



Effect of fermentation, aging and thermal storage on total glycosides, phenol-free glycosides and volatile compounds of White Riesling (*Vitis vinifera* L.) wines

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There is growing recognition of the significance of the products of glycoside hydrolysis to varietal wine aroma. White Riesling wines were produced from four strains of *Saccharomyces cerevisiae*. Wines underwent conventional aging or anaerobic thermal storage (20 days at 45°C) either 2 or 40 months post-fermentation to quantify influences on total glycosides, phenol-free glycosides and selected volatiles. Glycoside and free volatile concentrations were estimated by analysis of glycosyl-glucose and gas chromatography/mass spectrometry, respectively. Thermal storage of wines 2 months post-fermentation reduced the total glycosides by an average of 33% for all yeasts and increased the concentration of free benzyl alcohol while decreasing the concentration of free linalool and geraniol. Conventional aging for 40 months reduced the total and phenol-free glycosides equally among yeasts by an average of 60%, with phenol-free glycosides averaging 80% of the total. Thermal storage of aged wines reduced the total glycoside concentration by an additional 29%. The effect of thermal storage on selected volatile phenols, higher alcohols, esters, acids, terpenes, carbonyl compounds, C-13 norisoprenoids and six-carbon alcohols was variable depending upon the component.

Keywords: glycosides; thermal processing; White Riesling; volatile compounds; glycosyl-glucose (GG)

Introduction

Grape aroma compounds are present as free volatiles, which may contribute directly to odor, or as nonvolatile, sugar-bound conjugates (mainly glycosides). Glycoside hydrolysis can produce free volatiles, some of which are odor-active compounds. Initial research focused on the hydrolysis of monoterpenes glycosides and the role of various aglycones in floral varieties such as White Riesling [41]. Subsequent research demonstrated involvement of glycosides of C13 norisoprenoids and shikimic acid-derived metabolites in the aroma of non-floral varieties [49].

Grape monoterpenyl sugar conjugates include 6-O- β -1-rhamnopyranosyl-d-glucopyranoside, 6-O- β -1-arabinofuranosyl-d-glucopyranoside and 3-(hydroxymethyl)-d-erythofuranose [5,41,47]. Volatile compounds can be liberated from these bound sugar conjugated forms by slow chemical hydrolysis, by hydrolysis catalyzed by the acids present in wine [10,17,46], or by enzymes with glycosidase activity [6,15,17,46]. The composition and sensory properties of the volatiles released by acid or enzymatic mechanisms are different. Enzyme hydrolysis releases mainly alcohols by breaking the glycoside bond without the aglycone undergoing any further chemical transformation [27]. Acid-catalyzed hydrolysis of glycoconjugates of alcohols is believed to involve cleavage of the ether rather than the glycosidic linkage between the glucose and the aglycone

[27]. The resultant carbocation can react to give a range of compounds [27].

Several studies have been conducted on the composition of grape-derived volatile compounds liberated from glycosidically bound precursors [1,2,28–30,55], with hundreds of components identified to date [27]. The vast majority of these compounds are odorless or possess only weak aromas, although a significant number of compounds have not been identified [27]. However, the sensory significance of the products of glycoside hydrolysis to varietal aroma has been established [1,3,11,13,24,51], providing justification and rationalization for their quantitation and study [52]. Williams *et al* [53] suggested the possible sensory significance of the hydrolysis products of glycosides without phenol aglycones (phenol-free glycosides).

Many yeast genera and species, including *Saccharomyces* sp, the traditional wine yeast, possess glucosidase activities [18]. Although Laffort *et al* [20] suggested that yeast strains can influence wine aroma as a result of the hydrolysis of conjugated aroma precursors, Delcroix *et al* [9] demonstrated that glucosidase activity among three unidentified wine yeasts was limited. Zoecklein *et al* [57,59] showed that the effects of four commercial strains of *Saccharomyces cerevisiae* on grape glycosides were similar. It appears that in conventional winemaking a large percentage of the grape aroma potential remains in the conjugated, non-volatile form [18,48,58].

Heat has been used as a means of increasing or accelerating wine aging resulting in changes in color and flavor [35,36,38]. The extent and nature of these changes is a function of time, temperature and the presence or absence of oxygen. Compounds associated with aging are enhanced by mild anaerobic heating [32,39] possibly as a result of

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hydrolysis of grape glycosides [12,13,58]. Leino *et al* [21] and De la Presa-Owens and Noble [8] noted anaerobic heating of Chardonnay wines decreased the floral character, while increasing the perception of aromas described as oak, honey, and smokey.

The objectives of this study were to evaluate the influence of fermentation, age and post-fermentation thermal storage (20 days at 45°C for 2 or 40 months) on glycosides and free volatiles in White Riesling wines produced from four strains of *S. cerevisiae*. The evaluation involved quantitation of the total pool of glycosides, the total pool of glycosides of non-phenol origin (phenol-free glycosides), as well as selected individual glycosides and free volatiles.

Materials and methods

White Riesling grapes (340 kg) were hand harvested at 17.1°Brix (soluble solids), chilled to 9°C for 12 h and crushed. Skin contact occurred for 4 h at 9°C, followed by pressing to two bars in a Willmes 100-L bladder press. Free-run and press juices were combined, cold clarified (1°C for 24 h) and subsequently decanted from the sediment. The addition of dimethyldicarbonate (DMDC), at a concentration of 400 mg L⁻¹, was used to kill yeasts, lactic acid and acetic acid bacteria as described by Fugelsang [14]. Treated juice was divided into sixteen 11.5-L carboys with 7.3 L of juice per container.

Yeast cultures were grown from 5.0 g of dry active yeast rehydrated in sterile water at 37–40°C for 15 min, transferred to membrane-filtered (0.45 µm) juice and developed for 10 h at 30°C prior to inoculation. Four sterile carboys were randomly selected for each treatment and inoculated with a 3% (v/v) actively growing culture containing 2 × 10⁶ cells ml⁻¹ of a single strain of *Saccharomyces cerevisiae*. Fermentations were conducted at 10°C without sulfur dioxide addition.

The yeasts examined included the following strains: VL1 and D47 (Lallemant, Montreal, Canada); Fermiblanc, FB (Gist-brocades, Cedex, France); and Prise de Mousse, PDM (UCD 796, Universal Foods Corp, Milwaukee, WI, USA).

Each carboy was analyzed pre- and post-fermentation for °Brix, alcohol% (v/v), pH, and titratable acidity as described by Zoecklein *et al* [56]. Before and after fermentation and thermal storage, the tartaric and malic acid content was determined by HPLC using a BioRad (Richmond, CA, USA) isocratic system, model 1306 UV detector at 220 nm with an Aminex ion exclusion HPX-87 (300 mm × 7.8 mm) column. Glucose, fructose, glycerol, succinic and acetic acids were determined using the HPLC system with a model 1770 refractive index detector.

Following fermentation, 25 mg L⁻¹ sulfur dioxide was added to each replicate. All wines were membrane filtered (0.45 µm) into CO₂-sparged bottles (750 ml) and sealed. Wines were stored at 10°C (conventional storage) except those used for thermal storage. Three bottles of each yeast replicate were segregated for thermal storage for either 2 or 40 months post-fermentation. Thermal treatment involved storage at 45°C for 20 days.

The total and phenol-free glycoside concentrations were measured prefermentation, at 2 and 40 months post-fermentation and pre- and post-thermal treatment at 2 and 40

months. The total glycoside concentration was estimated by analysis of glycosyl-glucose (GG) as described by Abbott *et al* [1] and Williams *et al* [50] and modified by Iland *et al* [19]. The concentration of glycosides without functional groups ionizable at pH 10.0 was reported as phenol-free glycosyl-glucose (PFGG) [53]. The procedure requires sample adjustment to pH 10.0 prior to adsorption to C18 RP.

Wines pre- and post-thermal storage were also analyzed for selected free volatiles and glycosides by selective retention on Amberlite XAD-2 (Rohm-Hass, Philadelphia, PA, USA) as described by Gunata *et al* [15]. Free volatiles and bound compounds were eluted with 50 ml pentane : dichloromethane (2:1) and 50 ml ethyl acetate : methanol (9:1), respectively [44]. The pentane : dichloromethane eluate was dried and concentrated prior to gas chromatography/mass spectrometry. The ethyl acetate : methanol fraction was concentrated *in vacuo* to dryness, extracted with pentane : dichloromethane to eliminate traces of free compounds and re-dried prior to enzymatic hydrolysis. The eluate was dissolved in 0.2 ml of a 0.2-M citrate-phosphate buffer and 1.0 mg of pectic enzyme (Rohapect C, Rohm Tech, Malden, MA, USA) was added. The mixture was incubated at 40°C for 16 h. The released aglycones were extracted with pentane: dichloromethane, an internal standard (10 µl of 0.1% 2-octanol) was added and the sample was concentrated to 40 µl for analysis.

Analysis of volatiles was performed using a Hewlett-Packard (Richmond, VA, USA) model 5790 gas chromatograph, a DB-5, 0.25 µm × 30 m column (J & W Scientific, Folsom, CA 95630, USA) and a model 5972 electron impact mass spectrometer. Carrier gas flow (He) was 1.30 ml min⁻¹, split ratio 90 : 1, with the oven temperature: 40°C for 3 min, to 175°C at 6°C min⁻¹, 175°C for 3 min, advanced to 210°C at 6°C min⁻¹. Concentrations of individual compounds were determined assuming a 1:1 response factor between the analyte and internal standards. Results are, therefore, regarded as semiquantitative only.

One-way analysis of variance and least significant difference (LSD) comparison tests of SAS (SAS Institute, Cary, NC, USA) were used to interpret differences in means, if any, at the 95% confidence level.

Results and discussion

The ethanol concentration was similar among wines, although the glycerol concentration differed, ranging from 4.7 to 6.1 g L⁻¹ for the D47 and Fermiblanc yeast strains, respectively, as previously reported [57]. Wines made from the VL1 strain had a lower titratable acidity and, along with the Prise de Mousse, had the lowest pH. The Fermiblanc yeast showed a unique pattern of carbohydrate utilization; it had the lowest glucose and highest fructose remaining following fermentation [57].

Fermentation decreased the total glycosides, resulting in similar concentrations among yeasts, with the exception of the Fermiblanc strain (Table 1). The analysis of total glycosyl glucose (GG) provided an estimation of the total pool of glycosides, including monoterpenes, aliphatic residues, sesquiterpenes, norisoprenoids and shikimic acid-related

Table 1 Effect of four strains of *Saccharomyces cerevisiae* on total and phenol-free glycoside concentrations (expressed as μmol glycosyl-glucose and phenol-free glycosyl-glucose, GG and PFGG) in Riesling wines following fermentation and 20 days thermal storage (45°C)

Stage	Yeast strains							
	Prise de Mousse		D47		Fermiblanco		VL1	
	GG	PFGG	GG	PFGG	GG	PFGG	GG	PFGG
End of fermentation	369 ^a ± 76.56	226 ^a ± 12.5	374 ^a ± 47.39	230 ^a ± 18.8	352 ^b ± 6.98	216 ^b ± 5.1	379 ^a ± 48.05	233 ^a ± 13.0
Thermal storage	231 ^b ± 6.41	142 ^b ± 8.9	241 ^b ± 11.90	148 ^b ± 7.3	298 ^a ± 18.26	183 ^a ± 3.9	218 ^c ± 9.32	134 ^b ± 9.9

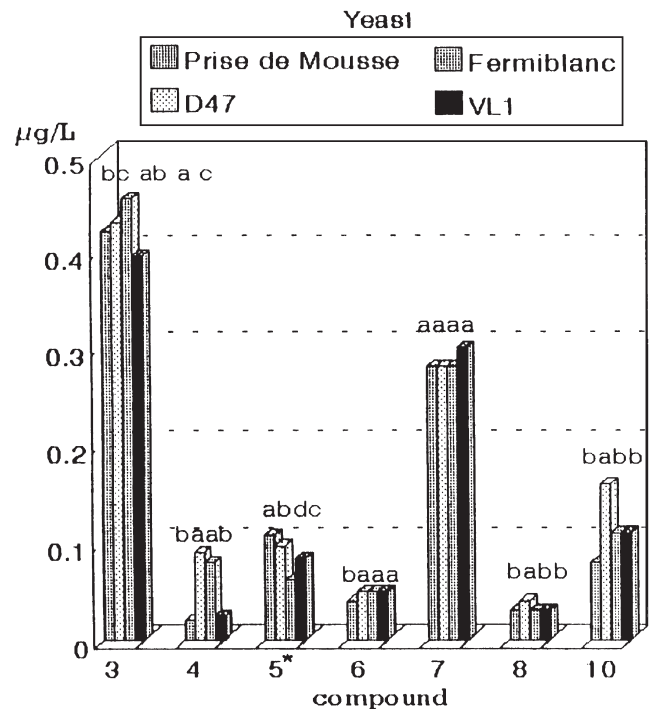
Significance of LSD test of treatment means at $P \leq 0.05$ and standard deviation. Means with the same letter are not significantly different.

compounds [50]. Thermal storage of wines for 2 months post-fermentation resulted in an average decline in the total glycoside content of 33.1%. The highest glycoside concentration after thermal storage was observed with the Fermiblanco strain, the yeast with the lowest concentration post-fermentation.

At the completion of fermentation, phenol glycosides averaged 38.5% of the total for all yeasts (Table 1). The ratio of total to phenol-free glycoconjugates was not influenced by thermal storage of the young wines. The size of the total pool of glycoconjugates may give little indication of the impact on aroma/flavor generation. Sefton [31] observed that despite the preponderance of benzenoid compounds from enzyme hydrolyzed C18 RP isolates, few were of sensory importance. The purpose of the phenol-free assay was to estimate the concentration of glycosides without functional groups ionizable at pH 10.0, leaving glycosidic compounds more directly related to aroma potential. While phenolic glycosides are important color and structural components, their impact on wine aroma may not be large [37]. In a related study, the analysis of the phenol-free Riesling glycoside fractions demonstrated they contained less than 9% of the total wine phenols, justifying use of the term (data not shown).

The principal free monoterpene and aromatic alcohols found in White Riesling grapes and wines are linalool, α -terpineol, geraniol, nerol, 2-phenylethanol and benzyl alcohol [43]. With the exception of α -terpineol, yeast strain had a significant effect on the concentration of these free volatiles 2 months post-fermentation (Figure 1). Additionally, both forms of free pyran linalool oxide were affected by yeast strain. However, yeast did not influence the concentration of bound monoterpenes or bound aromatic alcohols in the young wines (Figure 2). Free monoterpene and aromatic alcohol concentrations were not matched directly by decreases in the conjugate forms, supporting the findings of Gunata *et al* [16]. This may be the result of cyclization, isomerization and/or entrainment loss of free volatiles during fermentation.

Thermal storage of wines 2 months post-fermentation resulted in quantitative differences in free volatiles among yeasts, with the exception of free linalool (Figure 3). Comparing all yeasts, thermal treatment decreased the concentration of free linalool and geraniol while benzyl alcohol was increased. Thermal storage influenced the concentration of bound components, reducing linalool, α -terpineol, nerol, geraniol, and the bound forms of furan linalool


Figure 1 Effect of four strains of *Saccharomyces cerevisiae* on free monoterpenes and aromatic alcohols of Riesling wines 2 months post-fermentation. Aroma and flavor compounds analyzed: (1) Furan linalool oxide I; (2) Furan linalool oxide II; (3) Linalool; (4) Benzyl alcohol; (5) 2-phenyl ethanol; (6) Pyran linalool oxide I; (7) Alpha terpineol; (8) Pyran linalool oxide II; (9) Nerol; (10) Geraniol. Significance of LSD-test of treatment means. Different letters for each compound indicate significant difference at $P \leq 0.05$ level. *Concentration divided by 100.

oxides (Figure 4). There were no differences in bound components of heat-treated wines as a result of yeast strain. Thermal storage did not significantly influence the concentration of either isomer of pyran linalool oxide.

Aging wines for 40 months post-fermentation reduced the total glycoside concentration by an average of 60%, resulting in no difference among yeasts (Table 2). The portion of the total glycosides which did not contain phenolic compounds (phenol-free glycosyl-glucose) averaged 20.0% in the aged wines and was similar among yeast strains. Thermal storage of wines 40 months post-fermentation reduced the total glycosides by an average of 28.9% compared to conventionally stored wines of the same age. This was slightly less than the 33% reduction which occurred

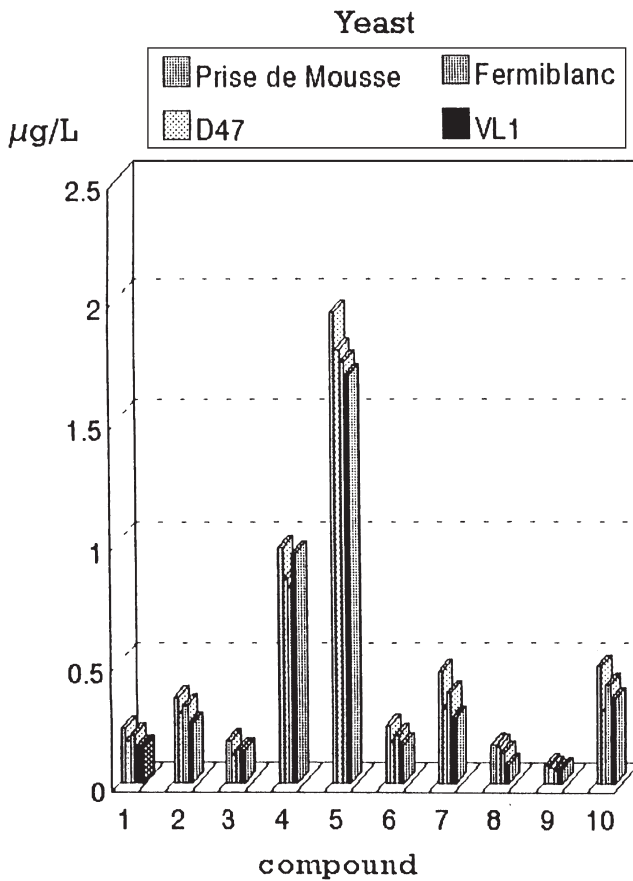


Figure 2 Effect of four strains of *Saccharomyces cerevisiae* on bound monoterpenes and aromatic alcohols of Riesling wines 2 months post-fermentation. Aroma and flavor compounds analyzed, see Figure caption 1. Significance of LSD-test of treatment means. Differences in each compound were not significant at $P \leq 0.05$ level.

from thermal storage of wine 2 months post-fermentation. The percentage of phenol-free glycosides to the total averaged 26.4% in aged wines thermally stored compared to 40% in conventionally stored wines. Wines aged 40 months had few significant differences in either free volatiles or bound components as a result of yeast treatment (data not shown). Comparing all yeasts, thermal storage of wines aged for 40 months had a variable effect on free volatiles depending upon the component (Table 3). The concentration of the free benzyl alcohol was increased as a result of thermal treatment while 2-phenolethanol was not affected. Reductions occurred in the concentrations of free linalool and geraniol that were similar to the reductions noted in the heat treatment of 2-month-old wines. Analogous changes were reported by Rapp and Mandery [26] in Riesling wines stored for a number of years at ambient temperature. A lower concentration of free diendiol-1 (3,7-dimethylocta-1,5-dien-3,7 diol) and higher concentrations of free hotrienol and nerol oxide were observed in heat-treated wines, corresponding to results reported by Williams *et al* [45] on heated juice. Significant decreases in the concentration of free diendiol-1 have been reported during conventional wine aging [22]. Thermal storage would be expected to convert a portion of this labile diol to both hotrienol and nerol oxide [45]. Elevated levels of free lin-

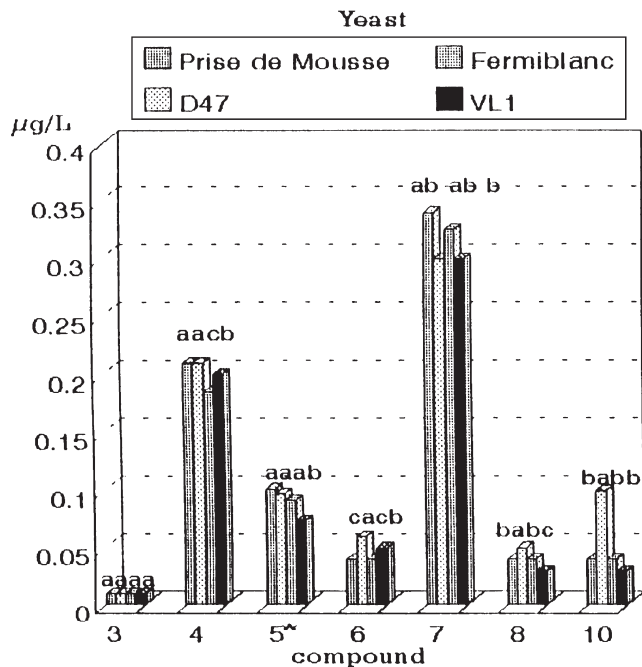


Figure 3 Effect of thermal storage (20 days at 45°C) on free monoterpenes and aromatic alcohols in Riesling wines produced by four strains of *Saccharomyces cerevisiae* 2 months post-fermentation. Aroma and flavor compounds analyzed, see Figure caption 1. Significance of LSD-test of treatment means. Different letters for each compound indicate significant difference at $P \leq 0.05$ level. *Concentration divided by 100.

alool oxide were not observed in the heat-treated wines but are believed to result, in part, from hydrolysis of labile triol 3 (3, 7-dimethyloct-1-en 3,6,7 triol) [45]. The concentration of endiol 4 (3,7-dimethyloct-1-en-3,7-diol) was similar in the control and treated wines (Table 3).

Monoterpenes can undergo considerable fluctuations as a result of isomerization and/or breakdown [7]. It is possible that some changes in the concentration of individual free monoterpenes resulted from biochemical rearrangement in addition to hydrolysis; linalool may be formed from nerol and/or geraniol, while α -terpineol, may be generated by linalool, nerol and/or geraniol [4,54]. With the exceptions of α -terpineol, linalool, nerol, geraniol and pyran linalool oxides, most heat-induced terpenes can be attributed to rearrangement products of polyols [45].

Thermal storage lowered the concentration of most esters. The concentrations of ethyl and hexyl hexanoate, ethyl octanoate, ethyl decanoate and ethyl-4-hydroxy butanoate were reduced by thermal storage as were ethyl, isoamyl, and 2-phenethyl acetate. Fatty acids and their esters may increase or decrease during storage due to chemical esterification or hydrolysis [25]. The extent of these reactions depends on the presence of the alcohol, acid and ester constituents at concentrations above or below that of their equilibrium values. Changes in the concentrations of the major fatty acid esters (C6–C10) did not individually exceed their reported flavor threshold values. Spirov and Goranov [40] reported that conventional storage for 8 months increased the concentration of ethyl formate, isobutyl acetate, hexyl acetate, ethyl octanoate and ethyl lactate. Marais and Rapp [23] noted that isoamyl, hexyl, and 2-

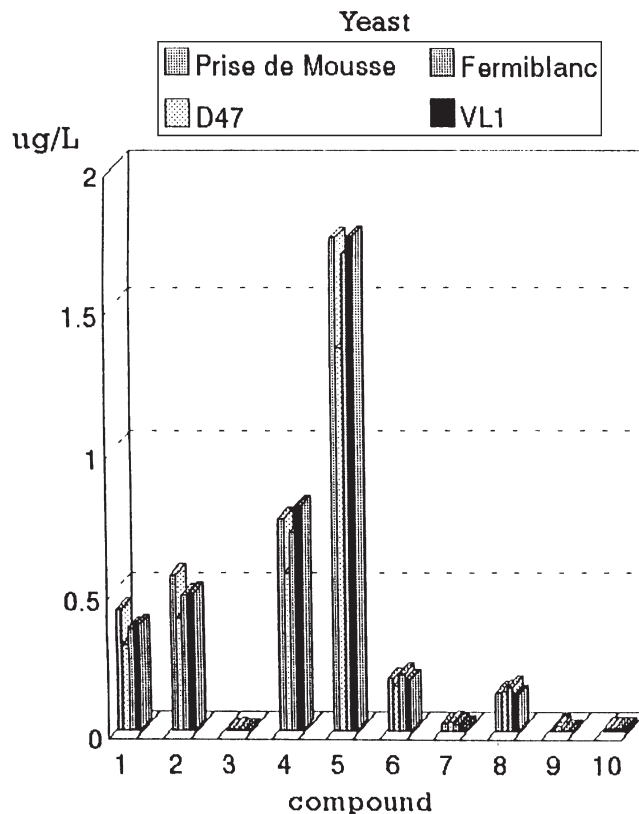


Figure 4 Effect of thermal storage (20 days at 45°C) on bound monoterpenes and aromatic alcohols in Riesling wines produced by four strains of *Saccharomyces cerevisiae* 2 months post-fermentation. Aroma and flavor compounds analyzed, see Figure caption 1. Significance of LSD-test of treatment means. Differences in each compound were not significant at $P \leq 0.05$ level.

phenylethyl acetates all decreased in concentration with time and, to a greater extent, with elevated temperatures. Similar reductions were noted in the thermally-treated wine in the current study. Unlike acetates, concentrations of the straight chain fatty acid ethyl esters remained fairly constant during storage due to the fact that ethyl esters hydrolyze more slowly than acetate esters [26]. Ethyl esters of diprotic acids increased significantly as a result of thermal treatment. These compounds generally show a consistent increase in concentration caused by chemical esterification during aging [26]. The concentration of benzaldehyde was lower in treated *vs* control wines, an indication that the thermal storage was anaerobic [32].

Some of the components resulting from carotenoid

degradation during aging are of interest because of their sensory significance. Increases in 1,1,6-trimethyl-1,2-dihydronaphthalene (TDN) and *trans*-vitispirane resulted from heat treatment, consistent with the results of Marais *et al* [22] for long-term storage of Weisser Riesling. Vitispiranes may contribute to bottle-aged character [32–34]. A number of TDN precursors are present in Riesling wines formed by acid-catalyzed rearrangement of glycosylated norisoprenoids and possibly non-glycosidic compounds [55]. Leino *et al* [21] also demonstrated that heat treatment altered the norisoprenoid composition of wines. Not all volatiles released from grape glycosides have a desirable effect on wine aroma, as demonstrated by TDN which is responsible for the kerosene-like odor in aged Riesling wines [32].

Several volatile phenols were increased by thermal treatment. These compounds are not present in the grape but form as a result of either yeast or bacterial metabolism or from the hydrolysis of wood-derived large molecular weight phenols. For example, 4-vinyl guaiacol and 4-vinyl phenol can originate by enzymatic or thermal decarboxylation from cinnamic acids such as *p*-coumaric and ferulic acids [42].

The change in concentration of aglycones from bound glycosides in wines aged 40 months compared to wines aged for the same period and then thermally stored was variable (data not shown). Reductions in bound components were not matched in all cases by concomitant increases in their corresponding free volatile.

In this study, the yeast strain conducting fermentation had limited influence on the hydrolysis of total and phenol-free grape glycosides. Post-fermentation thermal storage resulted in increases in the concentration of some ethyl esters of fatty acids and a general reduction in acetate esters, analogous with conventional aging. While contributions of these fermentation products to wine aroma and quality are important, products of grape glycoside hydrolysis are the principle contributors to the varietal character of wine. This study demonstrated that thermal storage of young and aged wines significantly lowered the pool of total and phenol-free glycosides. Thermal storage also changed the concentration of some volatile products of glycoside hydrolysis such as monoterpenes, norisoprenoids, phenols and aliphatic compounds. These changes approximated the transformations which occur with long-term conventional bottle aging and bouquet development. Understanding the hydrolytic mechanisms that convert odorless glycoconjugates to free volatiles and the sensory impact of

Table 2 Effect of 40 months cold storage and thermal storage of aged wines (20 days at 45°C) on total and phenol-free glycoside concentrations (expressed as μmol glycosyl-glucose and phenol-free glycosyl-glucose, GG, PFGG) in Riesling wines produced from four strains of *Saccharomyces cerevisiae*

Storage	Prise de Mousse		D47		Fermiblanco		VL1	
	GG	PFGG	GG	PFGG	GG	PFGG	GG	PFGG
40 months	152 ^a ± 4.21	119 ^a ± 1.99	150 ^a ± 9.71	118 ^b ± 4.51	147 ^a ± 8.01	121 ^b ± 6.7	146 ^a ± 10.27	117 ^b ± 4.57
Thermal storage	108 ^a ± 1.64	75 ^b ± 1.49	104 ^a ± 3.71	87 ^b ± 17.26	105 ^a ± 2.92	74 ^b ± 0.85	107 ^a ± 10.35	77 ^b ± 1.82

Significance of LSD test of treatment means at $P \leq 0.05$ and standard deviation. Means with the same letter are not significantly different.

Table 3 Effect of post-fermentation thermal storage of aged Riesling wine (20 days, 45°C) on selected free alcohols, terpenes, esters, fatty acids, norisoprenoids and phenols of Riesling wines

Free compounds ($\mu\text{g L}^{-1}$)	Control wine	Thermal storage	Significance
Alcohols			
2-Methyl-1-propanol	1850	1874	NS
3-Methyl-1butanol	11188	11363	NS
1-Pentanol	115	132	NS
3-Methyl-1-pentanol	23	25	NS
4-Methyl-1-pentanol	103	107	NS
1-Heptanol	2	3	NS
Benzylalcohol	50	202	*
2-Penylethanol	1834	1899	NS
1-Hexanol	115	115	NS
Terpenes			
Linalool	5	1	*
α -Terpineol	29	33	NS
Geraniol	14	44	*
Nerol	8	15	NS
Hotrienol	15	40	*
Nerol oxide	21	42	*
<i>cis, trans</i> Furan linalool oxide	20	30	NS
<i>cis, trans</i> Pyran linalool oxide	55	46	NS
2-Methyl-1,4-pentadiene	13	12	NS
Pyran linalool oxide 12	0.3	0.4	NS
3,7-Dimethyl-1,5-octadiene-3,7-diol	1.5	0.60	*
Hydroxylinalool	0.5	0.1	NS
3,7-Dimethyloct-1-en-3,7-diol	0.4	0.5	NS
Esters			
Isoamyl acetate	854	643	*
Isooctane	73	68	NS
Ethyl hexanoate	152	123	*
Ethyl lactate	101	124	NS
Ethyl acetate	82	53	*
Ethyl octanoate	110	16	*
Ethyl butanoate	16	14	NS
Hexyl ethanoate	20	1	*
Ethyl decanoate	28	2	*
Diethyl succinate	10	48	*
Diethyl 2-hydroxy pentanedioate	6	10	*
2-Phenylethyl acetate + Ethyl 4-hydroxybutanoate	109	7	*
Diethyl malate	14	84	*
Fatty acids			
Acetic acid	75	77	NS
4-Ethoxycarbonyl-g-butyrolactone	502	1012	*
Isovaleric acid	92	95	NS
Hexanoic acid	437	379	NS
Octanoic acid	625	515	NS
Dacenoic acid	48	12	*
9-Decenoic acid	26	11	*
Benzaldehyde	3	1	*
γ -Butyrolactone + Butanoic acid	18	19	NS
C-13 norisoprenoids			
β -Damascenone	tr	tr	NS
3-Hydroxy- β -damascone	0	0	NS
3-Oxo- α -ionol	28	47	NS
Vitispirane	nd	6	*
1,1,6-Trimethyl-1,2-dihydronaphthalene	nd	3	*
Phenols			
Phenol	22	20	NS
4-Ethylphenol	7	10	NS
4-Vinyguaiacol	117	153	*
4-Vinylphenol	47	53	*
Methyl vanillate	2	2	NS
Vanillin	85	22	*
Acetovanillone	1	1	NS

*Significance of *t*-test of treatment means at $P < 0.05$. ns denotes not significant.

such conversions may allow for the production wines with greater aroma intensity and quality.

References

- Abbott NA, BG Combe, MA Sefton and PJ Williams. 1990. The secondary metabolites of Shiraz grapes as an index of table wine quality. In: Proceedings of the Seventh Australian Wine Industry Technical Conference (PJ Williams, DM Davidson and TH Lee, eds), pp 117–120, Winetitles, Adelaide, South Australia.
- Abbott NA, BG Combe and PJ Williams. 1991. The contribution of hydrolysed flavour precursors to quality differences in Shiraz juice and wines: an investigation by sensory descriptive analysis. *Am J Enol Vitic* 42: 167–174.
- Abbott NA, PJ Williams and BG Coombe. 1993. Measure of potential wine quality by analysis of grape glycosides. In: Proceedings of the Eighth Australian Wine Industry Technical Conference (CS Stockley, RS Johnstone, PA Leske and TH Lee, eds), pp 72–75, Winetitles, Adelaide, South Australia.
- Banthorpe DV, BM Modawait, I Poots and MG Rowan. 1978. Redox interconversions of geraniol and nerol in higher plants. *Phytochemistry* 17: 1115–1118.
- Brillouet JM, YZ Gunata, S Bitteut, R Cordonnier and C Bosso. 1989. Terminal apiose: a new sugar constituent of grape juice glycosides. *J Agric Food Chem* 37: 910–912.
- Cordonnier R and C Bayonove. 1974. Mise en evidence dans la baie de raisin, variete Muscat d Alexandrie, de monoterpenes lies revelables par une ou plusieurs enzymes du fruit. *CR Acad Sci Paris, Ser D* 278: 3387–3390.
- Croteau R. 1984. Biosynthesis and catabolism of monoterpenes. In: *Isopentenoids in Plants* (WD Nes, G Fuller and L Tsai, eds), pp 31–80, Marcel Dekker, New York.
- De la Presa-Owens C and AC Noble. 1997. Effect of storage at elevated temperatures on aroma of Chardonnay wines. *Am J Enol Vitic* 48: 310–316.
- Delcroix A, YZ Gunata, JC Sapis, JM Salmon and C Bayonove. 1994. Glycosidase activities of three enological yeast strains during wine-making: effect on terpenol content of Muscat wine. *Am J Enol Vitic* 45: 291–296.
- Di Stefano R. 1989. Le determinazione dei profenoli totali nei mosti e nei vine. *Vigne Vini* 38: 216–220.
- Francis IL, MA Sefton and PJ Williams. 1992. Sensory descriptive analysis of hydrolysed precursor fractions from Semillon, Chardonnay, and Sauvignon Blanc grape juices. *J Sci Food Agric* 59: 511–520.
- Francis IL, MA Sefton and PJ Williams. 1994. The sensory effects of pre- or post-fermentation thermal processing on Chardonnay and Semillon wines. *Am J Enol Vitic* 45: 243–251.
- Francis IL, ME Tate and PJ Williams. 1996. The effect of hydrolysis conditions on the aroma released from Semillon grape glycosides. *Aust J Grape Wine Res* 2: 70–76.
- Fugelsang KC. 1996. *Wine Microbiology*. Chapman and Hall, New York, NY.
- Gunata YZ, CL Bayonove, RL Baumes and RE Cordonnier. 1985. The extraction and determination of free and glycosidically bound fractions of some grape aroma substances. *J Chromatog* 331: 83–90.
- Gunata YZ, CL Bayonove, RL Baumes and RE Cordonnier. 1986. Stability of free and bound fractions of some aroma components of grapes cv. Muscat during the wine processing: preliminary results. *Am J Enol Vitic* 37: 112–114.
- Gunata YZ, S Bitteut, JM Brillouet, C Bayonove and R Cordonnier. 1988. Sequential enzymatic hydrolysis of potentially aromatic glycosides from grapes. *Carbohydr Res* 184: 139–149.
- Gunata YZ, L Dugelay, JC Sapis, R Baumes and C Bayonove. 1994. Role of enzymes in the use of the flavor potential from grape glycosides in winemaking. In: *Progress in Flavor Precursor Studies*. Proceedings of the International Conference (P Schreier and P Winter-Halter, eds), pp 219–234, Wurzburg, Germany. Allured Publishing Corp, Carol Stream, IL.
- Iland PG, R Gawel, MG McCarthy, DG Botting, J Giddings, BG Coombe and PJ Williams. 1996. The glycosyl-glucose assay—its application to assessing grape composition. In: Proceedings of the Ninth Australian Wine Industry Technical Conference (CS Stockley, AN Sas, RS Johnstone and TH Lee, eds), pp 98–100, Winetitles, Adelaide, South Australia.
- Laffort JF, H Romat and P Darriet. 1989. Les levures et l'expression aromatique des vins blancs. *Revue des Oenologues* 53: 9–12.
- Leino M, IL Francis, H Kallio and PJ Williams. 1993. Gas chromatographic headspace analysis of Chardonnay and Semillon wines after thermal processing. *Z Lebensm Unters Forsch* 197: 29–33.
- Marais J, CJ van Wyk and A Rapp. 1992. Effect of sunlight and shade on norisoprenoid levels in maturing Weisser Riesling and Chenin blanc grapes and Weisser Riesling wines. *S Afr J Enol Vitic* 13: 23–32.
- Marais J and A Rapp. 1998. Effect of skin contact time and temperature on juice and wine quality. *S Afr Enol Vitic* 9: 22–30.
- Noble AC, CR Strauss, PJ Williams and B Wilson. 1987. Sensory evaluation of non-volatile flavour precursors in wine. In: *Flavour Science and Technology* (M Martens, GA Dalen and H Russwurm Jr, eds), pp 383–390, John Wiley & Sons, New York.
- Ramey DD and CS Ough. 1980. Volatile ester hydrolysis of formation during storage of model solutions and wines. *J Agric Food Chem* 28: 928–934.
- Rapp A and H Mandery. 1986. Wine aroma. *Experientia* 42: 873–883.
- Sefton MA, GK Skouroumounis, RA Massy-Westropp and PJ Williams. 1989. Norisoprenoids in *Vitis vinifera* white wine grapes and the identification of a precursor of damascenone in these fruits. *Aust J Chem* 42: 2071–2084.
- Sefton MA, IL Francis and PJ Williams. 1993. The volatile composition of chardonnay juices: a study by flavor precursor analysis. *Am J Enol Vitic* 44: 359–370.
- Sefton MA, IL Francis and PJ Williams. 1994. Free and bound volatile secondary metabolites of *Vitis vinifera* grape cv. Sauvignon Blanc. *J Food Sci* 59: 142–147.
- Sefton MA, IL Francis and PJ Williams. 1996. The free and bound volatile secondary metabolites of *Vitis vinifera* grape Semillon. *Aust J Grape Wine Res* 2: 179–183.
- Sefton MA. 1998. Hydrolytically-released volatile secondary metabolites from a juice sample of *Vitis Vinifera* grape cvs Merlot and Cabernet Sauvignon. *Aust J Grape and Wine Res* 4: 30–38.
- Simpson RF. 1978. Aroma and compositional changes in wine with oxidation, storage and aging. *Vitis* 17: 274–287.
- Simpson RF, CR Strauss and PJ Williams. 1977. Vitispirane: a C13 spiro-ether in the aroma volatiles of grape juice, wines, and distilled grape spirits. *Chem Ind* 663–674.
- Simpson RF and GC Miller. 1983. Aroma composition of aged Riesling wine. *Vitis* 22: 51–63.
- Singleton VL. 1962. Aging of wines and other spiritous products, acceleration by physical treatments. *Hilgardia* 32: 319–392.
- Singleton VL, CS Ough and MA Amerine. 1964. Chemical and sensory effects of heating wines under different gases. *Am J Enol Vitic* 15: 134–145.
- Singleton VL and AC Noble. 1976. Wine flavor and phenolic substances. In: *Phenolic, Sulfur and Nitrogen Compounds in Food Flavors* (G Charalambous and I Katz, eds), pp 47–70, ACS Symposium Series 26, American Chemical Society, Washington, DC.
- Somers TC and KF Pocock. 1990. Evolution of red wines: 111. Promotion of the maturation phase. *Vitis* 29: 109–121.
- Somers TC and ME Evans. 1977. Spectral evaluation of young red wines: anthocyanin equilibria, total phenolics, free and molecular sulfur dioxide. *J Sci Food Agric* 28: 279–287.
- Spirov N and N Goranov. 1977. Changes in readily volatile substances in white table wines during storage. *Lozar Vinir* 26: 22–30.
- Strauss CR, B Wilson, PR Goodley and PJ Williams. 1986. Role of monoterpenes in grape and wine flavor. In: *Biogenesis of Aroma* (TH Parliament and R Croteau, eds), pp 222–242, Am Chem Soc Washington, DC.
- Steinke RD and MC Pauson. 1964. The production of steam-volatile phenols during the cooking and alcoholic fermentation of grain. *J Agric Food Chem* 12: 381–387.
- Versini G, S Inama and G Sartori. 1981. A capillary column gas chromatographic research into the terpene constituents of 'Riesling Renano' (Rhine Riesling) wine from Trentino Alto Adige: their distribution within berries, their passage into must and their presence in the wine according to different wine-making procedures. *Organoleptic considerations*. *Vini Ital* 23: 189–211.
- Versini GS, I Orrilos and A Dalla Serra. 1994. Aroma components of Galician Albarino Loureira and Godello wines. *Vitis* 33: 165–170.
- Williams PJ, CR Strauss and B Wilson. 1980. Hydroxylized linalool



- derivatives as precursors of volatile monoterpenes of muscat grapes. *J Agr Food Chem* 28: 766–771.
- 46 Williams PJ, CR Strauss, B Wilson and RA Massey-Westropp. 1982. Studies on the hydrolysis of *Vitis vinifera* monoterpene precursor compounds and model monoterpene β -*d*-glucosides rationalizing the monoterpene composition of grapes. *J Agric Food Chem* 30: 1219–1223.
- 47 Williams PJ, CR Strauss, B Wilson and RA Massey-Westropp. 1982. Novel monoterpene disaccharide glycosides of *Vitis vinifera* grapes and wines. *Phytochemistry* 21: 2013–2020.
- 48 Williams PJ, CR Strauss, AP Aryan and B Wilson. 1987. Grape flavor—a review of some pre- and post-harvest influences. In: Proceedings of the sixth Australian wine industry technical conference (T Lee, ed), pp 111–116, Australian Industrial Publishers, Urrbrae, South Australia.
- 49 Williams PJ, MA Sefton and B Wilson. 1989. Flavor chemistry, trends and developments. In: ACS Symposium Series No. 388 (R Teranishi, RG Buttery and F Shahidi, eds), pp 35–48, American Chemical Society, Washington, DC.
- 50 Williams PJ, W Cynkar, IL Francis, JP Gray, PG Iland and BG Coombe. 1995. Quantification of glycosides in grapes, juices and wines through a determination of glycosyl-glucose. *J Agric Food Chem* 43: 121–128.
- 51 Williams PJ and IL Francis. 1996. Sensory analysis and quantitative determination of grape glycosides; the contribution of these data to winemaking and viticulture. In: Foods Biotechnology for Improved Flavors. ACS Symposium Series No. 637 (GR Takeoka, R Teranishi, PJ Williams and A Kobayashi, eds), pp 124–133, American Chemical Society, Washington, DC.
- 52 Williams PJ and MS Allen. 1996. The analysis of flavouring compounds in grapes. In: Modern Methods of Plant Analysis, Vol 18, Fruit Analysis (HF Linskens and JF Jackson, eds), pp 37–57, Springer-Verlag, Berlin.
- 53 Williams PJ, IL Francis and S Black. 1996. Changes in concentration of juice and most glycosides, including flavor precursors, during primary fermentation. In: The 4th International Symposium on Cool Climate Viticulture and Enology (T Henick-Kling, TK Wolf, and EM Harkness, eds), pp V1 10–V18, Rochester, New York.
- 54 Wilson B, CR Strauss and PJ Williams. 1984. Changes in free and glycosidically bound monoterpenes in developing muscat grapes. *J Agric Food Chem* 32: 919–924.
- 55 Winterhalter P, MA Sefton and PJ Williams. 1990. Volatile C₁₃-norisoprenoid compounds in Riesling wine are generated from multiple precursors. *Am J Enol Vitic* 41: 277–283.
- 56 Zoecklein BW, K Fugelsang, BH Gump and FS Nury. 1995. Wine Analysis and Production. Chapman and Hall, New York, NY.
- 57 Zoecklein BW, JE Marcy, JM Williams and Y Jasinski. 1997. Effect of native yeasts and selected strains of *Saccharomyces cerevisiae* on glycosyl glucose, potential volatile terpenes and selected aglycones of White Riesling (*Vitis vinifera* L.) wines. *J Food Comp Anal* 10: 55–65.
- 58 Zoecklein BW, JE Marcy and Y Jasinski. 1997. Effect of fermentation, storage *sur lie* on post-fermentation thermal processing of White Riesling (*Vitis vinifera* L.) glycoconjugates. *Am J Enol Vitic* 48: 397–402.
- 59 Zoecklein BW, Y Jasinski and H McMahon. 1998. Effect of fermentation, aging and aging *sur lie* on total and phenol-free Riesling (*Vitis vinifera* L.) glycosides. *J Food Comp Anal* 11: 240–248.