Volatile thiol formation by yeast during fermentation

The important role of yeast in modifying the chemical, mouth-feel and flavour complexity of wine is well established. The action of yeast on wine occurs at several levels: by producing yeast metabolites, by assisting in the extraction of compounds from solids present in grape must and by modifying grape derived compounds (Lambrechts and Pretorius, 2000). A class of compounds that have an important influence on wine aroma and flavour are the many yeast derived sulfur containing compounds, including hydrogen sulfide and the mercaptans. More recently, the group of researchers working under the direction of Professor Dubourdieu in Bordeaux, France has described a number of sulfur containing volatile thiols in wine. The presence of these volatile thiols was first discovered in wines made with *Vitis vinifera* cv. Sauvignon Blanc grapes, where they provided the basis for the varietal aromas of Sauvignon Blanc wines (Darriet et al., 1995). One of the most potent thiol compounds is 4-mercapto-4-methylpentan-2-one (4MMP), which has a perception threshold of 0.1 ng/L and 3 ng/L in water and wine respectively. Further odorants of Sauvignon Blanc wines were found to be 3-mercaptohexan-1-ol (3MH) and its acetate, 3-mercaptohexyl acetate (3MHA) (Tominaga et al., 1996); 3-mercapto-3-methylbutan-1-ol (3MMB), and 4-mercapto-4-methylpentan-2-ol (4MMPOH) (Tominaga et al., 1998a) (see Table 1).

Table 1: Grape derived sulfur containing volatiles produced by yeast.

Compound	Odour	Aroma threshold ($ng\ L^{-1}$)	Concentration range in wine ($ng\ L^{-1}$)	Reference
4-mercapto-4 -methylpentan-2-one (4MMP)	cat urine box tree/ blackcurrant, broom	0.05 (water) 3.3 (wine)	0–30	Darriet et al., 1995; Tominaga et al., 1996; Tominaga and Dubourdieu, 1997; Tominaga et al., 2000
4-mercapto-4 -methylpentan-2-ol (4MMPOH)	citrus zest	20 (water) 55 (water/ethanol)	0–86	Tominaga et al., 1998a; Tominaga et al., 2000
3-mercaptohexan-1-ol (3MH)	passionfruit grapefruit	17 (water) 60 (water/ethanol)	50–5000	Tominaga et al., 1998a; Tominaga et al., 2000
3-mercaptohexyl acetate (3MHA)	Riesling-type note exotic fruits box tree	4.2 (water) 2-4 (wine)	1–100	Tominaga et al., 1996; Engel, 1999; Tominaga et al., 2000; Dubourdieu et al., 2000
3-mercapto-3-methylbutan-1-ol (3MMB)	cooked leeks	1300 (water) 1500 (water/ethanol)	0–1322 al., 2000	Tominaga et al., 1998a, Tominaga et
3-mercapto-2-methylpropanol (3MMP)	broth, sweat	3000 (water)	250–10000	Bouchilloux et al., 1998

These volatile thiols have also been found in wines made with other cultivars of *V. vinifera* including Scheruebe (Guth, 1997a; Guth, 1997b), Gewürztraminer, Pinot Gris, Riesling, Muscat, Sylvaner, Pinot Blanc, Petit Manseng and Semillon (Tominaga et al., 2000), Cabernet Sauvignon and Merlot (Murat et al., 2001), and Rioja wines (Aznar et al., 2001). In Scheruebe wine, 4MMP was found to be the most potent aroma of 42 isolated odorants analysed. When each of the 42 odorants were individually excluded from a model wine, the absence of 4MMP produced a wine least like the original (Guth, 1997b; Guth, 1997a).

Consistent with their aroma descriptions (see Table 1), 4MMP was found to be present in extracts of box tree and broom (Tominaga and Dubourdieu, 1997) and 3MMB, 3MH and 3MHA were present in a passionfruit extract (Tominaga et al., 2000; Engel and Tressel, 1991). Additionally, 4MMP is a potent odorant of grapefruit. A reconstitution experiment showed that when 4MMP was omitted, the mixture was described as 'orange-like' rather than grapefruit; this was attributed to the interaction of 4MMP with other compounds present in the mix (Buettner and Schieberle, 2001). This finding contrasts with previous reports attributing 'blackcurrant', 'catty', and 'box tree' descriptors to 4MMP when present alone or in solution (Tominaga et al., 1998a; Guth, 1997b; Darriet et al., 1995), but shows consistencies with recent GC sniff investigations which describe the aroma of 4MMP as 'citrus fruit' and 'vinaigrette', as well as 'black currant', 'catty', and 'broom ' (Vermeulen et al., 2001).

Cysteine conjugated precursors present in grapes

Volatile thiols have not been isolated from grape juice. However, Professor Dubourdieu and his research team have elucidated that the sulfur-containing volatiles are present as a cysteine conjugate in grape juice and are released during fermentation by the action of yeast (Tominaga et al., 1995; Tominaga et al., 1998b). The role of the cysteine conjugated compounds in plants is unknown, but similar cysteine bound sulfoxides are present in onion and garlic, where their release by endogenous enzymes acts as a feeding deterrent in animals (Jocelyn, 1972; Luckner, 1990). In grapes, formation of the precursors has been hypothesised as being part of a detoxification pathway, via a glutathione precursor. Peyrot des Gachons et al. (2002) propose that in the grapevine, xenobiotics are inactivated by enzymatic conjugation to glutathione, then sequentially broken down to a S-cysteine conjugate. This has been demonstrated in other plants and animals.

With the lack of understanding of the role of the cysteine conjugated compounds in grapevines, it is not presently possible to predict the concentrations of flavour precursors in grapes. The concentrations vary from year to year and, in contrast to the concentrations of methoxypyrazines in Sauvignon Blanc grapes, the thiol precursors do not appear to accumulate or dissipate over ripening (Peyrot des Gachons, 2000). However, winemakers can utilise the results on the localisation of the thiol precursors in grapes to regulate their extraction. Between 60% (Merlot, Cabernet Sauvignon) and 90% (Sauvignon Blanc) of cysteine bound 3MH precursor is localised in the skin of the berry. In the production of Rosé wines made from Cabernet Sauvignon, Cabernet Franc and Merlot, it was shown that increased skin contact time (from 0 to 24 hours) correlated with a higher extraction of the 3MH precursor, as well as an

...continued on page 37

...continued from page 16

increased concentration of volatile 3MH following yeast fermentation. Extraction of 3MH also increased when the temperature was raised from 10ºC to 25ºC. (There was low correlation between the concentration of anthocyanins and the concentration of the 3MH precursor, indicating that extraction into the juice does not occur by similar mechanisms). In the vinification of Sauvignon Blanc, skin contact for 18 hours increased the concentration of the precursor in the pressed juice; a higher temperature (from 10ºC to 18ºC) made little difference (Murat et al., 2001). Similar experiments have not been performed with cysteine conjugated 4MMP, but the distribution of this precursor compound between the skin and the pulp appears to be more similar. For example, in Sauvignon Blanc grapes only 50% of the 4MMP precursor is localised in the skin, compared to 90% for the 3MH precursor, clearly demonstrating that differential extraction of these to potent aroma compounds from the grape can be achieved through established wine-making practices.

Release of volatile thiols by yeast

The conversion of the non-volatile grape precursors to volatile thiols requires the action of yeast. Release of these important aroma compounds during fermentation is thought to be by a family of enzymes known as the cysteine ß-lyases (also producing pyruvic acid and ammonium) (Tominaga et al., 1995; Tominaga et al., 1998b), of which there are thought to be several forms in *Saccharomyces cerevisiae*. Although the extraction of the cysteine conjugated precursors into the juice appears to be correlated to the final concentrations of the volatile thiols present in the wine, only a small and varying proportion of the precursor is converted to the active aroma compound during fermentation. Where this release has been studied, only 5% of the potential 4MMP was released during fermentation. Similarly, between 0–9% of 3MH, was released over the course of fermentation (Murat et al., 2001). The release of volatile thiols during fermentation appears to be strain dependent (Dubourdieu et al., 2000). In addition, a particular yeast's ability to release one thiol does not appear to be linked with its ability to release a second, different thiol. For example, commercial *S. cerevisiae* wine strains VL3c and EG8 release more 4MMP and 4MMPOH, but not 3MH, than strains VL1 and 522d (Dubourdieu et al., 2000). These results indicate that the activity of these enzymes during fermentation is strain dependent and that by using different strains, variation in the release of these enzymes can be achieved. Furthermore, separate yeast enzymes may be involved in the formation of different volatile thiols, allowing the levels of the aroma compounds to be altered independently. Post-fermentation practices could also affect the impact of the thiol aromas, with anecdotal evidence showing that aroma intensity is reduced as copper is added to Sauvignon Blanc wine (Darriet et al., 1999).

A project at the Institute has been initiated to provide winemakers with the ability to regulate the amounts of the volatile thiols released during fermentation. Together with a team of chemists: Gordon Elsey, Mark Sefton and Tracey Siebert, sensitive methods are being developed to measure accurately the concentrations of the most important volatile thiols present in wine. Using these techniques, the mechanisms of release of the volatile thiols from the cysteine bound conjugates are being studied. It is expected that this research will provide winemakers with the knowledge to select specific commercial yeast strains and to use various winemaking practices that will produce wines with the desired concentration of these important aroma compounds.

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