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The selection of yeast strains for the production of premium quality South African brandy base products

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One hundred seven yeast strains were screened for their ability to produce a brandy base wine of exceptional sensory quality. Volatile acids, esters and higher alcohols were quantified and the results were interpreted using a multivariate analysis of variance (MANOVA) and an average linkage cluster analysis. Significant differences between yeast strains for higher alcohol, fatty acid ester and acetate concentrations were observed. On the basis of their chemical profiles, 16 strains were selected and re-evaluated in larger-scale fermentations and subsequent double distillations. Results show that the yeast lees can have an important effect on the final concentration of higher alcohols and esters in the distillate. Highly elevated levels of ethyl acetate and *iso*-amyl acetate were found to be undesirable. Elevated levels of all the esters present contributed positively to the overall potential quality of the brandy base product. Too low higher alcohol concentrations were also not desirable. Sensory evaluations showed that, since the panel was composed of representatives of the three largest brandy-producing companies, each company preferred a different yeast strain most suitable for their style of brandy. For these reasons, three strains, B7, LL2 and 20-2, warranted further evaluation on a semi-commercial scale for each of the respective companies. *Journal of Industrial Microbiology & Biotechnology* (2000) **24,** 431–440.

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Introduction

Brandy is the soul of the wine from which it was distilled. Brandy flavour can be classified according to the source of different compounds that intervene in it. Varietal flavour is produced by those compounds already present in grapes, i.e. norisoprenoids, carotenoids, acid phenols and glycosidically bound precursors [1,4,5,10,24]. Pre-fermentative flavours are formed by those compounds that develop during extraction and conditioning of must [6,11,12,14]. Fermentative flavour is due to compounds produced by the action of yeast (*Saccharomyces cerevisiae*) and bacteria during alcoholic and malolactic fermentation [2,3,7,9,17,21,36]. Finally, post-fermentative flavour is brought about by compounds that appear during the aging process (wood aging or bottle aging) through enzymatic or physico-chemical reactions [20]. The majority of these post-fermentative flavour compounds present in brandy originate in barrels used for maturation of the beverage [8,16,25,29,35]. In the case of brandy, the intensive flavour and aroma research that has been carried out has shown that the aroma composition consists of several hundred chemical compounds [28]. Various acids and their esters along with higher alcohols (HA) form the main body of aroma compounds [24,34–36]. Although different beverages can readily be distinguished from one another through sensory perception, current analytical methods reveal surprisingly few differences in their chemical composition. The most important differences appear in the quantitative, rather than qualitative, composition of flavour and aroma compounds in beverages [28,30–32]. Some 1300 volatile compounds have been identified [28]. A few hundred more have since been identified. Taking non-volatile compounds into account, the number of identified compounds would be close to double. Accordingly, the composition of flavour is a very complex matter not only in brandy, but also in beer, wine and distilled alcoholic beverages. Because many compounds can take part in the formation of flavour, it is rare that a special component is identified which is responsible for nuances of a specific flavour.

The main focus of aroma research over the past years has been to ascertain where these aroma compounds have their origin and to what extent they are present in alcoholic beverages such as wine and brandy [5,6,26–28,30,36]. These authors have shown that the majority of aroma compounds are indeed formed by *S. cerevisiae* during alcoholic fermentation, and are not present in their aroma-active state in the grape berry [36]. The most important aroma-contributing volatile compounds, notably the volatile organic acids, esters and HA present in alcoholic beverages, are byproducts of yeast metabolism that are secreted into the fermenting medium [5]. The relative production and concentration of these compounds are highly yeast-strain-specific [3,23,33]. Thus, the choice of yeast strain for performing alcoholic fermentation in the production of wine and brandy can have a significant impact upon the ultimate sensory quality of the beverage and is crucial. Currently, only one yeast strain is used for the majority of commercial brandy base wine fermentations in South Africa.

The aim of this study was to identify the relationships between the composition of brandy base wine for use in brandy production, as well as its subsequent distillate, and the sensory perceptions of aroma intensity and quality that are coupled to this composition. Based on these observations, this study focused on the influence of yeast strain on the volatile aroma compound composition of a grape-derived spirit, with a view to finding a yeast strain that not

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only meets the technological specifications placed upon brandy base wines, but also possesses the most optimal volatile compound profile for the production of premium quality South African brandy.

Materials and methods

Microbial strains and media

Initially, 107 yeast strains, comprising commercially available strains, locally isolated strains and strains bred in this laboratory, were evaluated in triplicate small-scale fermentations. The final 16 yeast strains that were selected and further tested are listed in Table 1. Pre-cultures were grown at 30°C in rich medium (YPD) containing 1% yeast extract, 2% peptone and 2% glucose. Unsulphured grape juice that had been left to settle overnight was used for all fermentations. For the fermentations performed in 1997, French Colombard grape juice having a pH of 3.46, a total sugar content of 19.5°B and a free amino nitrogen content (FAN) of 1340 mg/l was used. In 1998, French Colombard juice having a pH of 3.42, a total sugar content of 20.2°B and a FAN of 1310 mg/l was used. Velcorin® (a dimethyldicarbonate preparation; Bayer) was added to all grape juice at 0.203 ml/l in order to eliminate yeasts and bacteria present in the grape juice at the time of pressing. This was done in order to ensure a homogenous population consisting only of the yeast strain being tested. Nitrogen supplementation was performed directly prior to inoculation with yeast in the form of 0.75 g/l diammonium phosphate. All fermentations were performed with a volume of 0.75 l juice, in triplicate, at 15°C. Each 0.75 l of grape juice was inoculated with a 10-ml pre-culture of the respective yeast strain. Fifteen-liter fermentations were performed in duplicate for 16 of the most promising, selected yeast strains tested. These fermentations took place in 18-l stainless steel, pressure-resistant canisters, equipped with fermentation bungs, at 15°C. At the end of all fermentations, natural sedimentation of yeast cells was allowed to take place for 14 days. Wines that could not be distilled immediately were stored (not more than 2 weeks) at a temperature of 4°C.

Technological specifications for a brandy base wine

Apart from an optimal volatile compound composition, yeast strains used in brandy production were evaluated based on their compliance with the following criteria: a concentration of less than 4 g/l of residual sugar in the base wine within 14 days of inoculation; a concentration of 10–12% (v/v) ethanol; less than 20 mg/l total SO_2 and a volatile acidity of less than 0.7 g/l in their resultant base wines.

Distillations

Distillations were performed in electrically heated ball flasks, capable of holding 4.5 l of liquid. Three grams of copper sulphate, as well as three thin strips of copper metal, were added in order to simulate the conditions of a copper pot still. Boiling stones ensured a homogenous heat distribution during the distillation process. In the first distillation, volatile components were concentrated over a period of approximately 8 h, at a flow rate of 5 ml/min, to a final concentration of 30% (v/v) alcohol. This first distillate underwent a second distillation, which reached a final concentration of 70% (v/v) alcohol. In the second distillation, the heads fraction, comprising exactly 1% of the volume being distilled, was removed as is customary in the traditional *charentais* method of distillation in Cognac. Only the so-called hearts fraction was retained for analysis. The flow rate for this distillation was maintained at 4 ml/min, and the distillation lasted for approximately 10 h.

Since the distillations were performed on such a small scale, the volatile compound profiles of the experimental distillates were compared with those of some industrially produced distillates. Commercial samples were compared to the distillates obtained from fermentation with *S. cerevisiae* WE228 as this is most likely strain to have been used in production of commercial distillates. No significant differences in any of the volatile compounds were found except ethyl lactate. Ethyl lactate is generally associated with the malolactic fermentation, which may occur upon prolonged storage of commercial wines due to bottlenecks of brandy base wine volumes at the distilleries.

Analysis

The wines were analysed in terms of: residual sugar (g/l), alcohol content (% v/v), total and volatile acidity (g/l), total SO_2 (mg/l), and pH. The Ripper method of determination was used in measuring the total SO_2 concentrations present in these wines [37].

Table 1 Final 16 yeast strains selected in this study

Strain	Relevant genotype	Source
15-1	Diploid derived from Uvaferm Vinaroma	This study
20-2	Diploid derived from Anchor Yeast VIN13	This study
20f	Diploid derived from Anchor Yeast VIN13	This study
WE228	Commercial wine yeast	Anchor Yeast, South Africa
WB1	Diploid derived from WE228	This study
32F	Flor yeast strain	This study
Aiii5	Isolated from a Chardonnay vineyard	This study
B7	Isolated from a fermenting Chardonnay barrel	This study
Bi9	Isolated from a fermenting Chardonnay barrel	This laboratory
Bi10	Isolated from a fermenting Chardonnay barrel	This laboratory
H2	Isolated from a Cabernet Sauvignon vineyard	This laboratory
H4	Isolated from a Cabernet Sauvignon vineyard	This laboratory
H9	Isolated from a Cabernet Sauvignon vineyard	This laboratory
I9	Isolated from a Shiraz vineyard	This laboratory
LL2	Commercial wine yeast Lalvin ICV D254	Lallemand, Canada
NT7	Commercial wine yeast	Anchor Yeast, South Africa

Volatile compounds were analysed by gas chromatography. Fifty milliliters of the wine samples were used in a liquid–liquid extraction procedure using 30 ml of diethylether and 4 ml of a 2.2 mg/l solution of 4-methyl-2-pentanol, which served as an internal standard. Samples underwent continuous liquid–liquid extraction at 60 rpm in a rotary evaporator (without vacuum) for 30 min before removing 1 ml of the diethylether layer for analysis of volatile components, which was run on a Hewlett Packard HP5890 gas chromatograph, coupled to an HP7673 auto sampler and injector, and an HP3396A integrator. Column type: DB wax column; dimensions 0.5 μm×32 mm; carrier gas: hydrogen; detector: FID by 250; injector temperature: 200°C; split ratio: 20 ml/min; temperature programme: 35°C for 10 min, thereafter increasing at 3°C/min to 230°C; run time: 75 min.

Sensory evaluations

Sensory evaluation of the resultant wines and distillates was based on quality using a six-point scale. The six judges were asked to allocate scores in such a manner that: 0=completely unacceptable; 1=poor quality; 2=below average quality; 3=average quality; 4=above average quality; 5=outstanding quality. The panel of judges comprised brandy producers from the three major brandy-producing companies in South Africa. All judges possessed extensive commercial brandy base wine and wine distillate tasting experience. As the three companies produce different styles of brandy, the judges were asked to evaluate the base wines and distillates according to their company's quality criteria in order to ensure that the yeast strains selected would be strains most liked and favoured by the South African brandy industry.

Samples of 50 ml were presented in random order at 15°C in randomly numbered, clear, 125-ml tulip-shaped glasses. Samples were evaluated at an ambient room temperature of 22°C±1°C under white light. Evaluations took place in mornings between 0800 and 0900 h. The wines were not diluted or pre-treated. The 70% v/v distillates were diluted using distilled water to an alcohol strength of 30–35% v/v.

Statistical analysis

Multivariate analyses of variance (MANOVA), principal component analyses and average linkage cluster analyses were calculated for all resultant base wines and distillates using the gas chromatographic data pertaining to their respective volatile compound compositions. These were calculated using the SAS statistical processing package (SAS PROC GLM, SAS PROC PRINCOMP and SAS PROC CLUSTER). Results of the sensory evaluations were processed using a generalised linear model method run on the GREMLIN program (developed by J.H. Randall, 1998) suitable for use on ordinal scale organoleptic data.

Results and discussion

Yeast strain selection according to technological specifications

One hundred of the 107 yeast strains tested completed fermentation (less than 4 g/l residual sugar) within 14–18 days (data not shown). All resultant wines showed volatile acidity levels well below the maximum 0.7 g/l allowed. The average volatile acidity was 0.22 g/l. A high level of volatile acidity, which is an indication of biological activity by spoilage bacteria such as acetic or lactic acid bacteria, may also have a detrimental impact upon the sensory quality of the resultant distillate, due to the concentration effect of the distillation process.

Of the 107 yeast strains tested, only 46 produced wines that contained less than or exactly 20 mg/l of total SO_2 (data not shown). Of these 46 strains, 16 had total SO_2 values less than or equal to 15 mg/l. Excess sulphur present in this bound form is capable of reacting with the copper present in all *charentais* type pot stills, leading to production of copper sulphate, which precipitates into the distillate. Certain strains of *S. cerevisiae*, so-called "SO_2-producing yeasts", are able to produce sulphite in excess of 100 mg/l [13]. Thus, low sulphite-producing yeast strains (producing less than 20 mg/l) are imperative for use in brandy base wine production.

Many of the high SO_2-producing yeast strains that Riponi *et al.* [33] worked with also produced high levels of *n*-propanol during fermentation. A plot of total SO_2 content *versus* the resultant concentration of *n*-propanol yielded a corrected scatterplot curve that was an increasing linear function. However, the correlation of actual data to this generated curve was very small (data not shown).

Yeast strain selection according to production of volatile aroma compounds

The volatile composition of any alcoholic beverage directly determines the manner in which it is sensorily perceived and thus determines the quality of its flavour and aroma. Therefore, with the aim of creating a superior brandy base product, a MANOVA was performed on the volatile compound data of all 107 wines produced in 1997 using the SAS statistical processing package. This analysis yielded a mean value for each of the compounds quantified in the wine samples (data not shown).

Several average linkage cluster analyses were performed: (i) based on all volatile compounds, including the ratios of total volatile acids, HA and esters; (ii) based on the total concentrations of HA, esters and fatty acids; (iii) based on the concentrations of hexanoic acid, octanoic acid and decanoic acid; (iv) based on the concentrations of ethyl butyrate, *iso*-amyl acetate, ethyl caprylate, ethyl caproate, ethyl caprate and hexyl acetate; and (v) based on the concentrations of 2-phenethyl alcohol and 2-phenethyl acetate.

The analysis created a dendogram of clusters for yeast strains exhibiting similar characteristics in terms of volatile compound concentrations. Dendograms of this size cannot physically be included in a publication of this format. Potentially promising yeast strains were selected by locating clusters in the dendograms where the yeast strains exhibited high concentrations of esters, low concentrations of HA and high concentrations of 2-phenethyl acetate, respectively. These strains were then checked for their fermentative performance.

Forty of the original 107 strains fulfilled all specified technological criteria and simultaneously possessed volatile compound profiles in which total ester concentrations were high and total HA concentrations were low. These 40 strains were re-evaluated on a small scale to verify this performance. From these 40 strains, the 16 exhibiting, comparatively, the highest total ester concentrations, the lowest concentrations of

Table 2 Larger-scale fermentation of 16 selected strains — averaged routine results from analyses of resultant brandy base wines

Number	Yeast strain	Residual sugar (g/l)	Ethanol % (v/v)	Volatile acidity (mg/l)	Total acidity (mg/l)	Total SO_2 (mg/l)	pH
1	15-1	1.25	12.00	0.24	7.30	17.50	3.50
2	20-2	0.65	11.80	0.18	7.10	19.00	3.48
3	20 f	1.30	12.08	0.16	6.45	16.50	3.56
4	WE228	0.10	11.79	0.34	7.00	19.00	3.55
5	WB1	0.10	11.19	0.16	7.00	16.00	3.53
6	32 F	1.30	11.60	0.24	7.15	16.50	3.51
7	Aiii 5	1.03	12.16	0.20	6.80	12.00	3.50
8	B7	1.75	11.68	0.18	7.10	10.00	3.48
9	Bi10	1.00	11.96	0.29	7.35	18.00	3.61
10	Bi9	1.70	12.30	0.25	6.60	18.00	3.57
11	H2	1.40	11.74	0.28	7.25	17.50	3.53
12	H4	1.25	12.10	0.26	7.15	12.00	3.56
13	H9	1.20	12.35	0.14	6.80	17.00	3.52
14	I9	1.02	12.15	0.15	7.10	19.50	3.49
15	LL2	0.85	11.53	0.22	7.10	19.50	3.46
16	NT7	0.70	12.27	0.24	7.20	19.50	3.53

HA and which complied to the stipulated technological criteria were selected.

Characterisation of the 16 most promising selected strains

These 16 strains then underwent further evaluation in larger-scale 15-l fermentations and subsequent double distillations. Data pertaining to the routine analysis of the resultant brandy base wines are depicted in Table 2. Results of the average linkage cluster analysis performed on the volatile compound composition of both the brandy base wines and final distillates resulting from fermentation with these 16 strains are depicted in Figures 1 and 2. It is clear that the volatile profiles of some of the brandy base wines and final distillates fermented with the 16 selected strains are significantly different from each other, especially strain WB1 which is significantly different from all the remaining base wines and distillates. Note also the change in linkages between the strains in the two figures. Table 3 lists the compounds of the 16 selected strains in which statistically significant differences in concentrations were yeast-strain-dependent.

HA production in the 16 selected strains

The production of HA during fermentation is usually not of enough significance (unless juice with a high turbidity is used) to be of influence in sensory evaluation of the wine produced. However, for distilled beverage production, the concentration of the HA fraction that takes place during distillation can be great enough to render the flavour of the product unpleasant. This is especially true of the component usually produced in the largest amounts, *iso*-amyl alcohol [5,28]. Therefore, in this selection program, we attempted to identify yeast strains exhibiting low overall concentrations of HA, in particular *iso*-amyl alcohol.

The average total HA production of the base wines and distillates of the 16 selected strains appears in descending order of production in Tables 4 and 5. It is evident that base wines

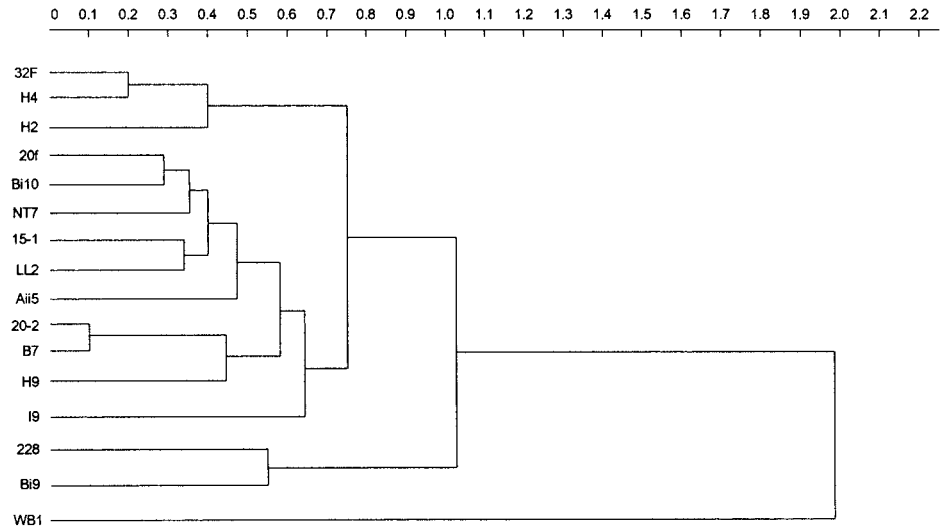

Figure 1 Average linkage cluster analysis of base wines resulting from fermentation with the 16 selected strains.

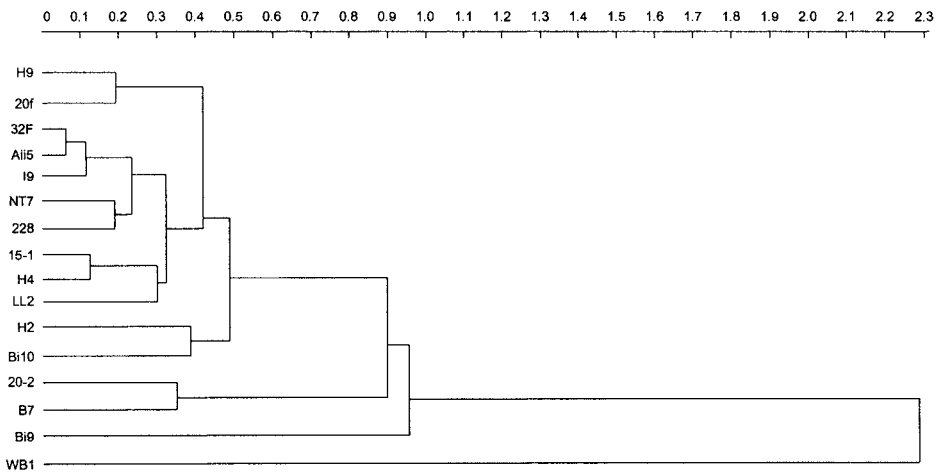

Figure 2 Average linkage cluster analysis of the 16 final distillates.

fermented with strains Bi10, Bi9 and H2 consistently exhibited the lowest relative HA concentrations. This trend was also evident in their resultant distillates. Strains WE228 and LL2, which produced the highest concentration of total HA in the brandy base wines, had high levels of total HA in their distillates. Strain B7 produced intermediate HA concentrations in the brandy base wine. The resultant second distillate then exhibited the highest concentration of HA. When one compares the total HA concentration between the base wine and the distillate for each strain, strain B7, there is a 6.3-fold increase, whereas strain I9 only showed a 3.7-fold increase. Strain WB1 showed a 3.2-fold, and strain WE228 a 6.1-fold increase in *iso*-amyl alcohol when comparing the base wine and the distillate, respectively. For propanol, the differences between the yeasts were more pronounced. The concentration of propanol from the base wine to the distillate increased 1.8-fold and 12.5-fold, respectively, when comparing strains I9 and WE228. The only explanation is that the different yeast lees are responsible for this effect. This might be attributed to the cell wall polysaccharides, which have the ability to bind to particular compounds. Mannoproteins and glucans from yeast can bind β-ionone, ethyl hexanoate and octanal and the hydrophobicity of the compounds plays an important role [22]. Hydrophobic links between aroma compounds and cell wall components, which are weak, will be cut during distillation at high temperatures. Thus, when evaluating yeasts for brandy production, sensory evaluation of the base wines can be misleading due to the change in ratios between the different volatile compounds that may take place during distillation. The HA quantified in this study (*n*-propanol, *iso*-amyl alcohol, *n*-butanol and *iso*-butanol) all have boiling points lower than 200°C and are soluble in alcohol and completely or partially soluble in water. They thus distill predominantly into the heart fraction of the distillate with only a small fraction, mainly methanol, distilling over earlier into the heads fraction. Our results show that the concentration of 2-phenethyl ethanol decreased from the base wines to the distillates, which is to be expected, because it is considered a tails fraction compound [19,30–32]. Most of the HAs quantified in this study (excluding methanol and 2-phenethyl ethanol) are not markedly eliminated through separation of the heads and tails fractions from the desired heart fraction of the distillate.

Table 3 Compounds in which statistically significant differences in compound concentrations were yeast-strain-dependent

Compound	Pr > *F* value
Ethyl butyrate	0.9938
n-Butanol	0.8865
Acetic acid	0.0707
Total volatile acids	0.0981
i-Valeric acid	0.2892
n-Butyric acid	0.6767

n-Butanol was the least abundant alcohol and *iso*-amyl alcohol was always the most abundant alcohol (Tables 4 and 5). In the distillate, 2-phenethyl ethanol values differed from 5.51 mg/l for strain WE228 to 12.50 mg/l for strain I9. *Iso*-butanol concentrations ranged from 54.13 mg/l in strain H2 to 193.98 mg/l in strain LL2. Significantly different concentrations of propanol were observed in the 16 strains. Thus, the differences present in the concentrations of the individual HA, even though amounting to relatively similar amounts of total HA concentrations among the distillates, should have the effect of a distinctly varying sensory perception of the distillates. For example, the distillates of strains I9 and WB1 contain 1051.40 mg/l and 1096.53 mg/l of total HA, respectively, but the total HA in strain I9 consists of 106.34 mg/l propanol, 95.32 mg/l *iso*-butanol, 825.14 mg/l *iso*-amyl alcohol, 10.57 mg/l hexanol and 12.5 mg/l 2-phenethyl alcohol. This is compared with strain WB1 which contains 232.17 mg/l propanol, 159.19 mg/l *iso*-butanol, 687.62 mg/l *iso*-amyl alcohol, 5.78 mg/l hexanol and 6.64 mg/l 2-phenethyl alcohol. HA formation, in particular that of *iso*-amyl alcohol, *n*-propanol and 2-phenethyl alcohol, is yeast-strain-specific [18,21]. Of these, 2-phenethyl alcohol is the most aromatic with a very low sensory threshold, making it highly influential to wine and spirit aroma even if present in low concentrations.

Strains Bi9 and Bi10 consistently yielded the lowest concentrations of *iso*-amyl alcohol in both the base wine and distillate phase. Strain LL2, Lalvin ICV D254, contained high levels of *iso*-amyl alcohol in its base wine and, comparatively, the highest levels of this compound in its distillate.

Table 4 Volatile compound profile of base wines made from the 16 selected yeast strains (mg/l)

Component	WE228	LL2	20f	20-2	15-1	I9	Aiii5	32F	H4	NT7	B7	H9	WB1	H2	Bi9	Bi10
Ethyl acetate	252.27	152.32	193.53	276.07	181.44	162.97	119.55	145.56	117.60	160.92	268.34	223.10	597.23	78.53	269.62	142.27
Ethyl butyrate	0.72	0.63	0.66	0.61	0.50	0.54	0.56	0.55	0.52	0.63	0.18	0.58	0.53	0.62	0.52	0.51
Iso-amyl acetate	12.09	5.14	16.33	20.67	13.37	9.23	7.52	8.70	7.93	13.15	17.36	15.63	43.11	9.05	9.08	6.69
Ethyl caproate	7.91	5.43	8.13	8.53	10.62	6.59	6.21	9.28	6.31	13.72	8.95	8.02	7.48	8.49	5.43	11.59
Hexyl acetate	0.81	0.58	0.96	1.10	0.83	0.97	0.51	0.63	0.58	0.89	0.95	0.89	0.96	0.72	0.73	0.58
Ethyl lactate	3.46	2.83	2.74	0.00	2.12	2.55	2.65	1.99	1.49	1.74	2.37	1.74	1.93	1.41	2.48	1.61
Ethyl caprylate	2.00	1.54	2.12	1.75	1.84	1.64	1.79	1.59	1.94	2.61	1.46	1.80	1.90	2.11	1.37	1.77
Ethyl caprate	2.84	2.49	2.85	2.23	2.14	2.27	2.35	2.20	2.07	2.63	1.98	2.31	2.36	2.62	1.79	2.19
Di-ethyl succinate	0.96	0.99	0.90	0.66	0.85	0.89	2.10	1.16	1.09	0.79	0.79	0.96	1.01	1.11	0.00	0.54
2-Phenethyl acetate	1.28	1.08	1.36	1.35	1.66	1.09	1.17	1.16	0.47	0.76	0.92	1.01	1.87	0.51	4.24	2.68
Total esters	284.34	173.01	229.55	312.95	215.34	188.72	144.39	172.79	139.98	197.81	303.28	256.02	658.38	105.14	295.24	170.41
Total ester-ethyl acetate	32.07	20.69	36.03	36.88	33.90	25.75	24.84	27.23	22.38	36.89	34.94	32.92	61.15	26.62	25.62	28.14
Methanol	237.39	217.10	211.49	174.09	165.36	179.13	116.90	132.02	176.47	197.47	166.90	153.33	175.58	136.17	211.51	105.32
Propanol	58.75	53.02	68.94	83.33	76.26	58.05	42.08	42.38	63.73	51.60	56.67	44.90	34.56	28.98	37.04	30.82
i-Butanol	46.82	45.65	34.67	28.98	23.16	34.10	35.31	31.16	24.94	22.70	10.87	20.97	37.19	20.13	28.30	26.05
n-Butanol	1.22	0.67	1.33	1.08	1.52	0.82	0.85	0.91	0.24	0.74	0.82	1.01	0.78	0.87	0.58	0.59
i-Amyl alcohol	216.83	204.01	188.76	164.57	169.34	173.48	174.18	158.73	141.63	151.58	154.90	148.73	132.54	141.60	116.58	121.01
Hexanol	2.11	2.30	1.65	1.26	1.40	1.85	2.01	1.71	1.46	1.35	1.09	1.22	0.67	1.49	1.55	1.77
2-Phenethyl EtOH	12.65	17.10	15.13	10.77	13.24	14.88	14.08	11.17	9.48	12.11	9.82	9.23	9.80	9.75	11.67	12.38
Total HAs	338.38	322.74	310.48	289.97	284.91	283.18	268.49	246.04	241.46	240.07	234.15	226.04	215.54	202.80	195.71	192.62
Acetic acid	416.78	243.63	180.78	179.92	305.48	143.31	228.72	316.99	330.72	256.43	190.97	127.76	166.64	384.09	422.87	366.04
Propionic acid	1.40	1.31	1.73	1.50	1.58	1.24	1.18	1.27	1.30	1.43	1.71	1.13	0.91	1.13	0.76	0.96
i-Butyric acid	1.36	1.03	0.78	0.67	0.75	0.81	0.92	0.81	0.92	0.63	0.65	0.66	1.31	0.84	0.86	0.77
n-Butyric acid	0.76	0.53	0.73	0.57	0.63	0.54	0.61	0.47	0.81	0.94	0.58	0.66	0.71	0.85	0.53	0.70
i-Valeric acid	0.00	0.00	0.34	0.00	0.26	0.16	0.30	0.26	0.00	0.24	0.24	0.00	0.00	0.25	0.00	0.32
n-Valeric acid	0.48	0.45	0.92	0.45	0.58	0.67	0.50	0.45	0.73	0.57	0.79	0.73	0.32	0.40	0.32	0.40
Hexanoic acid	7.23	5.79	7.37	5.73	6.32	5.91	6.46	5.71	6.39	8.23	4.72	6.11	6.74	6.58	5.04	5.92
Octanoic acid	11.14	9.06	12.56	10.48	11.61	10.07	11.07	11.12	10.91	16.59	11.35	10.70	11.00	12.10	8.24	10.62
Decanoic acid	6.55	3.65	5.38	4.33	5.39	4.05	5.07	5.15	5.11	6.69	4.82	4.65	4.96	6.20	3.95	55.58
Total volatile acids	445.70	265.43	210.58	203.62	332.59	166.73	254.82	342.21	356.88	291.72	215.83	152.38	192.59	412.42	442.55	391.30

SD < 10%.

Ester production in the 16 selected strains

The special fruity odour in white wines is primarily due to a mixture of hexyl acetate, ethyl caproate, and *iso*-amyl acetate in the ratio of about 3:2:1 [15]. Odorwise, hexyl acetate seems to be most important, and *iso*-amyl acetate least important to this bouquet. These compounds were examined in all the wines in order to ascertain whether any of the ratios was close to those reported in the literature. However, the ratios varies considerably and none of the individual wines possessed this ratio of the three volatile esters (data not shown).

There were significant differences in the volatile compound composition among the 16 strains. Ethyl acetate concentrations in the distillates varied from 390.07 mg/l for the distillate made from the base wine fermented with strain H2 to 1740.40 mg/l for the distillate made from the base wine fermented with strain WB1. *Iso*-amyl acetate concentrations also varied considerably, from 11.84 mg/l for the distillate made from base wine fermented with strain LL2 to 120.45 mg/l for the distillate made from base wine fermented with strain WB1. Ethyl caprate concentrations varied from 7.11 mg/l for the distillate made from base wine fermented with strain WB1 to 32.71 mg/l for the distillate made from the base wine fermented with strain H9. 2-Phenethyl acetate concentrations also varied considerably from 1.43 mg/l for the distillate made from base wine fermented with strain H2 to 10.96 mg/l for the distillate made from the base wine fermented with strain Bi9. The other esters, i.e. ethyl butyrate, ethyl caproate, hexyl acetate and ethyl caprylate, did not show significant differences among the 16 strains.

Strain WB1 was significantly different for ethyl acetate and *iso*-amyl acetate (Tables 4 and 5; Figures 1 and 2). The total ester concentrations, one including and the other excluding the ethyl acetate concentrations, consequently also reflect these differences. The wines were fermented with the same batch of grape juice under identical conditions. Thus, the only factor that such vast differences can be ascribed to is the yeast strain used. Since ethanol and acetic acid are the dominant compounds produced during fermentation, esters of ethanol and acetic acid predominate in the resultant wines, with ethyl acetate being the most abundant product being formed from these two compounds [5].

As in the case for HA, when the concentration of total esters between the base wine and the distillate is compared for each strain, there are significant differences between the strains. For example, strain WE228 shows a 2.1-fold increase compared to a 4.3-fold increase for strain 32f (Table 5). All yeasts yielded similar values for the ratio of concentration in the base wine to the distillate for the following esters: *iso*-amyl acetate, ethyl caproate,

Table 5 Volatile data on 70% v/v spirits obtained from distillation of base wines made from the 16 selected yeast strains (mg/l)

Component	B7	LL2	WE228	H4	15-1	20-2	20f	32F	H9	NT7	WB1	Aiii5	I9	Bi10	Bi9	H2
Ethyl acetate	803.79	422.48	532.07	406.37	473.55	570.62	649.58	689.65	640.45	500.38	1740.4	417.58	462.85	583.89	845.00	390.07
Ethyl butyrate	2.50	2.12	2.53	2.28	1.83	1.91	2.28	1.99	2.40	2.42	2.22	1.12	1.01	2.14	1.98	1.54
i-Amyl acetate	22.95	11.84	24.22	24.51	29.01	55.63	35.59	15.57	37.21	38.68	120.45	17.42	18.73	18.11	22.83	14.67
Ethyl caproate	7.68	7.84	8.71	10.56	8.06	8.99	8.39	7.11	9.05	12.21	9.26	8.18	8.07	7.74	6.72	9.26
Hexyl acetate	0.92	0.37	0.68	0.73	0.67	1.24	0.74	0.45	0.75	1.09	0.95	0.45	0.51	0.59	0.63	0.82
Ethyl lactate	13.62	15.57	7.1	10.67	7.33	4.56	17.51	8.37	7.68	5.03	9.08	10.78	10.96	11.34	37.50	5.44
Ethyl caprylate	10.85	8.18	9.56	14.42	12.75	11.72	8.77	8.13	13.26	12.82	9.14	9.54	9.40	7.75	10.52	10.14
Ethyl caprate	26.79	8.17	13.07	31.30	33.17	27.59	9.01	10.22	32.01	18.76	7.11	16.27	16.71	7.16	28.95	8.54
Di-ethyl succinate	1.61	3.21	0.91	1.61	0.61	0.64	3.06	1.26	0.77	0.48	1.18	1.43	2.99	2.56	9.54	0.78
2-Phenethyl acetate	2.82	3.14	3.43	1.77	3.85	3.93	3.03	3.09	2.57	2.30	6.12	2.83	2.60	7.45	10.96	1.43
Total esters	893.50	482.89	602.28	504.20	570.80	686.81	737.95	745.82	746.11	594.15	1905.91	485.56	533.81	648.72	974.62	442.67
Total ester-ethyl acetate	89.71	60.41	70.21	97.83	97.25	116.19	88.36	56.17	105.67	93.77	165.51	67.99	70.96	64.83	129.62	52.60
Propanol	576.33	274.88	432.8	393.44	412.80	448.20	341.22	255.15	339.46	254.61	232.17	128.28	106.34	190.62	186.06	70.98
i-Butanol	97.43	193.98	141.46	130.26	104.69	123.89	137.87	150.79	94.64	105.90	159.19	82.87	95.32	128.84	131.95	54.13
n-Butanol	9.03	3.81	7.64	3.57	8.51	5.41	6.25	5.51	6.55	4.03	5.13	2.32	1.54	3.67	3.37	2.72
i-Amyl alcohol	787.35	922.12	810.99	799.10	797.26	740.50	815.23	816.94	718.26	738.87	687.62	823.78	825.14	607.77	577.18	743.28
Hexanol	8.56	12.58	10.2	10.42	8.87	7.93	9.43	10.78	8.40	8.64	5.78	11.56	10.57	10.70	10.00	9.75
2-Phenethyl EtOH	7.00	9.63	5.51	8.05	9.18	7.88	8.56	7.16	7.73	7.82	6.64	8.52	12.50	8.69	10.08	6.32
Total HAs	1485.68	1416.99	1408.60	1344.82	1341.30	1333.80	1318.55	1246.32	1175.04	1119.85	1096.53	1057.32	1051.40	950.29	918.63	887.17
Acetic acid	25.47	19.36	18.47	25.12	18.97	11.42	17.58	20.92	13.15	16.47	12.25	16.86	12.29	24.09	44.90	25.49
i-Butyric acid	1.22	1.49	1.27	1.97	1.33	1.17	1.16	1.34	1.19	1.15	2.25	1.46	1.56	1.47	1.88	1.48
Hexanoic acid	8.63	8.49	7.58	12.60	10.58	10.38	9.44	8.87	11.00	12.04	11.51	9.12	9.92	10.70	9.73	10.93
Octanoic acid	28.74	25.74	25.59	39.49	33.15	32.93	27.15	27.82	32.52	37.73	34.44	28.56	29.39	30.60	29.43	34.55
Decanoic acid	26.11	18.76	22.05	32.59	23.62	27.93	18.11	20.03	26.22	27.60	27.89	23.60	19.36	22.42	24.97	20.74
Total volatile acids	90.16	73.83	74.96	111.76	87.64	83.83	73.42	78.97	84.07	94.98	88.34	79.58	72.51	89.28	110.90	93.18

SD < 10%.

ethyl caprylate, 2-phenethyl acetate, ethyl acetate, and hexyl acetate. Significant differences between the yeasts were observed for the ester ratios: ethyl caprate, ethyl butyrate, diethyl succinate and ethyl lactate. Thus, when evaluating different yeasts for their suitability for brandy production, sensory evaluation of the base wines can be misleading due to the change in ratios of concentrations for these volatile compounds due to effects of the yeast lees. As in the case of the HA, this change in the ratios between the different volatile compounds may have an impact on the sensory evaluation of the product. This would also explain the change in linkages between the yeast strains when comparing Figures 1 and 2.

Sensory evaluations

Panel variation: The opinions within the tasting panel were compared by fitting a generalised linear model to the ordinal data, obtained from sensory evaluations of both the brandy base wine and the resultant second distillates, with a logit link function using the GREMLIN program (J.H. Randall, 1998). GREMLIN found that the column model provided the best fitting for this type of data.

In the analysis of the sensory scores obtained from brandy base wines, the deviance of the column model was found to be 321.52 with 235 *df*. The approximate significance level for the goodness of fit of this model is thus 0.0002, indicating that the model provided a very good fit of the ordinal scale data. From sequential analysis of deviance table, it is evident that there were no significant variations in the opinions of the tasters involved (Table 6). The tasters, however, did find significant differences between the wines resulting from use of the different strains. An interaction between the covariates, namely the effect of taster variation (due to quality preference differences) and strain variation, is also apparent from these data. This strong interaction, in effect, nullifies the calculated significance level for the effect of taster variation. There are thus significant variations in the opinions of the tasters with regard to the differences in quality of the brandy base wines.

The perceived quality of the individual brandy base wines, as assessed by the tasters representing three brandy-producing companies, is depicted graphically by plotting the model means calculated for each brandy base wine on an axis along which the model-generated cutoff values for the quality gradients have been marked (Figure 3).

Table 6 Sequential analysis of deviance for (A) brandy base wines and (B) for final distillates assessed by tasters representing three brandy-producing companies

Effect	A			B		
	df	Deviance	Approximate significance level	*df*	Deviance	Approximate significance level
Company	2	2.240581	0.3262	2	14.066183	0.0009
Strain	15	75.325604	0.0001	15	34.194140	0.0032
Company* strain	30	121.77241	0.0001	30	95.984861	0.0001
Remainder	188	122.17818	0.9999	188	77.709139	0.9999

The same analysis was performed on scores awarded to the final distillates by the tasters (Table 6). From these values, it is clear that each of the producers also has different criteria in judging the quality of a brandy distillate. There is thus significant variation in the opinions of the tasters involved as to the quality of both a brandy base wine and distillate.

Statistical analysis of sensory scores of the three companies evaluating the brandy base wines: Having demonstrated that statistically significant variations exist in the opinions of the tasters representing three brandy-producing companies in South Africa, it was decided to view the scores allocated to the distillates individually for each company. Using the GREMLIN program, we again attempted to fit a generalised linear model to the scores with a logit link function.

In the case of Company 1, the deviance of the column model fitted to the scores allocated to the brandy base wines was 99.78 with 75 *df*. The approximate significance level for the goodness of fit of this model is 0.0295. As is evident from Figure 3, a graphic interpretation of the model means that brandy base wines from strains 20-2, H2 and H4 were evaluated as being above average quality and wines from strains WE228 and Aiii5 were judged to be of poor quality. No statistically significant differences exist between any of the other brandy base wines.

In the case of Company 2, the deviance of the column model was 70.57 with 60 *df*. The approximate significance level for the goodness of fit for this model is 0.1652. As is evident from Figure 3, brandy base wines resulting from strains 15-1, 20f, 32F, H2 and NT7 were judged to be of above average quality. The brandy base wine resulting from strain 20-2 was judged to be of poor quality.

For Company 3, the deviance of the column model was 129.74 with 75 *df*. The approximate significance level for the goodness of fit of this model is 0.0001. As is evident from Figure 3, Company 3 found none of the samples presented for evaluation to be of poor or below average quality. Brandy base wines resulting from strains NT7, H2 and WE228 were judged to be of above average quality, whereas strains 15-1, 20-2, 20f and H9 were borderline cases for average to above average quality.

Statistical analysis of scores allocated to final distillates by the three companies: It was not possible to interpret the scores of Company 1 in the manner used in this study, as the individual variation in scores allocated to the three replicates of each of the distillates was too large. The only distillate in which there was no variation in the score allocated was to the distillate of strain B7, which was awarded a score of 4 out of a possible 5 on each of the occasions tasted. This was the highest consistent score

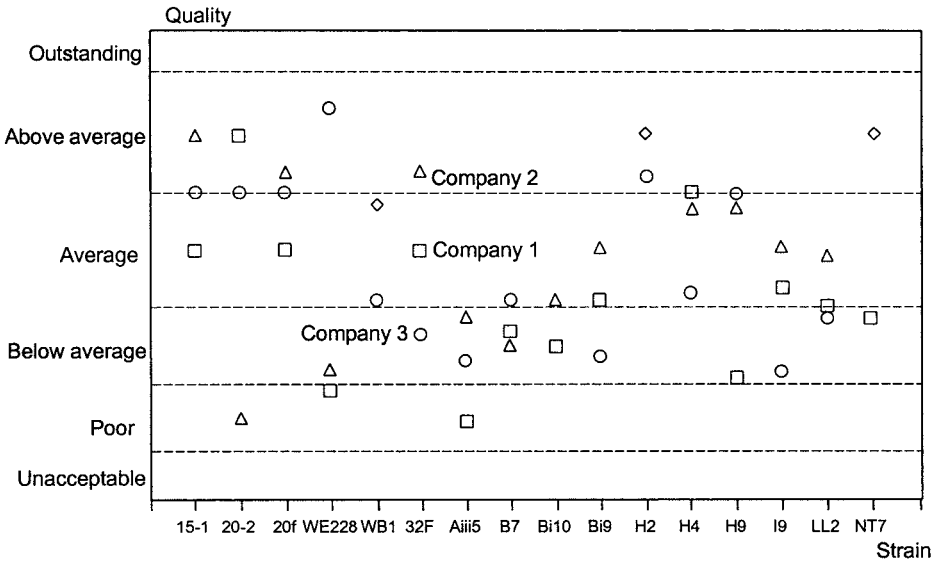

Figure 3 Brandy base wines assessed by three Companies. (◇ where the scores of two companies overlap).

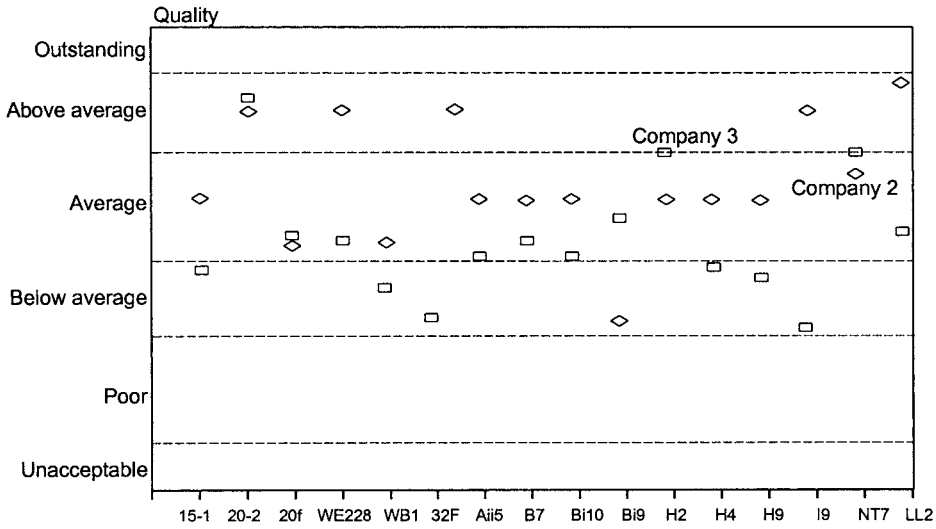

Figure 4 Quality of final distillates as assessed by Companies 2 and 3.

within the company as no other distillate samples were allocated a score of 5 in any one of the evaluations.

For Company 2, the deviance of the column model was 39.23 with 30 *df*. The approximate significance level for goodness of fit of the model was 0.1206. Significant differences in quality were found to exist between the distillate of strains LL2 and 20f, WB1 and Bi9, respectively. Distillates of strains 20f, WB1 and Bi9 were judged to be of lesser quality. The distillate of strain LL2 was judged to yield the highest quality distillate, although no statistically significant differences between it and the remaining strains were found (Figure 4).

The deviance of the column model fitted to the scores of Company 3 was found to be 92.59 with 75 *df*. The approximate significance level for the goodness of fit of this model was 0.0821. The distillate of strain 20-2 was judged to be of the highest quality when compared to the other distillates. However, only significant differences can be reported between the distillates of strains 20-2 and 32F, H9 and I9, respectively. Distillates from strains NT7 and H2 were judged to be of equal above average quality (Figure 4).

It is apparent from the sensory evaluations that each of the brandy-producing companies has differing opinions as to what constitutes a brandy spirit of above average quality. This can be attributed to the fact that each company produces a different style of brandy in order to meet the demands of diverse consumer tastes and preferences. The 16 selected strains possessed relatively similar characteristics as a result of our selection criteria. This is most likely the reason why no one strain produced a distillate of significantly higher quality than the rest of the wine yeast strains tested. Nevertheless, there are one or two strains noted by each company that warrant further attention.

When excluding the distillate made from base wine fermented with strain WB1, the distillate fermented with strain Bi9 possessed not only the highest total ester concentration (with and without ethyl acetate) but also a comparatively low total HA concentration. Thus, from the criteria stipulated in our selection procedure, strain Bi9 seems to be the most promising candidate as a potential brandy yeast strain for commercial use. However, Company 3 found the distillate made from base wine fermented with strain Bi9 to be of average potential quality, whereas Company 2, in fact, rated the Bi9 distillate as the lowest in quality (Figure 4). According to the sensory evaluation, three other yeasts (B7, 20-2 and LL2) showed potential for use in brandy production. The distillate made from the base wine fermented with strain B7 produced relatively high levels of total ester concentrations, even without consideration of ethyl acetate, yet it possessed the highest average concentration of HA. It was awarded consistently the highest marks by Company 1 (data not shown) and was found to be of average quality by Companies 2 and 3 (Figure 4). The distillate made from the base wine fermented with strain 20-2 possessed an intermediate HA concentration, a total ester concentration just below that of the average of the 16 strains, and the third highest ester-ethyl acetate concentration. On the other hand, the distillate made from the base wine fermented with strain LL2 had the second highest HA concentration, and an intermediate concentration of total esters.

Correlation between sensory evaluation results and volatile compound composition: HA, in particular *iso*-amyl alcohol, may have a more significant impact upon the sensory composition in the distillate than in the wine. As is evident when viewing Figure 4, the second distillate resulting from the brandy base wine fermented by strain LL2 was judged to be the best distillate by Company 2. This distillate contained comparatively the highest levels of *iso*-amyl alcohol. In contrast, Company 3 rated the distillate of strain LL2 to be only of average quality. The distillate of B7 averaged an intermediate *iso*-amyl alcohol concentration of 787.4 mg/l and was the only distillate awarded a consistently above average score from Company 1 (data not shown). It is clear that no direct correlation can be made between the concentrations of *iso*-amyl alcohol and sensory quality of a brandy base wine and distillate when comparing the results of these three brandy-producing companies.

Conclusions

The 16 selected strains exhibited significant differences in production of specific volatile compounds during fermentation. This had a direct effect on sensory evaluations of base wines and

distillates. As most of the volatile substances analysed in this work are of fundamental importance to the quality of wines and their resultant spirits, the choice of the strain is crucial. It is important to use the quality of the distillate as basis for evaluation of the yeast, due to the effect that the yeast lees have on the ratio of aroma compounds between the base wine and distillate. The cultivar used for the fermentation of base wines may also influence the choice of yeast strain. This point should be addressed in future work.

This study has proven that it is, at present, impossible to select one yeast strain for optimal use in brandy production on the basis of a sensory evaluation using a panel of brandy producers who produce differing styles of brandy. Perhaps, each of the noted yeast strains is suitable for production of a different style of brandy. It may thus be possible to select yeast strains for production of certain styles of brandy being produced within a particular company. On the basis of sensory evaluations, strains 20-2, B7 and LL2 should be evaluated on a semi-commercial scale and then presented to each of the companies in question for a further sensory assessment.

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