore the following points were modified, compared to the research work of Pripis-Nicolau et al. 2003:

- pH values were in the range of 2.9 to 3.8
- The methionine level was decreased to 200 mg/L
- Ethanol was added to a level of 10%/vol.

Per litre, the improved wine-like synthetic media contained 5 g yeast extract, 2.5 g casamino acids, 2 g glucose, 2 g fructose, 0.3 g citric acid, 4 g L-malic acid and demineralized water; pH values were adjusted with HCL or KOH.

The synthetic media were autoclaved at 121°C for 15 minutes. Ethanol (10%/vol) was added after the media were cooled to room temperature. Fermentation volume was 750 mL. The samples were stored at 20°C. The lactic acid bacteria *O. oeni* strain A was used for the trials (2 x 10^6 cfu/mL for inoculation).

Table 1 gives an overview of the experimental design of Trial I. Apart from a control with no additions, methionine (Met) and glutathione (GSH) were added, each in a concentration of 200 mg/L. Low levels of methionine and glutathione were also detected in the control with no supplements before MLF was started. As the trial was carried out with duplicates, each variant had one replicate (sample A and sample B). Average values are presented in the following table.

Table 1. Experimental design of Trial I synthetic wine-like media.

Variants
pH 2.9, control
pH 2.9, addition of 200 mg/L methionine (Met)
pH 2.9, addition of 200 mg/L glutathione (GSH)
pH 3.2, control
pH 3.2, addition of 200 mg/L methionine (Met)
pH value 3.2, addition of 200 mg/L glutathione (GSH)
pH 3.5, control
pH 3.5, addition of 200 mg/L methionine (Met)
pH 3.5, addition of 200 mg/L glutathione (GSH)
pH 3.8, control
pH 3.8, addition of 200 mg/L methionine (Met)
pH 3.8, addition of 200 mg/L glutathione (GSH)

Trial II was carried out on a synthetic must-like medium, created according to Costello et al. (2003) to improve the synthetic media composition. The pH was adjusted to 3.5. The medium was autoclaved at 121°C for 15 minutes. The must-like medium was fermented with the commercial yeast Uvaferm CM at 20°C.

MLF was conducted after the alcoholic fermentation of the must-like medium was completed. The wine-like medium contained only very low amounts of the organic S- compounds, which were in the normal range of concentrations that can be expected in wines (Henschke and Jiranek 1993). The samples were stored at 20°C. The lactic acid bacteria *O. oeni* strain A was used for the trials (2 x 10^6 cfu/mL for inoculation).

Table 2 gives an overview on the experimental design of Trial II. Apart from a control with no additions, methionine (Met) and glutathione (GSH) were added, each in a concentration of 50 or 200 mg/L. The trial was carried out with duplicates. Average values are presented in the following table.

Table 2. Experimental design of Trial II synthetic wine-like media

Variant	Abbreviation
Control	C
Methionine 50 mg/L	Met 50
Methionine 200 mg/L	Met 200
Glutathione 50 mg/L	GSH 50
Glutathione 200 mg/L	GSH 200
Methionine 50 mg/L and Glutathione 50 mg/L	Met 50/GSH 50

Analysis was done during MLF to control the development of malic acid decrease by the use of HPLC-measuring (Rauhut and Dost 2005). The S-compounds were determined by GC-PFPD and other detectors at the end of MLF (Rauhut et al. 2005, Rauhut et al. 2007, Irmler et al. 2008).

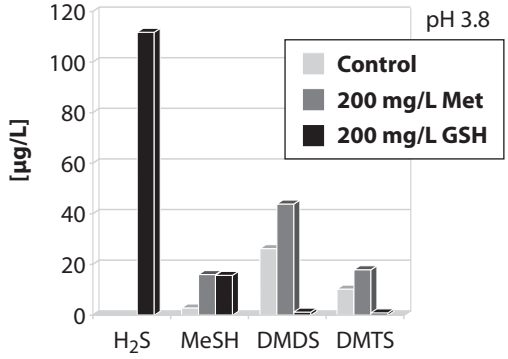
Results and discussion

The results of Trial I indicated that MLF could not be finished within adequate time at pH 2.9. No difference in the duration of MLF could be detected at pH 3.5 and pH 3.8. The addition of methionine (Met) and glutathione (GSH) seemed to accelerate the speed of MLF a little bit at pH 3.2 (results not presented).

Variations in the formation of volatile S-compounds were observed at the different pH levels, and also in the variants with methionine or glutathione addition. Figure 1 indicates the production of volatile S-compounds in the different variants at pH 3.8.

Hydrogen sulphide (H_2S) was synthesized far over the threshold value in the variant supplemented with glutathione. Methanethiol (MeSH), dimethyldisulphide (DMDS) and dimethyltrisulphide (DMTS) were increased in the variant with the addition of methionine (Met). MeSH, DMDS and DMTS were also produced in the control. The levels were near or above the threshold value.

Figure 1. Formation of low volatile S-compounds at pH 3.8 (Trial I).

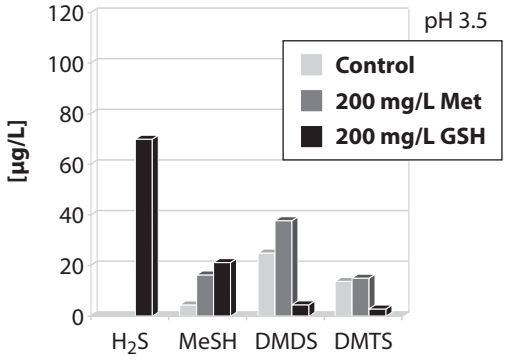


Legend: H_2S (hydrogen sulphide)
MeSH (methanethiol)
DMDS (dimethyldisulphide)
DMTS (dimethyltrisulphide)

Figure 2 demonstrates the measured concentrations of volatile S-substances in the variants at pH 3.5. The amounts of the S-substances were similar to the samples at pH 3.8. The concentration of hydrogen sulphide was a little lower but also over the threshold value in wine with glutathione

addition. The formation of MeSH, DMDS and DMTS was similar in the samples at pH 3.8.

Figure 2. Formation of low volatile S-compounds at pH 3.5 (Trial I).



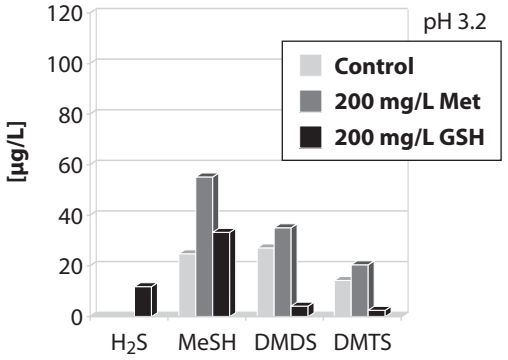
Legend: H_2S (hydrogen sulphide)
MeSH (methanethiol)
DMDS (dimethyldisulphide)
DMTS (dimethyltrisulphide)

Higher levels of MeSH were produced at lower pH (3.2), especially in the variant with methionine addition. The control showed also higher levels of MeSH in the variant at pH 3.2 than in the samples with higher pH values (Figure 3).

The results of Trial II showed no increase in the formation of volatile S-compounds that were mainly responsible for reduced or reductive off-flavours. Figure 4 indicates that the detected levels of MeSH in the samples with methionine and/or glutathione addition were at the same level after MLF as the control with no additions.

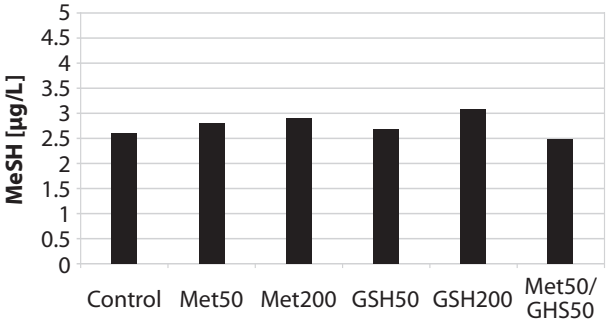
Further trials were conducted with an addition of methionine or glutathione in an amount of 50 mg/L^{-1} or less to different wines (data not presented). The results of the measuring of volatile sulphur compounds after MLF confirmed the results that were obtained with the use of synthetic must and wine-like media. No increased levels of sulphur compounds were detected after MLF if normal levels of organic S-sources like methionine and glutathione were supplemented (Henschke and Jiranek 1993, Rauhut et al. 2008).

Figure 3. Formation of low volatile S-compounds at pH 3.2 (Trial I).



Legend: H_2S (hydrogen sulphide)
MeSH (methanethiol)
DMDS (dimethyldisulphide)
DMTS (dimethyltrisulphide)

Figure 4. Formation of methanethiol (MeSH) in an improved synthetic wine-like media (Trial II).



Conclusions

Preliminary results showed that an addition of cysteine and glutathione to wine after alcoholic fermentation can promote the development of lactic acid bacteria and malolactic fermentation. This effect seems to be influenced by the added concentration and by the nutrient composition of the wine, which is also affected by the nutrient requirement of the chosen yeast strain for alcoholic fermentation and by the applied strain of *Oenococcus oeni*. On the other hand, reactions of the SH-group in cysteine and glutathione with other ingredients of wine can be supposed, which probably vary the effect of their addition to the different wines.

Furthermore, the research confirmed that *O. oeni* is able to produce methanethiol, dimethyldisulphide, methional and methionol at wine-like conditions from methionine. The addition of high levels of glutathione can lead to an increase of H_2S. The results indicate that the formation of volatile S-compounds depends on the concentration of methionine and glutathione, the pH, the ability of the

O. oeni strain, and the nutrient composition of the wine-like media used for the studies.

An increased production of volatile S-compounds was only observed at concentrations of methionine and glutathione that were far over the normal levels in wines after the end of alcoholic fermentation. Therefore, off-flavours due to volatile sulphur compounds after MLF seem to be unrelated to the metabolism of normal concentrations of methionine and glutathione in wine by *O. oeni*.

It is assumed that the chemical or biochemical transformation of other volatile or non-volatile S-precursors in wine are the cause for an increase of reduced or reductive off-flavours after MLF.

Future research is directed to further investigation of the composition of volatile S-compounds before and after MLF, and before and after the bottling of wine, with the addition of sulphite and/or ascorbic acid.

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OPTIMIZING WINE QUALITY THROUGH THE APPLICATION OF FLAVOUR-ACTIVE YEAST STRAINS AND NUTRIENTS

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Introduction

Deciding on the relative quality of a wine is a subjective process that can be influenced by many factors – previous experience, environment and branding, to name but a few. Ultimately, in the eyes of the consumer wine quality comes down to one simple question: "Do I like it?" The sensory properties of a wine in relation to its price point trigger the physiological and emotional responses required for a consumer to purchase a product repeatedly. In this sense, quality is something that can be manipulated, as the sensory characteristics of a wine are dependent on complex interactions among an array of grape-, yeast-, and oak-derived compounds, and the changes they undergo during maturation. The purpose of this article is to examine how the selection of appropriate yeast strains and the management of nutrients during fermentation might be used to produce wines with desirable sensory characteristics – wines consumers would consider of high quality.

Do we know what sensory characteristics drive consumer preference?

Relatively few studies have examined the sensory characteristics that drive wine preference, whereas the responses of consumers to extraneous factors have received more attention. For example, Marin and Durham (2007) found that the closure type influenced consumer perceptions of quality, while Mtimet and Albusi (2006) examined the relative importance of origin, price, wine aging and grape variety. Such information is important for the development of marketing strategies, and can infer sensory attributes that may be important – but if most wine is consumed while young a focus on age-related aroma compounds may not be instructive.

On the other hand, direct assessment of preference measured as the degree of liking for a product (Stone and Sidel 2004) has the potential to aid all stages of the wine product development cycle. It is important to assess consumer preference of relevant target-market segments, and it is critical to take into consideration the relative experience typical for these segments. A recent study demonstrated that neural processing in response to wine tasting differs for sommeliers and inexperienced wine-tasters (Castriota-Scanderbeg et al. 2005). Predictably, sommeliers processed wine sensory information through regions of the brain implicated in working memory and behavioural response, whereas inexperienced tasters responded via brain areas involved in emotional processing. Inexperienced tasters respond strongly in terms of "like" or "dislike," but cannot always enunciate the characteristics of the wine to which they are responding. To overcome this issue, consumer-preference testing can be coupled with quantitative sensory-descriptive analysis, chemical profiling and multivariate statistics. This approach can provide insight into the sensory characteristics consumers like and dislike, and the chemical compounds that correlate with these characteristics.

Extensive studies at the Australian Wine Research Institute (AWRI) have taken this approach to investigate drivers of consumer preference for commercial Riesling and unwooded Chardonnay wine styles (Lattey et al. 2004), and Shiraz and Cabernet wine styles (Lattey et al. 2007). In

both studies, consumers could be subdivided into groups which have similar preferences within the group, and different preferences between the groups, through cluster analysis. For example, in the red wine study, one cluster of consumers exhibited a strong preference for Cabernet Sauvignon varietal wines over Shiraz varietal wines. An interesting observation from the white wine study was that a Riesling wine exhibiting a strong passion-fruit aroma had a polarizing effect upon consumers – one cluster of consumers strongly liked the wine while another cluster disliked the wine relative to other styles.

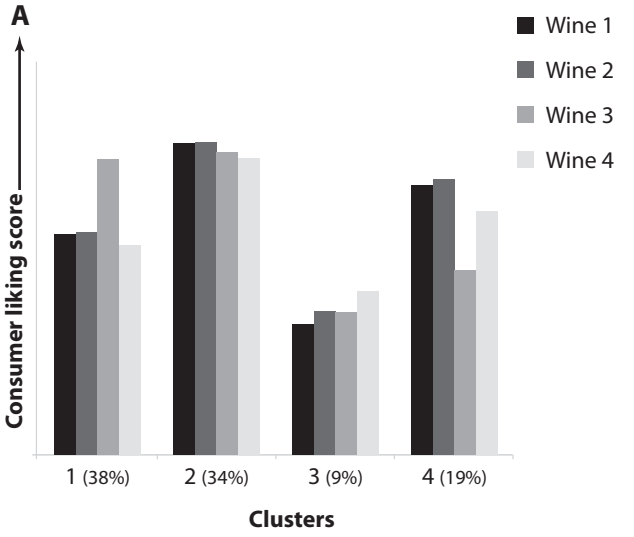
More recently, consumer sensory analysis has been integrated into a study of different yeast strains and inoculation strategies aimed at improving Sauvignon Blanc wine quality (King et al. 2008a). Figure 1 summarizes the results of consumer acceptance testing, where it was evident that consumer sub-groups have different wine-style preferences. In this case, one particular yeast treatment yielded wine with more box hedge character, and fewer ester or citrus aromas. This treatment polarized consumer clusters in the same manner as passion-fruit aroma in Riesling appeared to (Lattey et al. 2004). Importantly, King et al. (2008a) demonstrated that sub-groups of consumers could be targeted, based on the intrinsic wine characteristics they preferred, and that this could be achieved by the choice of yeast treatment. In other words, yeast-derived volatile compounds played a critical role in defining the eventual style of the Sauvignon Blanc wine produced and, by extension, the likely target consumer group to which the wine could be marketed.

Wine-volatile compounds derived from or modified by yeast

Several hundred volatile compounds contribute to wine aroma. Many of these are grape-derived compounds, such as terpenes, norisoprenoids and methoxypyrazines, which generally contribute varietal characters to wine (Ribéreau-Gayon et al. 2006). Depending on the variety, these compounds can exist in the grape predominantly as free, volatile compounds or as odourless, non-volatile precursors. The polyfunctional thiols, associated with the Sauvignon Blanc character, are also present in the grape as odourless precursors. The volatile thiol is released from the non-volatile conjugate during fermentation. Also during fermentation, a large number of yeast and bacterial fermentation products are formed – secondary metabolites, such as esters, higher alcohols, low molecular weight sulphur compounds and volatile acids (Fleet 2003). Of the fermentation-derived volatiles, esters are considered to have the greatest impact upon young wine aroma (Schreier 1979), while the positive polyfunctional thiols have received significant attention recently due to their critical role in such wine varietals as Sauvignon Blanc (Swiegers and Pretorius 2007).

Ester formation occurs predominantly via enzymatic reactions within the yeast cell (Mason and Dufour 2000). The enzymatic reactions for ester synthesis occur between higher alcohols and acetyl-CoA compounds (acetate esters) and ethanol and short-chain fatty acyl-CoA compounds (fatty-acid ethyl esters) during alcoholic fermentation (Fleet 2003, Mason and Dufour 2000, Swiegers et al.

Figure 1. Results of consumer acceptance testing.
(A) Mean liking scores of four consumer clusters for four wines made using different yeast inocula to conduct fermentation. Percentages indicate the proportion of consumers in each cluster group.
(B) Sensory descriptors of wines that were most liked and disliked by Clusters 1 and 4, as rated by trained panellists in a descriptive sensory analysis.



B

Cluster 1 likes: Wine 3
Box hedge
Cluster 1 dislikes: Wine 4
Estery, Fresh citrus

Cluster 4 likes: Wine 4
Estery, Fresh citrus
Cluster 4 dislikes: Wine 3
Box hedge

2005a). The concentration of ester compounds in wine is influenced by the yeast species and strains used for fermentation, and the balance of ester synthesis and hydrolysis during and after fermentation (Rojas et al. 2003, Swiegers et al. 2005a). Both types of esters contribute fruity characteristics to wines, reminiscent of apple, banana, perfume and rose (Mason and Dufour 2000), as shown in Table 1. Esters are present in all wines and are considered to significantly influence wine aroma and quality (Fleet 2003, Lilly et al. 2006, Marais 2001, Mason and Dufour 2000, Rojas et al. 2003, Swiegers et al. 2005a).

Three sensorially positive polyfunctional thiol compounds found in wine have been shown to significantly contribute to wine aroma: 4-mercapto-4-methylpentan-2-one (4MMP), 3-mercaptohexan-1-ol (3MH) and 3-mercaptohexyl acetate (3MHA) (Dubourdieu et al. 2006, Howell et al. 2004, Swiegers et al. 2005a, Tominaga et al. 1996, 1998a, 1998b, 2000, Tominaga and Dubourdieu 2000). The aroma descriptors assigned to these compounds vary (see Table 2), the most common being box tree for 4-mercapto 4-methylpentan-2-one, grapefruit and passion fruit for 3-mercaptohexan-1-ol, and grapefruit, passion fruit and box tree for 3-mercaptohexyl acetate; 3MH and 3MHA have been shown to exist in two enantiomeric forms, S- and R- (Tominaga et al. 2006), which may contribute to

Table 1. The most significant ester compounds formed by yeast during fermentation and their aroma descriptors.

Volatile Compound	Aroma Descriptors
Fatty-acid ethyl esters	
Ethyl propanoate	Fruity
Ethyl 2-methyl propanoate	Fruity
Ethyl butanoate	Acid fruit
Ethyl 2-methylbutanoate	Sweet fruit
Ethyl 3-methylbutanoate	Berry
Ethyl hexanoate	Green apple
Ethyl lactate	Strawberries
Ethyl octanoate	Sweet soap
Ethyl decanoate	Pleasant, soap
Ethyl dodecanoate	Soapy, estery
Acetate esters	
Ethyl acetate	Fruity, nail polish remover (high concentration)
2-methylpropyl acetate	Banana, fruity
2-methylbutyl acetate	Banana, fruity
3-methylbutyl acetate	Banana
Hexyl acetate	Sweet, perfume
2-phenylethyl acetate	Flowery

Table 2. Summary of three polyfunctional thiol compounds and their reported aroma descriptors and perception thresholds, as described in the literature.

Polyfunctional Thiol Compounds	Current Literature	Reported Aroma Characteristics	Reported Perception Thresholds
4-mercapto-4-methylpentan-2-one (4MMP)	Tominaga et al. (1998a)	Box tree, broom	0.8 ng/L[a]
	Tominaga et al. (2000)	Box tree	
	Howell et al. (2004)	Box tree, blackcurrant; cat's urine (high concentration)	0.1 ng/L[a] 3 ng/L[b]
	Swiegers et al. (2005)	Box tree	3 ng/L[b]
3-mercaptohexan-1-ol (3MH)	Tominaga et al. (1998a)	Fruity	
	Tominaga et al. (1998b)	Citrus zest, grapefruit	17 ng/L[a] 60 ng/L[b]
	Tominaga et al. (2000)	Grapefruit, passion fruit	
	Swiegers et al. (2005)	Passion fruit, grapefruit, gooseberry, guava	60 ng/L[b]
3-mercaptohexyl acetate (3MHA)	Tominaga et al. (1996)	Grapefruit, passion fruit, box tree	2-4 ng/L
	Tominaga et al. (1998a)	Fruity	4.2 ng/L
	Tominaga and Dubourdieu (2000)	Passion fruit	
	Tominaga et al. (2000)	Box tree	
	Swiegers et al. (2005)	Passion fruit, grapefruit, gooseberry, guava	4 ng/L[b]

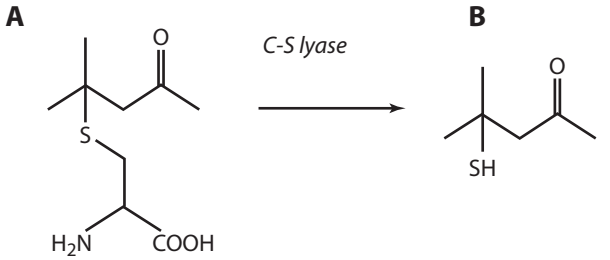
[a] Measured in aqueous solution
[b] Measured in alcohol solution

variability in sensory perceptions. The (R)-3MH form was reported to exhibit the zesty, grapefruit characteristics of 3MH, whereas the (S)-3MH was found to exhibit the classical passion-fruit polyfunctional thiol character. Perception thresholds for both 3MH enantiomers were similar, whereas (S)-3MHA was more potent than (R)-3MHA and contributed boxwood aromas as opposed to passion fruit (Tominaga et al. 2006). Understanding the factors that favour formation of one enantiomer over another is critical in developing winemaking strategies aimed at maximizing desirable varietal characters.

Polyfunctional thiol release from amino acid and peptide conjugates

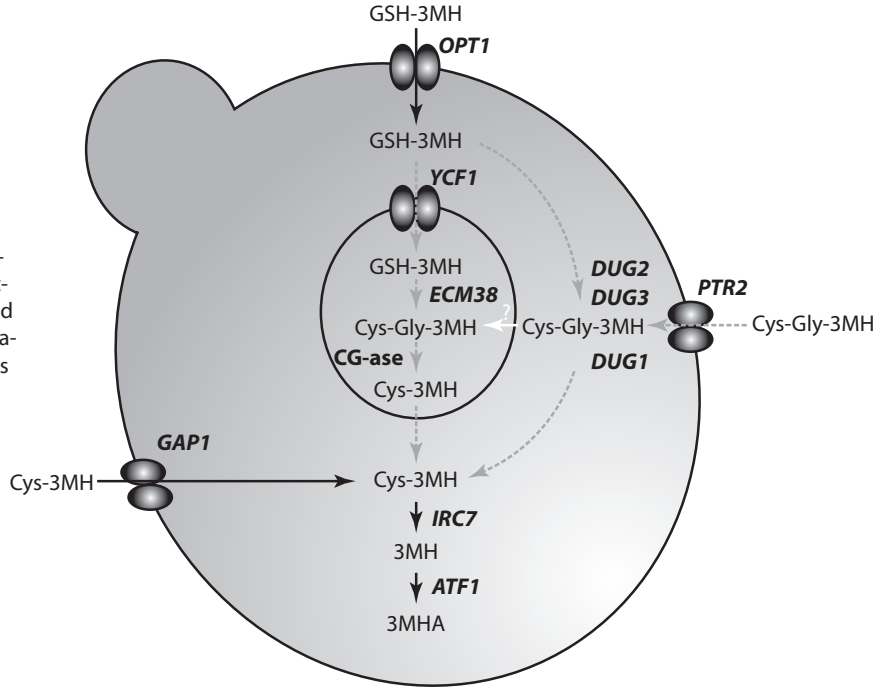
Simplistically, the polyfunctional thiols 4MMP and 3MH are found as amino-acid conjugates in grape berries (Tominaga et al. 1998a), and are released through the activity of yeast carbon sulphur-lyase (CS-lyase) enzymes (Howell et al. 2005, Swiegers et al. 2006), as illustrated in Figure 2. Subsequent acetylation of 3MH yields the sensorially potent 3MHA compound (Swiegers et al. 2005b). Recent studies have indicated the likelihood of a more complex, compartmentalized biochemical network leading to polyfunctional thiol release, conversion and sensory perception. Figure 3 depicts this network for 3MH and 3MHA formation, with many elements inferred from related cellular processes. Clearly, if we are to fully realize the potential aroma and flavour relating to polyfunctional thiol release and conversion, this system requires extensive further study.

Figure 2. Representation of polyfunctional thiol release during fermentation by carbon sulphur (CS) lyase enzymes. Compound A is Cys-4MMP and compound B is the volatile form of 4MMP. Adapted from Howell et al. (2004).



Of key importance in the quest to unlock maximum aroma and flavour is the relationship between all potential precursors, and the eventual quantity of polyfunctional thiol released. The existence of odourless grape-derived precursors for polyfunctional thiols was first demonstrated by Darriet et al. (1993) while observing the liberation of 4MMP during alcoholic fermentation. Subsequently, Tominaga et al. (1998a) identified cysteine conjugates of both 4MMP (Cys-4MMP) and 3MH (Cys-3MH) present in Sauvignon must. An S-glutathione conjugated precursor of 3MH (S-3-(hexan-1-ol)-glutathione) has also been identified in Sauvignon Blanc juice by Peyrot des Gachons et al. (2002), and recent results have inferred that as much as 50% of total 3MH in wine may be derived from such a precursor (Subileau et al. 2008a). It is also likely that a cysteinyl-glycine conjugated 3MH precursor (Cys-Gly-3MH) exists in grape berries and juice as an intermediate breakdown product of the glutathione conjugate.

Figure 3. Schematic of 3MH and 3MHA formation taking into account transport of the inferred glutathione conjugated precursor and potential routes for its degradation.
Pathways in solid black have been investigated in the context of polyfunctional thiol formation, those in dashed gray are inferred from studies of glutathione turnover, while white indicates a hypothetical transport route.



As depicted in Figure 3, conjugated precursors have been shown to enter the yeast cell via amino-acid and peptide transporters. Subileau et al. (2008b) found that transport of cysteinylated thiol precursors occurs in part via Gap1p, a general amino acid permease, by observing decreased 3MH and 3MHA production in a *GAP1* gene deletion strain. As will be discussed, this gene is repressed in response to preferred nitrogen sources (Beltran et al. 2005). Several other permeases have been implicated in cysteine uptake and could also be involved in uptake of Cys-3MH. Of particular interest is the recently characterized protein encoded by *YCT1* (Kaur and Bachhawat 2007), a high-affinity cysteine transporter which appears to be the main route of cysteine uptake under both nitrogen-rich and nitrogen-poor conditions. In contrast to the complexity of cysteine uptake, glutathione uptake by *Saccharomyces cerevisiae* appears to be mediated exclusively by the oligopeptide transporter Hgt1p (Bourbouloux et al. 2000), also known as Opt1p. Deletion of the *OPT1* gene decreased the release of 3MH during grape must fermentation by 50% (Subileau et al. 2008b), inferring that the major 3MH precursor(s) entered the yeast cell via this transporter and that they are most likely glutathione conjugates. The potential involvement of Ptr2p in the transport of Cys-Gly-3MH can be inferred from experiments where cysteinylglycine uptake was completely blocked by the deletion of the *PTR2* gene, but not by the deletion of the *OPT1* gene (Ganguli et al. 2007).

Having entered the yeast cell, various biochemical pathways may be involved in the catabolism of conjugated precursors and the eventual release of polyfunctional thiols. The most studied pathway is also the most simple, being the cleavage of 3MH and 4MMP from their cysteinylated precursors. Howell et al. (2005) investigated the effect of potential CS-lyase genes on the release of 4MMP, by using laboratory deletion strains that encode putative yeast carbon-sulphur lyases. Four genes were identified, *BNA3, GLO1, CYS3* and *YFR055c* (*IRC7*), which influence the release of 4MMP, suggesting that the mechanism of release involved multiple genes. The *BNA3* gene deletion strain released 40% less 4MMP than the wild-type strain, and the other genes released 50% less. Thibon et al. (2008) examined some of these genes in an experimental system that contained wine-like concentrations of the Cys-3MH and Cys-4MMP precursors. The results conflicted with those of Howell et al. (2005), explained as being a consequence of the different precursor concentrations. Importantly though, *IRC7* was again found to be involved in release of the polyfunctional thiols by yeast; an *IRC7* gene deletion strain accumulated less than 5% of the total 4MMP released by the wild-type strain, whereas deletions of the *BNA3* and *CYS3* genes did not affect thiol release (Thibon et al. 2008). Furthermore, in this study the *IRC7* mRNA transcript was up-regulated under conditions where thiol release was enhanced. Final confirmation of the role this gene plays in thiol release from cysteinylated precursors requires an overexpression study and the biochemical characterization of Irc7p.

The only study to date where the release of polyfunctional thiols has been assessed in response to the overexpression of a CS-lyase encoding gene was conducted at the AWRI by Swiegers et al. (2007). In this study, the *Escherichia coli tnaA* gene, encoding a tryptophanase with strong cysteine-β-lyase activity, was incorporated into the genome of a commercial wine yeast strain. This modified strain released up to 25 times more 4MMP and 3MH in model ferments than the control strain, and the ferments exhibited an intense passion-fruit aroma.

The profound impact of overexpressing *tnaA* renders the argument that cysteinylated precursors are not the main source of polyfunctional thiols released during fermentation as somewhat academic. If intracellular precursor concentrations were limiting, it would be expected that such an overexpression would have minimal impact, thus sufficient Cys-4MMP and Cys-3MH was present in the cytosol of the yeast to allow the *E. coli* tryptophanase to release significantly more 4MMP and 3MH than the wild-type strain. However, in order to develop novel wine yeasts with enhanced thiol-releasing capability in the absence of recombinant technology, all biochemical steps leading to thiol formation need to be understood. Therefore, if the effect of *OPT1* gene deletion on thiol formation is due to decreased transport of glutathione-conjugated precursors (as hypothesized by Subileau et al. 2008b), the fate of such precursors once in the yeast cytosol must be considered.

Glutathione (GSH) itself is a multi-functional tri-peptide (L-γ-glutamyl-L-cysteinyl-glycine), critical to redox homeostasis in eukaryotic cells (Sies 1999, Meyer and Hell 2005, Wheeler and Grant 2004). GSH also plays a key role in detoxification, as a substrate for glutathione-*S*-transferase (GST) catalyzed conjugation to a wide range of reactive compounds (Sheehan et al. 2001). Presumably one or more GSTs may be involved in the formation of glutathione-conjugated precursors for 3MH and 4MMP in grape berries, although the biosynthetic pathways for these compounds have not been elucidated. Furthermore, post-crushing reactions may be involved, similar to those which occur during formation of 2-*S*-glutathionyl-caftaric acid (du Toit et al. 2006).

While no studies have addressed the formation or fate of glutathione-conjugated precursors for 3MH and 4MMP, intracellular processing of glutathione in yeast has received considerable attention. Glutathione plays a role in several yeast organelles (Perrone et al. 2005), and multiple cellular functions affect intra- and extra-cellular glutathione concentration and redox status. To exert fine control over glutathione concentrations, yeast cells are able to synthesize, degrade, import and export glutathione (Ganguli et al. 2007). Of relevance to polyfunctional thiol release are the processes through which glutathione (and its conjugates) might be degraded. In *S. cerevisiae* the vacuolar membrane protein Ycf1p mediates the transport of glutathione conjugates into the vacuole (Li et al. 1996), where the enzyme γ-glutamyltranspeptidase (γ-GT) cleaves the γ-glutamyl moiety of GSH leaving cysteinyl-glycine (Mehdi et al. 2001). Plant and animal γ–GTs are known to have high-affinity and broad substrate specificity against both glutathione and glutathione-conjugates (Martin et al. 2007). This would also appear to be the case in yeast, as a single gene, *ECM38*, encodes this enzymatic activity. Direct evidence of *ECM38* involvement in conjugate degradation was demonstrated through deletion experiments where it was essential in the vacuolar degradation of glutathione-conjugated xenobiotics through to cysteine and *N*-acetylcysteine-bound products (Ubiyvovk et al. 2006). Following the γ–GT cleavage of glutamate, cysteinyl-glycine is then further degraded in the vacuole to its amino acid components, through cysteinylglycine dipeptidase (CGase) (Jaspers et al. 1985), although no open reading frame has been assigned to this function in yeast to date.

Surprisingly, loss-of-function mutations in the *ECM38* gene did not impart a deleterious growth defect in the presence of glutathione as the sole source of sulphur (Kumar et al. 2003). This result inferred the existence of an alternative glutathione-degradation pathway. The genetic and biochemical basis of this pathway was demonstrated in a study by Ganguli et al. (2007), where three previously uncharacterized open reading frames were found to encode the enzymes Dug1p, Dug2p and Dug3p. These proteins interact with one another to form a cytosolic glutathione degradosomal complex. Dug1p alone is capable of degrading cysteinyl-glycine to its constituent amino acids, so it may be able to cleave Cys-Gly-3MH to Cys-3MH. On the other hand, all three proteins are required to degrade glutathione through to cysteine. Thus, two cellular processes may be relevant in the formation of Cys-3MH from glutathione-3MH, with Cys-3MH ultimately cleaved by enzymes with CS-lyase activity. The possibility that 3MH is cleaved from glutathione directly cannot be excluded, although conventional knowledge of glutathione-conjugate detoxification suggests the more likely scenario would be degradation via the vacuolar catabolic pathway.

Completing the pathway shown in Figure 3 is the final conversion of 3MH into 3MHA, which has not been studied in great detail. Swiegers et al. (2005b) demonstrated that the overexpression of the yeast alcohol acetyltransferase *ATF1* increased the synthesis of 3MHA, while the overexpression of the esterase encoding *IAH1* decreased 3MHA formation from 3MH. The ratio of 3MH and 3MHA in wine might represent the balance between Atf1p and Iah1p activities. However, little information is available on the transport of 3MH and 3MHA across the plasma membrane or whether other esterification/de-esterification reactions are involved.

The compartmentalized metabolic network encompassing amino acid- and glutathione-conjugated precursor release and conversion extends beyond the role of yeast. A recent study has also shown the ability of salival bacteria to convert the *S*-cysteine-conjugated precursors to their corresponding volatile compounds (Starkenmann et al. 2008). Results demonstrated that within 20 to 30 seconds of exposure to cysteine conjugates, polyfunctional thiols are produced in the mouth, retaining their odour for up to 3 minutes. Thus, a key aspect of sensorial perception for thiol-driven characters occurs during the process of wine tasting, rather than during winemaking. It is conceivable, then, that the full release of all bound thiol precursors may not be desirable. Unfortunately, current knowledge of aroma and flavour perception involving in-mouth interactions is insufficient to guide winemaking strategies. With the current focus still on maximizing polyfunctional thiol release, two variables amenable to manipulation are the selection of yeast strains and the management of nutrients during fermentation.

Effect of yeast strain on polyfunctional thiol release and conversion

Studies have shown that the choice of yeast strain has a clear effect on the aroma and flavour of Sauvignon Blanc wines, including the fermentation product profile and the polyfunctional thiol profile. Investigations by Howell et al. (2004) showed variation in 4MMP release by fermentation in small-scale laboratory ferments with different commercial wine yeast strains and the addition of the Cys-4MMP precursor to the medium. A preliminary trial in Sauvignon Blanc wine was conducted in 2005 (Swiegers et al. 2008) to study the effects of seven commercial yeast strains on fermentation flavours and aromas. Differences in the chemical profiles of fermentation products and polyfunctional thiols were observed, along with dif-

ferences in sensory profiles for such polyfunctional thiol characters as passion fruit. It was concluded that some yeasts are more efficient at releasing the polyfunctional thiol from the *S*-cysteine-conjugated precursor than others. Similarly, other yeast strains were more efficient at converting 3MH to 3MHA, to create the sensorially more potent acetate ester.

Subsequently, the impact of coinoculating commercial yeast strains on the volatile composition and sensory profile of Sauvignon Blanc wines was also demonstrated (King et al. 2008b) in small-scale fermentations using single-strain and coinoculations of Vin7 with QA23 and with Vin13. These strains were selected based on their differing abilities to release and convert polyfunctional thiols during fermentation. The results demonstrated that the chemical and sensory profiles of the coinoculated wines were different from both the single-strain wines and equal blends of the single-strain wines. Polyfunctional thiol analysis indicated that the Vin7/QA23 coinoculated wines were highest in 3MH and 3MHA, although this pattern was not observed for the Vin7/Vin13 yeast combination. Due to the complexity of thiol-releasing and -converting pathways discussed in the previous section, the determinants of these phenotypes are yet to be determined.

Influence of nutrients on polyfunctional thiol release

From an evolutionary perspective, yeasts may benefit by taking up non-volatile precursors from their environment and converting them to volatile aroma and flavour compounds. Insect attraction to fruit-volatile compounds has been shown in several studies, for example Natale et al. (2004). Therefore, an efficient releaser of polyfunctional thiols, delivering strong fruit-like aromas, may attract more insects. Such yeasts may "hitch a ride" and spread to other environmental niches that the insect visits. Nevertheless, it is likely that natural selection would favour the primary benefit yeasts derive from the catabolic degradation of amino acid and glutathione conjugates, namely access to important cellular building blocks.

Yeasts maintain strict control over nitrogen uptake and metabolism via a process referred to as nitrogen catabolite repression (NCR), which ensures that yeast consume rich nitrogen sources when they are present and that they can switch into nitrogen scavenging mode when they are not. This switch occurs during typical wine fermentation (Beltran et al. 2004), unless nitrogen is added in the form of diammonium phosphate (DAP), which extends the period of NCR (Beltran et al. 2005).

NCR is transcriptionally mediated. For example, the transcription factors Gln3p and Gat1p are deactivated by Ure2p when in the presence of rich nitrogen sources, preventing expression of an array of genes involved in nitrogen metabolism. One of the target genes transcriptionally repressed by Ure2p is *GAP1* (Jauniaux and Grenson 1990), which, as discussed earlier, is at least partially responsible for the transport of Cys-3MH into yeast cells (Subileau et al. 2008b). In this study, mutants exhibiting relief of NCR released twice as much 3MH. Similarly, the deletion of *URE2* by Thibon et al. (2008) enhanced the release of 3MH and 4MMP three-fold, although the authors concluded only a small portion of this increase could be attributed to the enhanced uptake of precursors. Instead, it appeared the increased efficiency of release by yeast CS-lyase enzymes was responsible, and through deletions and gene expression analysis Irc7p was strongly implicated. Interestingly, *URE2* deletion also caused an imbalance in release of the (R)- and (S)-3MH enantiomers, favouring the (R)-form, perhaps indicating an enantiomeric preference of the Irc7p enzyme.

With at least two genes in the thiol-releasing pathways linked to NCR, the implications of nitrogen availability become abundantly clear. The addition of DAP to a fermentation, triggering NCR, will result in lower concentrations of polyfunctional thiols being released. That said, the desire to express maximal varietal character in a Sauvignon Blanc wine by minimizing DAP additions must be tempered by the risk of hydrogen sulphide (H_2S) production, which is a feature of low nitrogen winemaking. As suggested by Swiegers and Pretorius (2007), sulphur compounds in wine are a double-edged sword.

Implications of nitrogen availability for the modulation of other yeast-derived aroma compounds

Recent studies have investigated the effects of nitrogen supplementation on yeast-derived volatile metabolites in highly clarified white grape juice and/or model grape juice (Rapp and Versini 1991, Hernández-Orte et al. 2005 and 2006, Vilanova et al. 2007, Carrau et al. 2008). In fermentations deficient in yeast-assimilable nitrogen (YAN), the addition of DAP was found to be an extremely powerful tool to modulate the production of esters. Torrea et al. (2004) found that DAP had a positive effect on ester production in Chardonnay wines, while decreasing the formation of higher alcohols. However, wines obtained with moderate nitrogen supplementation of the juice were preferred by panellists, compared to those obtained without or with high DAP addition (Bell and Henschke 2005). This preference might be due to a synergy between high

acetates and ethyl fatty-acid ester concentrations, and moderate levels of ethyl acetate; the latter associated with unwanted solvent-like characteristics and the masking of other aromas when present at very high concentrations.

These results suggest that, at least for Chardonnay, the flavour and style of wine is strongly modulated by the initial YAN concentration of the grape juice. Most of the studies reported to date have focused on DAP supplementation of low YAN juices, as DAP is widely used as a nutrient supplement. However, recent research suggests that ester production is less variable in juices in which the nitrogen content has been varied with amino acids, compared to DAP (Miller et al. 2007). The impact of high initial juice amino-acid content, such as that which results from high nutrient vineyards, on polyfunctional thiol production is largely unknown, but might be less than that produced by an equivalent nitrogen content in the form of DAP. The effects of nitrogen content on thiol production are also likely to be yeast-strain-dependant, as is the case for hydrogen sulphide (Bell and Henschke 2005, Spiropoulos et al. 2000) and ester production (Vilanova et al. 2007, Carrau et al. 2008). Similar effects can be expected in other white varieties, particularly where fermentation-derived volatiles, such as esters and higher alcohols, are a major component of the wine aroma profile. In contrast, relatively few studies have examined nitrogen supplementation and red wine flavour. In a recent study (Ugliano et al. 2008) it was shown that DAP supplementation of a low-nitrogen Shiraz must resulted in higher production of ethyl fatty-acid esters and acetate esters, although higher alcohols were scarcely affected. Extension of the generally observed trend regarding nitrogen concentration and ester formation into experiments with red wine suggests that for a wide range of wine styles, the guided addition of nitrogen may be used by winemakers as a powerful tool to optimize quality and meet consumer preferences.

Conclusions

Wine sensory characters associated with the polyfunctional thiols 4MMP, 3MH and 3MHA polarize Australian wine consumers with regard to their preferences in blind tastings. The careful management of winemaking practices for varieties such as Sauvignon Blanc are required in order to produce wine that meets the requirements of target markets.

The choice of yeast strain, and indeed the coinoculation of multiple yeast strains, have proven to be valuable tools for winemakers seeking to maximize the contribution of polyfunctional thiols to their wines. Recent studies highlighting the role of nitrogen-catabolite repression in polyfunctional thiol formation now require consideration of appropriate nitrogen management strategies. Standardized additions of DAP in the absence of YAN measurements risk limiting polyfunctional thiol formation, and in some cases may lead to excessive production of ethyl acetate. Furthermore, with DAP costs increasing rapidly, wineries should revisit the economics of YAN measurement and consider the potential cost savings of informed DAP usage.

The way forward is to develop strain-specific nutrient management strategies that are coupled with YAN measurement of juices and musts. It is critical that trials to refine such strategies include consumer acceptance testing, to ensure the ultimate benefit is wine that consumers prefer.

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