Impact of Yeast Breeding for Elevated Glycerol Production on Fermentative Activity and Metabolite Formation in Chardonnay Wine

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Glycerol in wine originates mainly as a by-product during fermentation by yeast and is thought to add to the body and smooth mouth-feel. We evaluated the properties of Chardonnay wine produced using various wine yeast strains of *Saccharomyces cerevisiae* and hybrid strains that were bred to produce elevated glycerol concentrations in laboratory trial experiments. The wine yeast strains (commercial strains or strains from culture collections) produced a mean glycerol and ethanol concentration of 4.38 and 101.2 g/L (12.8% v/v; n=26) respectively, whereas the glycerol and ethanol concentrations in wine made using the hybrid strains was 7.18 g/L and 96.0 g/L (12.2% v/v; n=15). Considerable variability in the glycerol-producing ability of the wine yeast and hybrid strains was apparent. Coupled to the higher glycerol levels formed by the hybrid strains, acetic acid, volatile acidity, acetoin, acetaldehyde and 2,3-butanediol levels were higher than the levels produced by the wine yeast strains. The levels of some of these metabolites were strongly linked to elevated glycerol production. The hybrid strains fermented the Chardonnay grape juice more slowly than the wine yeast strains, but in most instances dryness was achieved. The concentrations of miscellaneous metabolites (alcohols, acids and esters) were in most instances similar in the wine made with the wine yeast strains and hybrid strains, indicating that the breeding of yeast to produce higher glycerol levels has a minor influence on the production of these compounds. In a wine production experiment one hybrid yeast strain producing elevated glycerol levels yielded a Chardonnay wine with a better or equivalent body than wine made with commercial wine yeast strains, although the aroma and general quality were worse. These results suggest that further breeding and selection might yield yeast strains for fermentation that improves the body of wine without impacting on the overall balance of wine.

Glycerol is an important alcohol with a slightly sweet taste formed as a by-product in wine during the fermentation process and is the most abundant constituent except for ethanol and carbon dioxide (Scanes *et al.*, 1998). The levels of glycerol in must from healthy grapes is low, but during fermentation between 4 and 10% of the sugar in must is converted to glycerol (Radler & Schütz, 1982), depending upon the yeast strain, medium and process conditions. The final glycerol levels in wine are generally found to be between 7 and 10% those of ethanol (Ciani & Ferraro, 1996). Typically glycerol levels in wine are approximately 5-7 g/L (Mattick & Rice, 1970; Rankine & Bridson, 1971), but the levels in red wine are generally higher than those found in white wines. The relationship between wine quality and glycerol levels is uncertain, although it is thought that at the concentrations found in wine it may contribute to smoothness (Eustace & Thornton, 1987) and enhance flavour components in beverages (Eustace & Thornton, 1987; Omori *et al.*, 1995).

Many environmental factors influence the production of glycerol by yeast (Scanes *et al.*, 1998). These factors include fermentation temperature and pH, sugar, nitrogen and sulphur dioxide concentrations in the must, the grape variety and aeration during fermentation. However, there are limits to the increases in the glycerol concentration that the winemaker can achieve by manipulating the fermentation conditions. The amount of glycerol produced is also influenced by the *S. cerevisiae* strain used in the fermentation (Rankine & Bridson, 1971). This points to considerable genetic diversity in the ability to synthesise glycerol and the possibility that breeding of yeast would be the most successful way to increase glycerol levels in wine (Radler & Schütz, 1982). Two approaches have been attempted to attain this goal. Many of the *S. cerevisiae* genes involved in glycerol synthesis and retention have been cloned and characterised (Prior & Hohmann, 1997; Hohmann, 1998). Over-expression of some of these genes in yeast strains has resulted in glycerol concentrations greater than 15 g/L being achieved (De Barros *et al.*, 1996; Michnick *et al.*, 1997; Remize *et al.*, 1999). However, the use of yeast strains manipulated using molecular techniques has not yet gained wide acceptance among winemakers and alternative ways to increase glycerol production using classical genetic techniques have been considered. Breeding programmes have yielded *S. cerevisiae* strains that produced glycerol levels at least two-fold greater than those found in the parent strains (Eustace & Thornton, 1987; Prior *et al.*, 1999). Little information is available on the performance of hybrid yeast strains producing elevated glycerol levels. Therefore the purpose of this study was to investigate the fermentative activity and metabolite formation by these strains

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under oenological conditions and to compare these with wine produced using commercial wine yeast strains and strains from culture collections.

MATERIALS AND METHODS

Yeast Strains: The hybrid strains of *S. cerevisiae* were bred for production of elevated levels of glycerol by back-crossing three times a Premier Cuvée strain with yeast strain Ba25 isolated from a spontaneous wine fermentation as described by Prior *et al.* (1999) or by further crossing as described in Table 2. Other strains were obtained from wine yeast collections (Table 1) or from commercial sources. Strains were maintained in glycerol at –80°C.

Medium and cultivation: The yeast were cultivated in YPD broth (2% glucose, 2% peptone and 1% yeast extract) at 30°C overnight. a) **Laboratory trial experiments.** The culture (5 mL; approximately 10^6 cells/mL) was transferred to triplicate bottles (750 mL with fermentation caps) containing 500 ml previously frozen grape must prepared from Chardonnay grapes harvested in February 1998. The must composition was 22.8°B, 8.7 g/L total acidity and pH 3.18. Fermentation was conducted at 15°C until carbon dioxide loss ceased (approximately 30 days). b) **Wine production experiments.** Three cultures were investigated in greater detail during small-scale wine production. For this 180 mL of the culture was inoculated into duplicate stainless steel canisters containing 18 L of freshly prepared Chardonnay must (22.8°B, 7.7 g/L total acidity, pH 3.65, 18 mg/L free SO_2 and 50 mg/L total SO_2) harvested in February 1999. Di-ammonium phosphate (0.5 g/L final concentration) was added and the fermentation was conducted at 15°C (approximately 53 days). After fermentation, 50 mg/L SO_2 was added and after racking off the yeast lees, the free SO_2 was adjusted to 35 mg/L. Bentonite (0.75 g/L) was added to the wine which was then cold stabilised at 0°C for one week, filtered and transferred to five bottles according to standard practices for white wine production.

Analyses: Carbon dioxide loss from the fermentation containers was determined by daily measurement of the weight reduction. Glucose, fructose and glycerol concentrations were determined by high-performance liquid chromatography (Dionex DX 500 system with a GP50 gradient pump and an ED 40 pulsed amperometric detector with a gold electrode). The compounds were isocratically separated with a CarboPac PA10 column and a PA10 guard column with 50 mM sodium hydroxide as eluent. Glycerol concentrations were also determined in some instances using a glycerol test kit (Boehringer-Mannheim Cat No 148270). Reducing sugar, volatile acidity and free and total sulphur dioxide concentrations were determined as described by Amerine & Ough (1980). 2,3-Butanediol was extracted (Michnick *et al.*, 1997) and together with ethanol was quantified by gas chromatography (Hewlett-Packard Model 6890 gas chromatograph with a flame ionisation detector and INNOwax capillary column; 30 m length; 0.25 mm internal diameter; 0.25 µm film thickness) with helium as carrier gas. The temperature of the injection block and detector was maintained at 250 and 300°C respectively. Succinic acid was determined by using a succinic acid test kit (Boehringer-Mannheim Kit no 176281). The concentrations of acetic acid, acetoin, acetaldehyde and other miscellaneous metabolites were determined by adding 4 mL of a solution (2.2 mg/L) of 4-methyl-2-pentanol (internal standard) and 30 mL of diethyl ether to 50 mL of the Chardonnay wine. Following mechanical agitation for 30 min, the top ether layer was separated. The extracts were analysed by gas chromatography using a Hewlett-Packard model 5890 series II gas chromatograph with a Lab Alliance capillary column (60 m length; 0.32 mm inside diameter; 0.5 µm film thickness) with hydrogen as carrier gas and a split ratio of 1:20. The temperature of the injection block and detector was maintained at 200 and 250°C respectively. The column temperature programme was: 35°C (10 min)-3°C/min-230°C (0 min). The peaks of the separated compounds were quantified using a Hewlett-Packard 3396A integrator by using standard solutions.

Sensory evaluation: Batches from the three wines produced using yeast strains VIN13, N96 and XPB3-5C were ranked for aroma, body (mouth-feel) and general quality by a panel of six experienced judges in a randomised fashion according to standardised statistical procedures.

RESULTS

Wine yeasts (26 strains) obtained from various sources were found to produce a mean glycerol concentration of 4.38 g/L in Chardonnay must (Table 1). The glycerol concentrations produced by the strain UCD765 was 43.8% higher than the mean value, whereas the lowest glycerol concentration (strain UCD51) was 27.2% less than the mean value. The ethanol concentrations (mean value of 101.2 g/L) in the Chardonnay wine are typical of those found in wine. The mean ratio of ethanol to glycerol was 23.1:1. With strain UCD765, the ratio was as low as 16.1:1 because of the high glycerol level produced, whereas a ratio of 33.7:1 was observed in the wine produced using strain UCD51 as the strain produced a low glycerol concentration.

Wine produced by 15 *S. cerevisiae* hybrid strains bred for elevated glycerol production (Prior *et al.*, 1999) resulted in a mean glycerol concentration of 7.18 g/L (Table 2) that was 64% greater than the mean concentration produced by the 26 wine yeast strains (Table 1). The highest glycerol concentration of 9.95 g/L produced by strain XPD3-4D was, however, lower than the level of 15.7 g/L formed by the same strain in a glucose synthetic must at 23°C (Prior *et al.*, 1999). This observation suggests that the medium composition and temperature of fermentation might influence the glycerol levels in wine (Scanes *et al.*, 1998). The mean ethanol concentration of 96.0 g/L fermented by these hybrid strains was lower than the mean value obtained with the wine yeast strains (101.2 g/L). Furthermore, the ratios of ethanol to glycerol concentration were much lower (Table 2) than those observed in wine fermented with the wine yeast strains (Table 1). The ethanol levels were markedly lower in wines with elevated glycerol concentrations (Table 2).

A comparison of the fermentation products of wine produced from Chardonnay must by wine yeast strains and hybrid strains with elevated glycerol concentrations are shown in Table 3. The hybrid strains took approximately 30% longer to attain the maximum carbon dioxide production than the wine yeast strains and the maximum rate of carbon dioxide production was 27% lower. Breeding of the yeast strains for elevated glycerol production also affected the concentrations of the other metabolites. Levels of volatile acidity, acetic acid, acetoin and 2,3-butanediol and to a lesser extent acetaldehyde were markedly greater in the wine pro-

TABLE 1

Glycerol and ethanol concentrations (mean values of triplicate independent fermentations ± standard deviation) in Chardonnay produced using various wine yeast strains conducted in laboratory trial experiments.

Strain No.	Alternative name, number or original source	Glycerol concn: (g/L)	Ethanol concn: (g/L)	Ratio (ethanol/glycerol)
WE372	S. African commercial strain	4.46 ± 0.53	100.4 ± 6.3	22.5
N96	S. African commercial strain	5.05 ± 0.52	107.4 ± 11.9	21.3
VIN13	S. African commercial strain	4.49 ± 1.37	101.5 ± 5.5	22.6
UCD[a]51	ATCC36024	3.19 ± 0.52	107.6 ± 12.1	33.7
UCD529	ATCC42941	4.24 ± 0.32	105.2 ± 2.7	24.8
UCD530	ATCC36027	4.36 ± 0.44	99.6 ± 3.7	22.8
UCD585	Australia (Rankine 729)	3.66 ± 1.19	108.3 ± 12.2	29.6
UCD586	ATCC36029	3.66 ± 0.98	105.3 ± 4.4	28.8
UCD680	Germany	4.20 ± 0.27	103.3 ± 3.4	30.4
UCD753	Germany	4.80 ± 0.72	84.3 ± 7.3	17.6
UCD756	Germany	5.14 ± 0.64	104.5 ± 6.4	20.3
UCD758	Germany	3.26 ± 0.56	108.9 ± 13.1	33.4
UCD760	Italy	5.20 ± 0.20	98.5 ± 2.2	18.9
UCD765	Australia	6.30 ± 1.38	101.2 ± 1.1	16.1
UCD766	SIHA - 1	4.78 ± 0.52	99.6 ± 1.1	20.8
UCD773	Germany	5.06 ± 1.55	102.3 ± 0.7	20.2
UCD778	France	5.07 ± 1.26	93.1 ± 10.3	18.4
UCD812	Denmark	4.21 ± 0.17	99.0 ± 7.8	23.5
UCD813	Germany	3.57 ± 0.95	89.4 ± 8.5	25.0
UCD829	Bayanus strain	4.62 ± 0.55	108.4 ± 15.4	23.5
UCD866	Australia	4.97 ± 0.32	102.3 ± 12.6	20.6
UCD889	Italy	4.16 ± 0.88	97.4 ± 3.7	23.4
UCD890	Italy	3.12 ± 0.82	94.2 ± 3.3	30.2
UCB[b]2	Prise de Mousse	3.45 ± 1.13	101.1 ± 3.4	29.3
UCB4	Premier Cuvee	4.00 ± 0.89	101.9 ± 15.8	25.5
UCB8	Italy	4.75 ± 1.10	107.4 ± 16.2	22.6

[a] UCD: University of California (Davis) wine yeast collection.

[b] UCB: University of California (Berkeley) wine yeast collection (R.K. Mortimer).

TABLE 2

Glycerol and ethanol concentrations (mean of triplicate independent fermentations ± standard deviation) in Chardonnay wine produced using hybrid yeast strains in laboratory trial experiments.

Strain No.	Reference or genetic cross	Glycerol concn: (g/L)	Ethanol concn: (g/L)	Ratio (ethanol/glycerol)
XPB3-1C	Prior et al. (1999)	5.67 ± 1.50	97.1 ± 7.4	17.1
XPB3-1D	Prior et al. (1999)	6.41 ± 1.57	99.1 ± 2.9	15.5
XPB3-2C	Prior et al. (1999)	7.52 ± 1.16	89.5 ± 2.8	11.9
XPB3-2D	Prior et al. (1999)	5.78 ± 0.59	101.2 ± 2.4	17.5
XPB3-3A	Prior et al. (1999)	8.94 ± 1.43	93.5 ± 13.2	10.5
XPB3-3D	Prior et al. (1999)	9.09 ± 1.58	84.5 ± 12.5	9.3
XPB3-4C	Prior et al. (1999)	8.08 ± 1.67	93.8 ±.2.0	11.6
XPB3-4D	Prior et al. (1999)	9.95 ± 0.08	94.4 ± 15.8	9.5
XPB3-5B	Prior et al. (1999)	6.79 ± 2.90	96.2 ± 13.0	14.2
XPB3-5C	Prior et al. (1999)	6.68 ± 1.89	101.7 ± 9.9	15.2
XMB2	XPB 3-2B x XPB 3-2C[a]	5.98 ± 0.88	86.9 ± 4.7	14.5
XMB3	XPB 3-3A x XPB 3-3D	6.74 ± 1.33	93.6 ± 6.2	13.9
XMB4	XPB 3-4C x XPB 3-4D	5.66 ± 1.61	99.3 ± 3.9	17.5
XMB5	XPB 3-4C x XPB 3-4D	6.33 ± 1.45	109.5 ± 11.2	17.2
XMB6	XPB 3-5B x XPB 3-5C	8.10 ± 1.23	100.4 ± 4.5	12.4

[a] Strains crossed by C. Baccari and R.K. Mortimer as described in Prior *et al.* (1999).

TABLE 3

Fermentation products of wine from Chardonnay must produced by wine yeast strains and hybrid strains selectively bred to increase glycerol levels in laboratory trial experiments.

Product (concentration)	Must (n = 1)	Wine Yeast Strains (n = 26)	Hybrid Strains (n = 15)
Ethanol (g/L)	0	101.2 ± 6.0	96.0 ± 6.3
Glycerol (g/L)	0	4.38 ± 0.76	7.18 ± 1.37
Days to reach max CO_2 production	ND	5.6 ± 1.1	7.48 ± 1.4
Maximum CO_2 production rate (g/day)	ND	4.08 ± 0.95	2.98 ± 0.81
Total SO_2 (mg/L)	10.7	32.6 ± 14.8	28.3 ± 6.9
Volatile acidity (g/L)	0.14	0.57 ± 0.16	1.35 ± 0.38
Acetic acid (g/L)	0.08	0.66 ± 0.22	1.53 ± 0.43
Acetoin (g/L)	0.026	0.007 ± 0.004	0.047 ± 0.072
Acetaldehyde (g/L)	0.026	0.066 ± 0.045	0.103 ± 0.023
2,3-Butanediol (g/L)	ND	0.81 ± 0.40	4.24 ± 1.71
Glucose (g/L)	144	0.91 ± 1.58	1.22 ± 1.75
Fructose (g/L)	136.8	3.64 ± 4.75	2.53 ± 3.75

ND: Not determined.

duced using the hybrid strains than the wine yeast strains. The hybrid strains produced only slightly lower total SO_2 levels than the wine yeast strains and this might reflect the possibility of interaction between the SO_2 and the higher acetaldehyde concentration produced by the hybrid strains (Table 3). The wine strains and hybrid strains were similar in their ability to ferment the must to dryness.

No clear relationship between glycerol levels produced by the wine yeast strains (commercial and from culture collections) and levels of acetic acid, volatile acidity, 2,3-butanediol and acetoin was apparent (Fig. 1 A, C, E, I). However, acetaldehyde levels appeared to increase with glycerol levels (Fig. 1G). On the other hand, a clearer relationship between the levels of glycerol and acetic acid, volatile acidity and 2,3-butanediol was evident in the hybrid strains (Fig. 1 B, D, F). This suggests that the breeding of hybrid strains to produce elevated glycerol concentrations might not only select for the genes responsible for glycerol synthesis. Acetaldehyde and acetoin levels in these strains did not appear to be affected by the higher glycerol production (Fig. 1 H, J).

The concentrations of only some of the miscellaneous metabolites differed significantly between wine produced by the 26 wine yeast strains and the 15 hybrid strains producing elevated glycerol levels (Table 4). Especially notable are the higher concentrations of propanol and propionic acid and the lower levels of hexanoic and octanoic acids produced by the hybrid strains. Many of these metabolites are implicated in the bouquet and odours of wine, and the results suggest that the selection of wine strains for elevated glycerol production might affect some of the sensory properties.

TABLE 4

Miscellaneous metabolites (mg/L) in wine from Chardonnay must (n = 1) produced using various wine yeast strains (n = 26) and hybrid strains bred for elevated glycerol levels (n = 15) in laboratory trial experiments.

Metabolite	Must	Wine Yeast	Hybrids
ALCOHOLS			
methanol	60.5	72.20 ± 7.43	69.34 ± 4.45
propanol	0.65	34.46 ± 20.93	63.03 ± 21.59
n-butanol	0	0.78 ± 0.83	0.87 ± 0.33
iso-butanol	0.42	13.92 ± 5.56	14.81 ± 3.37
iso-amyl alcohol	2.1	101.67 ± 26.28	96.97 ± 26.02
hexanol	0.53	0.36 ± 0.10	0.35 ± 0.10
2-phenyl ethanol	0.75	24.46 ± 26.82	21.53 ± 4.92
Total miscellaneous alcohols	***64.9***	***248.0***	***266.8***
ACIDS			
propionic acid	0.65	1.50 ± 0.65	3.84 ± 1.07
iso-butyric acid	0.18	1.13 ± 0.42	0.88 ± 0.20
n-butyric acid	0	1.74 ± 0.40	2.81 ± 1.71
hexanoic acid	0.57	6.17 ± 1.72	2.94 ± 1.15
octanoic acid	0.71	8.36 ± 3.11	3.85 ± 2.0
iso-valeric acid	0	0.65 ± 0.19	0.80 ± 0.37
n-valeric acid	0	0.041 ± 0.15	0
decanoic acid	0.31	4.01 ± 3.73	2.41 ± 2.28
Total miscellaneous acids	***2.42***	***23.6***	***17.5***
ESTERS			
ethyl acetate	1.6	84.66 ± 32.76	89.01 ± 20.08
2-phenyl ethyl acetate	0.75	0.75 ± 0.79	0.60 ± 0.41
hexyl acetate	0	0.097 ± 0.093	0.041 ± 0.087
iso-amyl acetate	0	4.03 ± 2.52	2.67 ± 2.12
ethyl butyrate	0	0.517 ± 0.296	0.295 ± 0.230
ethyl caproate	0	5.62 ± 1.81	2.89 ± 1.70
ethyl caprate	0	0.75 ± 0.36	2.37 ± 2.45
ethyl caprylate	0	1.17 ± 0.35	0.52 ± 0.28
ethyl lactate	0	0.82 ± 0.96	1.18 ± 0.75
di-ethyl succinate	0.38	0.48 ± 0.43	1.05 ± 0.44
Total miscellaneous esters	***2.73***	***99.8***	***100.5***

Strains WE372, N96, VIN13, XPB3-5C, XMB6, UCD756 and UCB8 were chosen to ferment fresh Chardonnay must under wine-production conditions in stainless steel canisters. Only strains N96, VIN13 and XPB3-5C fermented the must to dryness and the wine produced by these strains were subjected to detailed metabolite and sensory evaluation (Table 5). Strains VIN13 and N96 are commercial yeast strains commonly used in South Africa and these strains, together with hybrid strain XPB3-5C, were found to have a rapid fermentation rate (maximum 3.5 g carbon dioxide per day) compared to the strains evaluated in this study (data not shown). The three strains fermented the must to produce similar levels of ethanol and residual reducing sugar (Table 5). The glycerol concentration produced by strain XPB3-5C was greater than that formed by the two commercial wine strains. The level was higher than that found in initial evaluation under laboratory trial conditions (Table 2), but was lower than the value reported for this strain in glucose synthetic must (12.7 g/L; Prior *et al.*, 1999). As noted above, the breeding of yeast strains for ele-

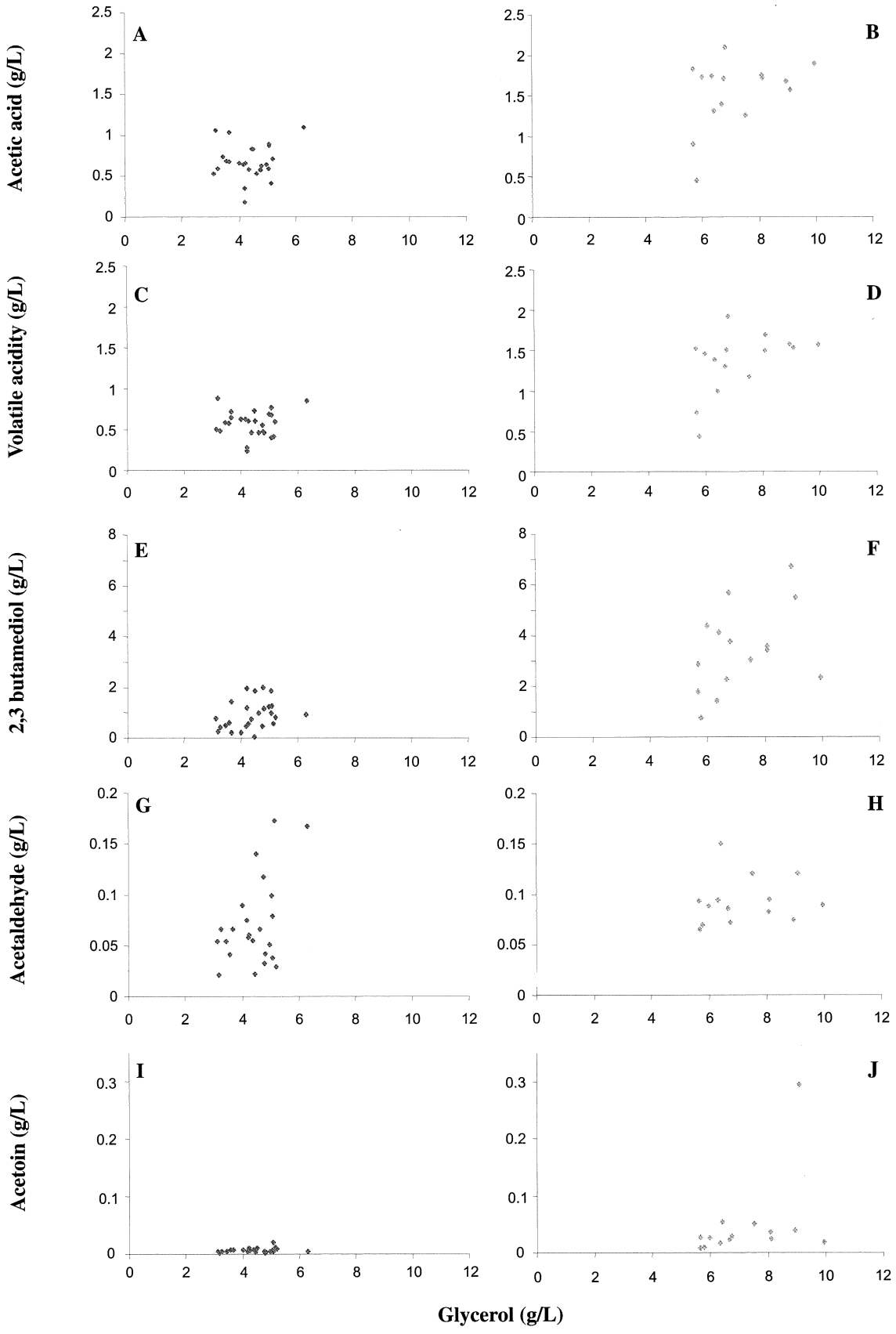

FIGURE 1
Relationship between glycerol concentration and acetic acid (A, B), volatile acidity (C, D), 2,3-butanediol (E, F), acetaldehyde (G, H) and acetoin (I, J) concentrations in Chardonnay wine produced using wine yeast strains (n = 26; A, C, E, G, I) and hybrid strains bred to produce elevated glycerol concentrations (n = 15; B, D, F, H, J) in laboratory trial experiments.

TABLE 5

Evaluation of Chardonnay wine produced by selected *S. cerevisiae* strains (mean of two determinations) in wine production experiments.

Item	Strains VIN13	N96	XPB3-5C
Ethanol (g/L)	116.9	118.1	116.0
Ethanol (% v/v)	14.81	14.96	14.70
Glycerol (g/L)	6.60	6.07	8.46
Reducing sugar (g/L)	4.2	2.5	4.0
Volatile acidity (g/L)	0.62	0.52	0.88
Free SO_2 (mg/L)	26	35	29
Total SO_2 (mg/L)	94	106	96
Total acidity (g/L)	5.70	5.80	6.20
pH	3.64	3.60	3.68
Succinic acid (g/L)	0.216	0.248	0.332
Acetic acid (g/L)	0.508	0.377	0.820
Acetoin (mg/L)	2.54	2.6	7.57
2,3-Butanediol (g/L)	0.48	0.42	1.12
Miscellaneous Alcohols			
Methanol (mg/L)	5.6	16.3	18.0
Propanol (mg/L)	38.8	47.5	41.4
iso-Butanol (mg/L)	11.2	12.8	12.6
n-Butanol (mg/L)	1.39	1.24	1.19
iso-Amyl alcohol (mg/L)	91.8	113.0	99.4
Hexanol (mg/L)	0.541	0.446	0.424
2-Phenyl ethanol (mg/L)	3.92	3.82	3.86
Total miscellaneous alcohols (mg/L)	153.3	195.1	176.9
Miscellaneous Esters			
Ethyl acetate (mg/L)	208	156	186
Ethyl butyrate (mg/L)	1.17	1.86	1.14
iso-Amyl acetate (mg/L)	10.5	8.9	8.2
Ethyl caproate (mg/L)	5.05	7.26	4.73
Hexyl acetate (mg/L)	0.260	0.196	0.270
Ethyl lactate (mg/L)	3.97	5.57	3.40
Ethyl caprolate (mg/L)	2.02	2.82	2.30
Total miscellaneous esters	231.0	182.6	206.1
Miscellaneous Acids			
Propionic acid (mg/L)	1.82	2.1	2.42
iso-Butyric acid (mg/L)	0.652	1.08	0.855
Ethyl caprate (mg/L)	3.61	4.75	3.11
n-Butyric acid (mg/L)	1.80	2.02	1.70
Di-ethyl succinic acid (mg/L)	1.13	1.24	1.01
n-Valeric acid (mg/L)	0.94	0.94	1.11
2-Phenyl ethylacetate (mg/L)	0.33	0.39	0.36
Hexanoic acid (mg/L)	6.52	9.06	6.39
Octanoic acid (mg/L)	7.08	9.17	7.74
Total miscellaneous acids (mg/L)	23.9	30.8	24.7
EVALUATION[a]			
Aroma (rank)	1	2	3
Body (mouthfeel) (rank)	1.5	3	1.5
General quality (rank)	1.5	2	2.5

[a] Evaluated on a scale from 1 (best) to 3 (worst).

vated glycerol production resulted in increased levels of acetic acid, acetoin, 2,3-butanediol and volatile acidity in the wine and some of these components might have a negative impact on the sensory properties. In addition, the hybrid strain yielded higher succinic acid concentrations than the two commercial strains. No relationship between the concentrations of glycerol and miscellaneous alcohols, esters and acids was evident.

When the sensory properties of wines produced in two batches each by the three yeast strains were ranked, it was evident that the strain producing elevated glycerol levels produced a wine not rated as highly as the other two in terms of aroma and general quality (Table 5). However, the wine produced using strains VIN13 and XPB3-5C were ranked the same in terms of body and better than wine produced by strain N96. The body of the Chardonnay wine from one batch produced using strain XPB3-5C was ranked higher than the other two wines, suggesting that the glycerol level of this batch (8.66 g/L) might have improved the overall body of the wine sufficiently to result in the higher ranking. Interestingly, the wine made with the strain VIN13 had notably lower levels of methanol, propanol and iso-butyric acid and higher levels of hexanol, ethyl acetate and iso-amylacetate when compared with the wine made with strains N96 and XPB3-5C. Whether these differences are significant to explain the differences in the sensory properties of the wine is uncertain.

DISCUSSION

The levels of glycerol found in the Chardonnay wine produced by various wine yeast strains (commercial strains and from culture collections) are typical of those reported to occur in white wines such as Australian dry white (Rankine & Bridson, 1971) and Californian white table wines (Ough *et al.*, 1972). However, the marked variation of the ability of certain strains to produce higher glycerol levels has also been observed in other studies (Rankine & Bridson, 1971; Radler & Schütz, 1982). This genetic variability was used by Prior *et al.* (1999) as the basis for the breeding and selection of strains able to produce higher glycerol concentrations. The glycerol concentrations found in this study are lower than those commonly found in red wines (Bridson & Rankine, 1971; Ough *et al.*, 1972) and also lower than values obtained in laboratory experiments using synthetic grape must as fermentation broth (Radler & Schütz, 1982; Prior *et al.*, 1999). This suggests that, apart from the strain genetic variability, environmental factors also affect the amount of glycerol synthesised by a yeast strain. For example, factors such as form of nitrogen, concentrations of phosphorus, sugars and other minerals can influence the amount of glycerol produced by a yeast strain (Scanes *et al.*, 1998).

The percent increases in the levels of glycerol formed by the strains bred to produce elevated glycerol levels using genetic crossing techniques were similar to those reported previously using a similar breeding strategy. This is apparently due to an increase in glycerol-3-phosphate dehydrogenase activity (Eustace & Thornton, 1987). This suggests that the use of genetic crossing techniques to increase glycerol in yeast strains suitable for wine fermentation might be limited to a 50 to 100% increase. Some studies have focused on the use of specific manipulations of yeast strains to increase glycerol levels (Michnick *et al.*, 1997; Remize *et al.*, 1999). For example, over-expression of the *GPD1* gene (encoding glycerol-3-phosphate dehydrogenase) in laboratory

S. cerevisiae strains can result in glycerol levels greater than 25 g/L being obtained (up to five-fold increase). However, the over-expression of *GPD1* in commercial wine strains resulted only in a 1.5- to 2-fold increase in glycerol level (Michnick *et al.*, 1997). Similar increases were observed in strains subjected to genetic crossing and selection (Eustace & Thornton, 1987; Prior *et al.*, 1999).

The increase in glycerol levels in wine made with hybrid yeast strains was coupled to increases in levels of other metabolites, some of which could be deleterious to the overall organoleptic properties of the wine (Table 3). Particularly notable were the levels of acetic acid, volatile acidity and 2,3-butanediol. Acetic acid contributes a major part of the volatile acidity component and is a desirable flavourant at normal levels in wine (< 0.3 g/L; Jackson, 1994). The acetic acid and volatile acidity levels observed in the wine made with the hybrid strains is in excess to those acceptable in wine. This must be considered to be a major disadvantage of these hybrid strains bred to produce elevated glycerol concentrations as the organoleptic properties of wine are spoilt. Metabolites such as 2,3-butanediol are thought not to have a significant impact on the sensory properties of wine (Boulton *et al.*, 1996). Studies with yeast strains specifically over-expressing *GPD1* also produced higher amounts of these metabolites. For example, Remize *et al.* (1999) found that the glycerol levels produced by a strain transformed with *GPD1* increased 2.2-fold, whereas the levels of acetic acid and 2,3-butanediol increased 2.8- and 8-fold respectively. The levels of succinic acid also increased by 2.8-fold. By comparison, the hybrid strains in our study produced 1.6-fold higher glycerol levels than the wine yeast strains, but the acetic acid and 2,3-butanediol levels were respectively 2.3- and 5.4-fold greater. We only measured the succinic acid concentration in wine produced by one hybrid strain (XPB3-5C) and this was also higher than the concentrations produced by the commercial wine strains (Table 5). Succinic acid contributes a bitter or salty taste to wine, although the upper limit of acceptance level in wine has not been firmly established (Jackson, 1994). The levels of acetaldehyde and acetoin in wine produced using the hybrid strains were higher than in wine made with wine yeast strains, but these levels together with succinic acid are within the acceptable concentrations found in wine (Remize *et al.*, 1999). Recently attempts have been made to reduce acetic acid production by disrupting the *ALD6* gene encoding an isoform of acetaldehyde dehydrogenase. This resulted in the production of lower acetic acid concentrations, but also led to higher concentrations of other metabolites such as 2,3-butanediol (Remize *et al.*, 2000).

The increases in glycerol concentrations produced by yeast are thought to be linked to acetaldehyde and acetate accumulation resulting from redox imbalances (Fig. 2; Jones, 1989). Interestingly, the increased level of 2,3-butanediol produced from

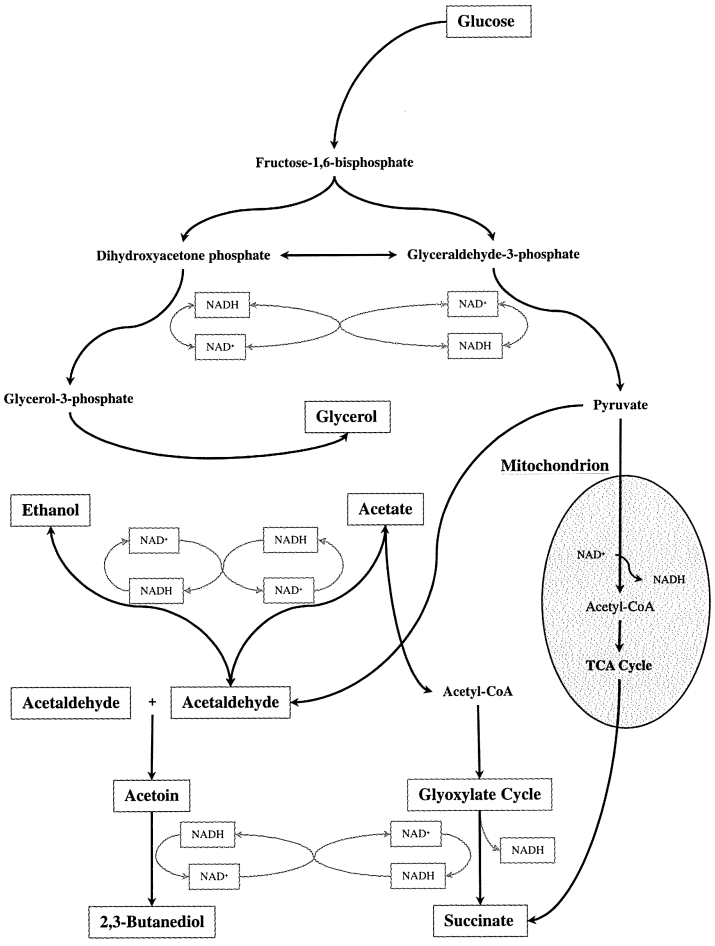

FIGURE 2
The putative pathway in *Saccharomyces cerevisiae* involved in the metabolism of sugar to various products.

acetoin would also result in an increase in NAD^+, suggesting that this reaction might also contribute to the redox imbalance. This reaction might respond as a mechanism to detoxify acetaldehyde (Remize *et al.*, 1999) as was observed in higher eucaryotes (Otsuka *et al.*, 1996), although the activity of the pathway is apparently low in yeast (Jones, 1989). These observations point to a complex interaction between the concentrations of metabolic intermediates and cofactors within the yeast cell that is not fully understood at present.

Breeding of the strains for elevated glycerol production apparently only affected the concentration of a few miscellaneous alcohols and acids and none of the esters (Table 4) that contribute to the sensory properties in wine (Jackson, 1994). This suggests that the pathways leading to the synthesis of these compounds are not influenced to a significant extent by the manipulation of the glycerol-producing ability of the yeast. This result was somewhat surprising since the sensory properties of wine produced by the hybrid strain were judged to be not as desirable as the wine produced with the established commercial strains (Table 5; data not shown). However, the elevated concentrations of metabolites such as acetic acid found in the wines produced using these hybrid strains might be the major factor affecting the sensory properties. Future strain breeding experiments should focus on ways to reduce acetic acid concentrations while maintaining the higher glycerol levels.

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