JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY

Selection of Yeast Starter Culture Strains for the Production of Marula Fruit Wines and Distillates

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Juice of the *Sclerocarya birrea* subsp. *caffra* (marula) fruit was fermented by indigenous microflora and different commercial *Saccharomyces cerevisiae* yeast strains at different temperatures, namely, 15 and 30 °C. Volatile acids, esters, and higher alcohols were quantified in the wine and distillates, and the results were interpreted using a multivariate analysis of variance and an average linkage cluster analysis. Significant differences between 15 and 30 °C and also among yeasts with respect to volatile compounds were observed. Yeast strains VIN7 and FC consistently produced wines and final distillates significantly different from the other strains. A panel of tasters and marula and brandy producers was asked to select wines and distillates that had an acceptable and typical marula "nose". They were also asked to detect the differences among wines and distillates fermented with the same yeast strain at different temperatures.

KEYWORDS: Marula juice; commercial yeast strains; volatile compounds; wine, distillate

INTRODUCTION

The use of selected yeast cultures as starters for wine fermentation has led to the production of more consistent wines; yeast and fermentation conditions are claimed to be the most important factors that influence the flavors in wine. Several authors have studied the influence on wine quality of yeast added to an alcoholic fermentation (*1*−*7*), because higher alcohol and ester contents in the wine depend on yeast and fermentation temperature (*8*, *9*). Dubourdieu and Chatonnet (*10*) reported that the enzyme activities of different yeast strains act differently on the precursors. Yeasts form and modify the important components of fermented beverages: volatile organic acids, aldehydes, alcohols, and esters (*11*). The production levels of these byproducts are variable and yeast strain specific. The yeast strain used during fermentation can have a great influence on the ultimate quality of the end product, making the choice of yeast strain crucial if good quality fruit wines and distillates are to be assured.

The literature on the influence of yeast on volatile composition of wines shows that yeast strains vary greatly in volatile compound production (*6*, *11*, *12*). The concentration of wine aroma compounds can be influenced by various factors; among these are the environment (climate, soil), grape variety, degree of ripeness, fermentation conditions (pH, temperature, yeast flora), wine production (enological methods, treatment substances), and aging (bottle maturation) of wine (*13*). Pretorius et al. (*14*) studied the volatile flavor components of marula (*Sclerocarya birrea* subsp. *caffra*) juice and showed that sesquiterpene hydrocarbons and benzyl alcohol are the major aroma components. The marula aroma extracts could be separated into 153 compounds. An odor assessment after GC separation of the aroma compounds showed the absence of a character impact compound. They concluded that the constituents of the aroma extracts contribute to the overall flavor according to their aroma value.

The aim of this work was to study the effect of different commercial yeast starter cultures on the flavor of marula wines and distillates. This study focused on significant differences among alcoholic fermentation secondary products, in particular, volatile composition. The aromas of the wines obtained by fermentation of marula fruit pulp were evaluated by a panel of experienced judges. This study contributes toward a collaborative program aimed at the enhancement of the quality of products derived from one of Africa's most popular wild fruits, the marula.

MATERIALS AND METHODS

Fruit Juice. Chilled marula pulp was collected from the Northern Province of South Africa in the 1999 and 2000 seasons. The pulp had on average sugar levels of 5 °Balling and a pH of 3.7. The marula pulp was diluted with water in a 1:1 ratio to reduce the turbidity of the juice.

Experimental Design. Two investigations were carried out: the first to determine the effect of fermentation temperature (15 and 30 °C) and the second to determine the effect of the yeast strain on the resultant wine and distillate. Ten randomly selected commercial yeast strains

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10.1021/jf0111514 CCC: $22.00
Published on Web 02/12/2002

Table 1. Commercial Yeast Strains Used in This Study

Saccharomyces cerevisiae strain	source
DY10	Anchor Yeast, South Africa
DY502	Anchor Yeast, South Africa
Fermol Clariferm (FC)	AEB Africa
VIN7	Anchor Yeast, South Africa
VIN13	Anchor Yeast, South Africa
N96	Anchor Yeast, South Africa
WE14	Anchor Yeast, South Africa
228	Anchor Yeast, South Africa
WE372	Anchor Yeast, South Africa
NT116	Anchor Yeast, South Africa

and spontaneous fermentations were studied. To compare the effects of yeast, fermentation temperature, and distillation process, the fermentations were done in triplicate and twice for each analysis during the 1999 and 2000 seasons. The yeast strains used are shown in **Table 1**. Yeast strain FC was obtained from AEB Africa (Cape Town, South Africa). All other strains were obtained from Anchor Yeast (Cape Town, South Africa).

Analysis. Conventional parameters such as specific gravity (°Balling), reducing sugars (RS), alcohol, volatile acidity (VA), total acids (TA), and pH were measured (*15*).

Wine-Making. Sugar was added to the diluted juice at a concentration of 35% per liter of water added. Diammonium phosphate (DAP; Lab Scientific Equipment, Cape Town, South Africa) was added to adjust the nitrogen concentration of the juice depending on the FAN concentration of the pulp. The amount of DAP added was calculated using the following formula:

$$\frac{43.9 - \text{FAN}/°\text{B}}{0.108} \times 0.5 = \text{g/hL DAP}$$

The juice was inoculated with a *Saccharomyces cerevisiae* yeast strain at 0.2 g/L concentration and fermented at the desired temperature (as recommended by supplier). The yeast was weighed and dissolved in 30 mL of marula juice and was incubated at 30 °C for 10 min. Fermentation was done in 4.5 L bottles. The fermentation process was followed by measuring the decrease in weight of the bottles, and alcoholic fermentation was considered to be complete when the weight of the bottles stabilized. Upon completion of alcoholic fermentation, the wines were racked and centrifuged at 2500 rpm for 10 min; the clear wine was stored at 4 °C prior to distillation and chemical and sensory analyses.

Distillations. The wines were double distilled in electrically heated round-bottom 4.5 L flasks. To simulate the conditions of a copper pot still, 0.67 g/L copper sulfate and two strips of copper metal were added. To ensure homogeneous heat distribution during the distillation process, boiling stones were added. In the first distillation, the wines were brought slowly to evaporation, after which the flow rate was maintained at 5 mL/min throughout the 10-h distillation process. Distillation was stopped when distillate reached 30% (v/v) alcohol. The second distillation was divided into two phases. The first phase was the collection of 1% of the first distillate at a flow rate of 1 mL/min; this fraction was discarded. The second phase then proceeded with an increased flow rate of 5 mL/min. Distillation proceeded until the distillate (or the heart fraction) reached an alcohol concentration of 70% (v/v). This fraction was collected and kept at 4 °C until analysis.

Extraction of Volatile Compounds and Their Analysis. A wine sample (50 mL) was combined with 4 mL of a 2.2 mg/L solution of 4-methyl-2-pentanol (internal standard) and 30 mL of diethyl ether in a round-bottom flask. This was mixed by rotating the contents at 60 rpm for 30 min. An aliquot (1 mL) of the ether layer was collected and analyzed for volatile compounds. For 70% (v/v) alcohol distillates, volatile extraction was done by taking a 5 mL sample to which 0.25 mL of internal standard was added and mixed before analysis. The analysis of volatile compounds was carried out on a Hewlett-Packard HP5890 series II gas chromatograph coupled to an HP7673 autosampler and injector and an HP3396A integrator. The column used was a Lab Alliance organic-coated fused silica capillary with dimensions of 60 m × 0.32 mm i.d. with a 0.5 μm coating thickness; hydrogen was used as the carrier gas for an FID detector held at 250 °C. The injector temperature was 200 °C, the split ratio 20:1, and the flow rate 15 mL/min. The oven temperature program was as follows: 35 °C for 15 min, thereafter increasing at 6 °C/min to 230 °C; run time, 75 min. For each of the compounds measured, a specific amount was measured for the standard used to calibrate the machine. The internal standard and the chemicals for calibration of each measured compound were sourced from Merck, Cape Town, South Africa.

Table 2. Means of Routine Analyses of Marula Base Wines Fermented at 15 °C

yeast strain	residual sugar (g/L)	ethanol (%)	volatile acidity (mg/L)	total acidity (mg/L)	pH	ascorbic acid (mg/L)
DY502	2.70	11.00	0.17	6.60	3.92	412.50
WE372	3.50	11.40	0.19	6.40	3.99	434.50
WE228	2.80	11.40	0.17	6.50	3.95	440.00
DY10	2.70	12.30	0.19	6.60	4.04	440.00
WE14	1.90	11.40	0.19	7.50	3.77	440.00
VIN13	2.90	11.90	0.20	6.60	4.00	440.00
NT116	2.10	12.30	0.19	6.90	4.03	448.30
N96	2.70	11.60	0.15	6.60	3.99	453.80
SPONT	3.20	11.00	0.17	6.40	4.01	448.00
VIN7	2.40	13.80	0.26	6.40	3.97	423.50
FC	6.80	13.80	0.20	6.90	3.63	192.50

Sensory Evaluation. A panel of 10 judges was formed from brandy and marula liqueur producers; all possessed extensive commercial brandy and marula base wine and distillate tasting expertise. The judges were asked to determine the acceptability of the samples by considering the "nose" and to mark on an unmarked line scale the intensity of the flavor profile. Sensory evaluation of the wines and distillates was based on flavor quality and intensity on a line scale; depending on acceptability, the score was negative or positive for not acceptable and acceptable situations, respectively. 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 a room temperature of 22 ± 1 °C under white light. Evaluations took place in the mornings between 9:00 and 10:00 a.m. The wines were not diluted or pretreated. The 70% distillates were diluted with distilled water to an alcohol strength of 23% v/v alcohol.

Statistical Analysis. Analysis of variance (ANOVA), principal component analysis (PCA), and the UPGMA (unweighted pair group method with arithmatic mean) Euclidean distance cluster analysis were calculated for all of the base wines and distillates using the gas chromatographic data pertaining to their respective volatile compound compositions. Cluster analysis and PCA are useful for finding natural groups among the samples. These were calculated using the STATISTICA program (STATSOFT Inc., Tulsa, OK). Results of the sensory evaluations were processed by the SYSTAT (SYSTAT Inc.) program using the Kolmogorov−Smirnov test for normality of distributions. A triangular test was done on the wines and distillates to determine the effect of fermentation temperature, and results were analyzed using a triangular testing program (*16*).

RESULTS AND DISCUSSION

Effect of Fermentation Temperature on Volatile Compounds. Means of routine analysis of the resultant wines fermented at 15 °C are shown in **Table 2**. All yeast strains but Fermol Clarifiant (FC) completed fermentation (<4 g/L residual sugar). All resultant wines showed volatile acidity levels well below the maximum of 0.7 g/L allowed. 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.

Table 3. Volatile Flavor Compound Contents (Milligrams per Liter) of Marula Wines Produced with Fermentations of 15 and 30 °C

	acetaldehyde		total esters		total esters – ethyl acetate		propanol		total HAs		total volatile acids	
yeast	15 °C	30 °C	15 °C	30 °C	15 °C	30 °C	15 °C	30 °C	15 °C	30 °C	15 °C	30 °C
DY502	0.00	21.72	52.08	26.84	20.25	5.17	101.17	189.48	896.8	571.63	1508.44	241.23
WE372	0.00	2.75	43.9	26.59	13.41	5.22	81.8	169.93	542.16	531.14	342.4	177.42
WE228	0.00	7.51	42.53	29.6	13.05	5.96	100.4	193.08	725.39	646.47	545.62	206.95
DY10	0.00	6.49	30.48	33.86	10.27	6.88	80.84	180.39	614.23	753.92	490.48	253.83
WE14	0.00	7.88	37.01	37.07	11.49	8.83	88.74	216.11	655.06	941.58	488.66	299.5
VIN13	0.00	9.38	30.7	49.54	11.14	10.67	72.89	341.79	581.4	1201.37	458.95	402.79
NT116	0.00	12.03	48.56	34.79	15.82	6.91	110.19	232.01	886.18	972.29	676.52	300.04
N96	0.00	10.4	28.82	31.68	11.35	6.93	73.55	264.25	560.34	1110.16	455.11	229.38
SPONT	0.00	6.16	39.2	28.05	11.08	5.87	88.82	166.27	635.73	654.47	654.4	201.48
VIN7	13.26	8.27	28.92	39.62	8.71	7.11	69.27	215.88	499.77	632.25	357.06	343.42
FC	0.00	0.00	63.22	33.73	14.93	6.39	325.25	191.07	674.95	643.36	386.96	272.45

Several authors have reported on the influence of fermentation temperature on the volatile concentration and hence the quality of wines (*8*, *9*, *17*). In this study, the concentrations of acetaldehyde and higher alcohols increased in 30 °C fermentations compared to 15 °C fermentations (**Table 3**). Total esters and total volatile acids increased at lower fermentation temperatures. The production of acetaldehyde increased at a fermentation temperature of 30 °C for all of the yeast strains used in this experiment except the VIN7 yeast strain, where 13.26 mg/L acetaldehyde was produced at 15 °C and 8.27 mg/L was produced at 30 °C. In wine samples fermented with FC no acetaldehyde was detected at either 15 or 30 °C. For the rest of the yeast strains no acetaldehyde was detected at 15 °C. At 30 °C the concentrations varied considerably, from 2.75 mg/L in wines fermented with strain WE372 to 21.72 mg/L in wines fermented with strain DY502. These results disagree with those of Amerine and Ough (*17*), who reported that temperature does not have an effect on acetaldehyde formation.

There was a clear reduction of total esters − ethyl acetate at a higher fermentation temperature. At least a 2-fold higher level of the esters was observed at 15 °C except for VIN13, which produced more esters at 30 °C than at 15 °C. The production of ethyl acetate did not show much variation due to temperature.

Wine fermented with yeast strains other than DY502, WE372, and FC exhibited higher levels of higher alcohols (HA) at 30 °C. Propanol concentration was higher at higher temperature. Lower propanol concentrations were recorded at 15 °C; the exception was FC, with a lower concentration at 30 °C.

Total volatile acid concentrations were higher at 15 °C than at 30 °C for all of the strains. At 15 °C the highest recorded concentration of volatile acids, 1508.44 mg/L, was exhibited in the wine fermented with strain DY502. The lowest concentration, 342.4 mg/L, was recorded in the wine fermented with strain WE372. At 30 °C the total volatile acids ranged from 177.42 mg/L exhibited in wine fermented with strain WE372 to 402.79 mg/L in wine fermented with VIN13. This may be due to interactions among temperature, yeast strain, and other factors. From these results the more suitable fermentation temperature would be 15 °C, as this produced wines with more esters and fewer higher alcohols, with their attendant undesirable odors when in excess.

A triangular test was done on the wines fermented at 15 and 30 °C and their resultant distillates. Wines and distillates that were fermented at the two temperatures varied significantly, as shown in **Table 4**. The significant level values shown in **Table 4** show the confidence level at which tasters could differentiate between the wines fermented at different temperatures. For the yeast strains VIN7, VIN13, DY10, DY502, N96, WE228, and WE14 the preferred temperature was 15 °C. Strain WE372 was preferred at the 30 °C fermentation temperature. For strain FC the differentiation between the two fermenting temperatures was not significant. Distilled wine fermented with strain FC at 30 °C had a virtually 99.99% confidence level of preference. Samples with native microflora behaved irregularly with respect to temperature; this correlates with work done by Aragon et al. (*8*) on grape wine. Due to the preference of the tasters for wines and distillates fermented at 15 °C, a decision was made to make a comprehensive analysis of the volatile compounds in those wines; therefore, the following results are based on the 15 °C fermentations.

Table 4. Sensory Preference for Marula Wines and Distillates Produced with 15 or 30 °C Fermentation Temperatures

	wine		distillate	
strain	SL value	preferred treatment	SL value	preferred treatment
VIN7	96.14	15 °C	99.12	15 °C
DY10	96.14	15 °C	99.99	15 °C
N96	99.12	15 °C	99.84	15 °C
VIN13	96.14	15 °C	87.79	15 °C
DY502	99.12	15 °C	99.84	15 °C
FC	87.79	15 °C	99.99	30 °C
NT116	99.99	30 °C	89.65	15 °C
WE228	99.99	15 °C	99.98	15 °C
WE14	99.99	15 °C	99.84	15 °C
WE372	99.12	30 °C	99.99	30 °C
SPONT	96.14	15/30 °C	96.53	15/30 °C

Influence of Yeast Type on Higher Alcohols. Higher alcohols themselves have little impact on the sensory properties of wine; however, high concentrations of the HA fraction during distillation can render the flavor of the product unpleasant, due to their strong, pungent smell and taste. This is particularly true for isoamyl alcohol, which is the component usually produced in largest amounts (*18*, *19*). The average HAs of wines fermented at 15 °C and their distillates are shown in **Tables 5** and **6**. It is clear from these results that different yeast strains produced different concentrations and ratios of HAs. Strains VIN7, WE372, and N96 exhibited the lowest relative total HA concentrations. DY502 and NT116 produced the highest total HAs in the base wines and in turn had relatively high levels of total HAs in their distillates. The spontaneous fermentation exhibited intermediate concentrations of total HAs in the base wine, which consequently produced a relatively low concentration of total HAs in the final distillate. Strains VIN7 and WE372 were among the lowest producers of total HAs and, in turn, their corresponding distillates had low total HA concentrations.

Strains VIN7 and N96 showed a 6-fold increase in total HA concentration from the wine to the distillate concentration. These

Table 5. Volatile Compound Profile of Marula Base Wines Made from the 10 Commercial Strains and a Spontaneous Fermentation (Milligrams per Liter)

component	VIN7	N96	SPONT	WE14	WE228	DY10	VIN13	WE372	DY502	NT116	FC
acetaldehyde	13.63	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
ethyl acetate	20.22	17.47	28.12	25.52	29.48	20.21	19.56	30.48	31.83	32.74	48.30
ethyl butyrate	0.57	0.96	0.96	0.83	0.90	0.00	0.78	1.06	1.11	1.13	1.33
isoamyl acetate	0.22	0.46	0.46	0.34	0.39	0.00	0.21	0.36	0.38	0.37	1.79
ethyl caproate	0.36	0.37	0.42	0.47	0.51	0.35	0.35	0.51	0.58	0.52	1.01
hexyl acetate	0.13	0.41	0.00	0.00	0.00	0.00	0.00	0.50	0.00	0.00	0.00
ethyl lactate	3.26	3.84	3.74	3.79	4.56	4.06	3.86	5.72	9.49	5.56	5.90
ethyl caprylate	0.32	0.33	0.39	0.44	0.47	0.33	0.33	0.49	0.54	0.49	0.99
ethyl caprate	0.48	1.02	0.99	1.19	1.32	1.00	0.97	1.54	1.80	1.66	2.70
diethyl succinate	1.96	3.84	3.84	4.20	4.65	4.30	4.43	2.97	6.10	5.82	1.21
2-phenethyl acetate	1.41	0.13	0.26	0.25	0.25	0.23	0.20	0.27	0.25	0.27	0.00
total esters	28.92	28.82	39.20	37.01	42.53	30.48	30.70	43.90	52.08	48.56	63.22
total esters – ethyl acetate	8.71	11.35	11.07	11.49	13.05	10.27	11.14	13.41	20.25	15.82	14.93
methanol	151.81	116.53	181.79	165.52	182.17	131.51	124.28	123.04	243.94	221.70	206.99
propanol	69.27	73.55	88.82	88.74	100.39	80.84	72.89	81.80	101.17	110.20	325.25
isobutanol	35.28	40.63	36.43	38.04	43.00	44.53	42.18	81.04	44.36	56.12	23.73
n-butanol	1.15	1.20	1.58	1.33	1.47	1.26	2.01	1.12	1.84	1.91	1.47
isoamyl alcohol	242.26	251.82	250.39	278.54	309.57	269.91	266.77	191.61	387.32	382.81	104.81
hexanol	0.00	0.00	0.00	0.00	0.00	0.00	0.00	5.72	0.00	0.00	0.00
2-phenethylethanol	84.41	76.60	76.72	82.89	88.79	86.19	81.26	57.83	118.17	113.45	12.70
total HAs	499.77	560.34	635.73	655.06	725.38	614.23	589.40	542.16	896.80	886.18	674.95
acetoin	1.55	1.43	1.36	9.17	24.28	1.74	1.16	21.93	145.18	3.82	0.97
acetic acid	348.05	442.97	646.80	479.83	535.86	482.61	450.69	331.44	1495.81	665.97	369.81
propionic acid	0.73	1.48	1.22	1.32	1.36	1.42	1.43	1.86	2.07	1.92	1.53
isobutyric acid	1.27	1.29	1.18	1.43	1.57	1.39	1.30	1.77	1.57	1.68	0.71
n-butyric acid	0.22	2.04	0.12	0.13	0.12	0.00	0.10	0.75	0.17	0.17	0.28
isovaleric acid	0.92	0.14	0.00	0.09	0.12	0.11	0.12	0.13	0.11	0.12	0.12
n-valeric acid	0.10	0.23	0.24	0.22	0.20	0.17	0.19	0.19	0.24	0.28	0.34
hexanoic acid	1.41	1.79	1.43	1.91	2.14	1.41	1.95	1.19	2.99	2.06	4.13
octanoic acid	2.02	2.67	1.90	2.54	2.90	2.07	1.97	2.90	3.89	2.80	5.22
decanoic acid	2.34	2.49	1.51	1.19	1.35	1.31	1.20	2.17	1.59	1.52	4.82
total volatile acids	357.06	455.11	654.40	488.66	545.62	490.48	458.94	342.40	1508.44	676.52	386.96

Table 6. Volatile Data on 70% v/v Spirits Obtained from Distillation of Marula Base Wines Made from the Different Yeast Strains (Milligrams per Liter)

component	VIN7	N96	SPONT	WE14	WE228	DY10	VIN13	WE372	DY502	NT116	FC
acetaldehyde	69.38	205.97	96.40	101.97	90.08	102.63	100.48	93.76	89.58	91.08	115.10
ethyl acetate	26.86	38.64	42.26	38.04	41.55	33.96	32.38	40.75	35.48	41.06	64.93
isoamyl acetate	1.37	0.99	1.70	1.92	1.62	1.39	1.60	1.78	1.13	0.90	4.19
ethyl caproate	1.44	1.89	2.31	1.71	1.56	2.01	1.93	2.11	2.08	2.28	3.04
hexyl acetate	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.11	0.00
ethyl lactate	17.38	19.92	19.99	19.39	17.48	18.63	18.34	18.00	19.03	16.53	24.20
ethyl caprilate	1.53	1.77	2.20	2.23	2.09	1.92	1.89	1.92	2.00	2.16	2.55
ethyl caprate	1.23	1.34	1.65	1.11	1.50	1.40	1.49	1.34	1.48	1.42	1.56
diethyl succinate	1.49	1.40	1.43	1.32	1.37	1.46	1.32	1.32	1.35	1.44	0.82
2-phenethyl acetate	0.76	0.79	1.02	0.87	1.48	0.89	0.93	0.87	0.90	0.85	0.63
total esters	52.06	66.74	72.58	66.60	68.65	61.65	59.88	68.09	63.45	66.76	101.91
total esters – ethyl acetate	25.20	28.10	30.31	28.56	27.10	27.69	27.50	27.34	27.97	25.69	36.98
propanol	1339.87	2060.62	1410.64	1468.84	1496.38	1448.74	1557.45	1367.07	1501.49	1898.76	1879.74
isobutanol	135.01	140.56	148.29	165.85	175.76	156.42	145.27	136.92	157.13	136.54	140.88
n-butanol	8.80	7.46	8.75	7.57	7.91	9.06	8.98	9.78	8.69	8.91	9.39
isoamyl alcohol	1440.25	1399.40	1417.25	1606.81	1648.64	1549.07	1517.75	1425.35	1520.00	1449.45	659.89
hexanol	0.34	0.41	0.65	0.56	0.68	0.43	0.49	0.50	0.62	0.51	0.27
2-phenethylethanol	54.25	63.81	58.59	54.89	54.34	55.43	59.46	57.11	62.71	49.37	16.29
total HAs	2978.52	3672.26	3044.16	3304.52	3383.71	3219.14	3289.40	2996.73	3250.64	3543.54	2706.47
acetic acid	9.74	7.12	6.12	13.93	11.98	13.97	10.54	10.81	7.16	14.48	11.53
isobutyric acid	0.82	1.66	5.06	3.94	3.90	1.99	2.43	2.77	3.13	2.64	0.96
hexanoic acid	7.01	7.55	8.51	6.09	8.39	6.68	7.57	6.30	7.86	5.88	7.16
octanoic acid	0.62	0.71	0.77	0.92	0.67	1.05	0.93	0.84	0.74	0.76	0.58
decanoic acid	4.02	4.91	5.84	5.99	5.08	4.11	7.09	5.35	5.40	4.57	5.39
total volatile acids	22.22	21.95	26.30	30.87	30.01	27.81	28.56	26.06	24.29	28.34	25.62

two strains produced wines with 19.3- and 28-fold increases, respectively, in propanol levels. The spontaneous fermentation and strain WE228 showed 4.8- and 4.7-fold increases in their total HA concentrations, respectively. Strains DY10, WE372, and VIN13 showed 5.2-, 5.5-, and 5.6-fold increases in their HA concentrations, respectively. Strains NT116 and FC exhibited 3.4-fold increases in their total HAs. Strain DY502 produced the lowest increase, 3.6-fold, in the total HAs on distillation. The wines fermented using the yeast strain WE14 exhibited a 5-fold increase of total HAs on distillation.

Methanol concentrations ranged from 116.53 mg/L for wines fermented with N96 to 243.94 mg/L for wines fermented with strain DY502. The boiling point for methanol is 65 °C; hence, on distillation it was collected in the head fraction, explaining its absence in the heart fraction.

The increase in propanol from the base wine to the concentration in the distillate varied from a 5.8-fold increase in strain FC to a 28-fold increase in distillate resulting from wine fermented with N96. The magnitude of difference in the increase in propanol can be attributed to the individual strain characteristics: different yeast lees exhibit different effects on higher alcohols (*20*). This can be attributed to the cell wall polysaccharides with the ability to bind to particular compounds (*6*). The yeast cell wall is made up of 50% glucans and 50% mannoproteins; these are able to bind with compounds such as β-ionone, ethyl hexanoate, and octanal, the hydrophobicity of which plays an important role. Strain N96 produced wine with 73.55 mg/L propanol, whereas strain FC produced wine with 325.25 mg/L. However, after distillation, strain N96 had a higher propanol concentration (2060.62 mg/L) than did strain FC (1879.76 mg/L).

These results clearly show how misleading it could be to choose a yeast strain for the production of a distillate on the basis of the performance of the yeast in the wine. The process of distillation and yeast lees present in the wine at the time of distillation contribute greatly to the resultant product. The HAs quantified in this study (*n*-propanol, isoamyl alcohol, *n*-butanol, and isobutanol) all have boiling points <200 °C and are soluble in alcohol. They are also completely or partially soluble in water. They therefore distill mainly into the heart fraction of the distillate with only a small amount, mainly methanol, distilling first into the head fraction (*6*).

Isobutanol production also varied with the yeast strain, and an increase in its concentration was observed after the second distillation of the base wines. Strain WE372 gave the lowest increase of 1.7-fold on distillation, whereas strain FC produced a 5.9-fold increase. The concentrations for *n*-butanol observed were very low compared to the other HAs. The lowest concentration of *n*-butanol (1.12 mg/L) was observed in the wine fermented with strain WE372, and the highest concentration (2.01 mg/L) was observed for the wines fermented with strain VIN13. Trace concentrations of hexanol were observed in the wines fermented from pulp collected in the 2000 season with only wines made with strain WE372 exhibiting some hexanol. Hexanol concentrations were not detected in the wines: on distillation these concentrations increased to detectable amounts. The highest concentration of hexanol (0.68 mg/L) was detected in the wine fermented with strain WE228. Concentrations of 2-phenethyl alcohol ranging from 12.7 mg/L in the distillate resulting from wine fermented with strain FC to 118.17 mg/L in the distillate resulting from DY502 fermentation were observed. The 2-phenethyl alcohol concentrations decreased after distillation, which is in agreement with the fact that its boiling point is 219 °C (*21*) and is therefore more likely to go into the tail fraction.

The results showed how fruit fermented under the same conditions gave a completely different volatile profile of a product, due to the use of different yeast strains that produced different proportions of the various volatiles. The proportions of volatile increases observed during the two harvest seasons 1999 and 2000 were roughly the same. However, the 1999 season fruit was highly fragrant compared to that of 2000, which was exposed to floods at the end of maturation. This resulted in the dilution of the fruit, giving it a low aromatic intensity; the heavy character of the HAs was thus more pronounced.

Ester Production by the Different Strains. Esters impart a pleasant smell. Young wines derive their fresh, fruity aroma characteristics largely from the presence of a mixture of esters produced during fermentation. The most significant esters are those of higher alcohols: ethyl acetate, isoamyl acetate, isobutyl acetate, ethyl hexanoate, 2-phenethyl acetate, and esters of straight-chain saturated fatty acids.

Ethyl acetate has been reported by various authors to be the main ester in wine (*6*, *21*). The same trend was observed in the marula wines fermented at 15 and 30 °C. The concentrations, however, varied significantly from season to season; 1999 had concentrations ranging from 100 to 193 mg/L, whereas in the year 2000 ethyl acetate concentrations varied from 17.50 to 48.30 mg/L.

Low concentrations of ethyl butyrate ranging from undetectable in DY10 to 1.33 mg/L in FC were observed in the wines; ethyl butyrate was undetectable in the distillates for all of the yeast strains. The heart fraction was analyzed for volatiles, however, and ethyl butyrate might have been included in the head fraction and hence was not detected. Ribereau-Gayon et al. (*21*) have reported the olfactory perception threshold of ethyl acetate as being $\sim$160 mg/L; at high levels it can spoil the wine bouquet with an unusual, unpleasant, pungent tang, whereas at very low doses (50−80 mg/L), ethyl acetate contributes to a wine's olfactory complexity and thus has a positive impact on quality. Isoamyl acetate levels in DY10 were undetectable; FC had the highest concentration with 1.79 mg/L. The second distillation of FC exhibited the highest concentration of isoamyl acetate of 4.19 mg/L. Ethyl caproate concentrations did not vary much among the different strains in the wine except for FC with 1.01 mg/L, at least double the concentration contributed by the other strains. The distillate also gave a high concentration of 3.04 mg/L compared to the lowest, exhibited in wine fermented with strain VIN7 with a concentration of 1.44 mg/L.

Hexyl acetate was recorded at very low levels in the wines and was not detectable in the second distillates except for strain NT116, with a concentration of 0.11 mg/L. Diethyl succinate concentrations also varied considerably, from 1.21 mg/L for the base wine fermented with strain FC to 6.10 mg/L in wine fermented with strain DY502. The resultant second distillates portrayed a similar trend with diethyl succinate levels from wine fermented with strain FC having a low concentration of 0.82 mg/L and that of strain DY502 having a relatively high concentration of 1.35 mg/L. Other esters (ethyl caprylate, ethyl caprate, and 2-phenethyl acetate) did not vary greatly in their concentration among the different yeast strains. The presence of ethyl lactate in the wines and distillates may be linked to malolactic fermentation, and the involvement of an esterase of bacterial origin in this case cannot be ruled out. Total esters varied considerably, from N96-derived wines with 28.82 mg/L to 63.22 mg/L obtained in wines fermented with strain FC.

Selection of High-Performance Yeast Strains. *UPGMA Cluster Analysis.* UPGMA cluster analysis of the volatiles was done on both base wines and final distillates resulting from fermentation with the different strains. **Figures 1** and **2** show the average clusters for volatile compounds of wines fermented with the different strains and their final distillates, respectively. From the clusters it was clear that some of the base wines and distillates fermented with the different strains differed significantly from each other. It is interesting to note that strains FC and VIN7 consistently produced wines and final distillates significantly different from the other strains. The rest of the

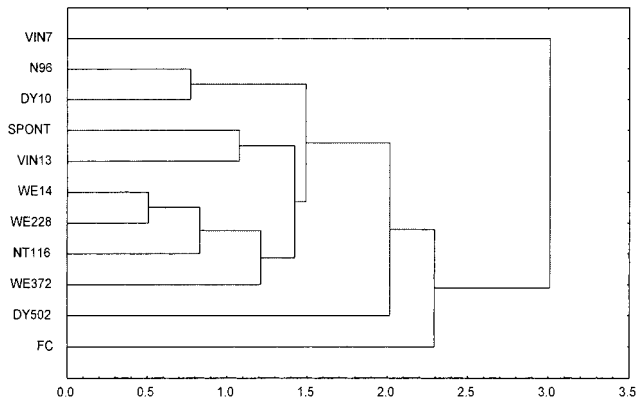

Figure 1. Average linkage cluster analysis of volatile compounds in marula base wines resulting from fermentation with 11 strains.

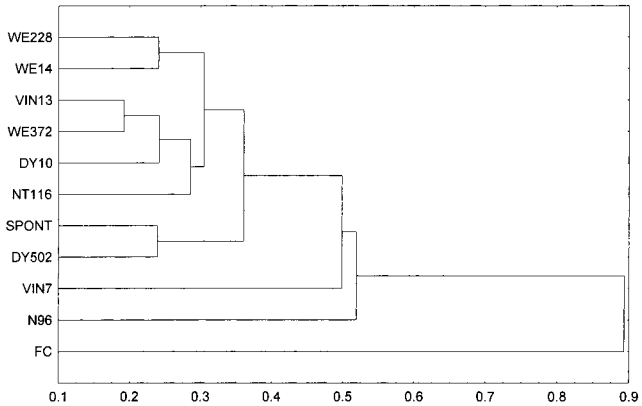

Figure 2. Average linkage cluster analysis of volatile compounds in the 11 final distillates of marula wines.

strains were clustered differently for the wines and the final distillates, presumably reflecting the effect of yeast lees present in the wine during the distillation process on the volatile profiles of the distillates.

It is also interesting to note that strains WE14 and WE228 consistently produced wines and distillates with similar profiles: hence, they were clustered together (**Figures 1** and **2**). The volatile profiles of the wines changed on distillation, hence the differences in the clustering, with the exceptions of those derived from strains WE14, WE228, and FC. Thus, in the selection of a yeast strain for the production of a distilled beverage, it is recommended that the performance of the strains after distillation be judged. This correlates with the report of Steger and Lambrechts (*6*), who selected yeast strains for the production of premium quality South African brandy base products. In their study, they concluded that it was important to use the quality of the distillate as a basis for evaluation of the yeast.

PCA Analysis. PCA was performed on the volatile compounds, esters, higher alcohols, and volatile acids of all the wines fermented with different strains to find yeast grouping at each temperature. PCA of base wines from 15 °C fermentations showed that volatile compounds, higher alcohols, esters, and volatile acids yielded a similar grouping of yeast strains for all of the wines (**Figure 3**). For wine, the first two principal components (PC) accounted for 42.7 and 26.6% of the variance, respectively. The first PC (PC1) separated WE372 from the rest of the strains, and the second PC separated VIN7 and FC. Ethyl caprate explained most of the variability between the yeast strains. For the distillates from 15 °C fermentations, PCA of the esters yielded a grouping with FC separated from the other

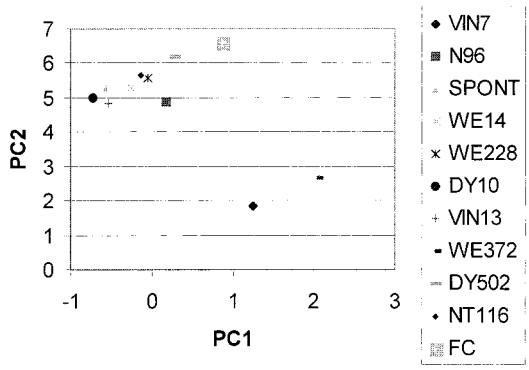

Figure 3. PCA of ester contents in the marula wines produced with 15 °C fermentations.

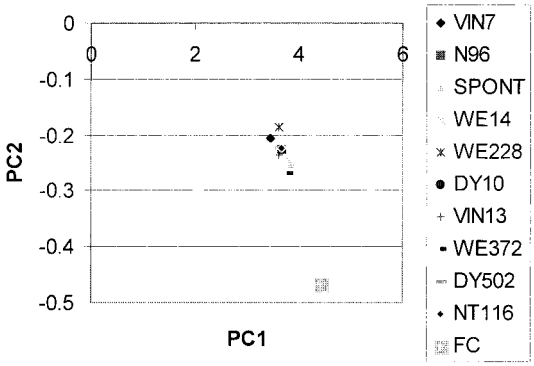

Figure 4. PCA of ester contents in the marula distillates produced with 30 °C fermentations.

strains (data not shown). No apparent grouping was observed in the volatile acids.

With PCA of 30 °C fermentations no apparent grouping was observed with any of the volatile components in the wines. With the corresponding distillates, no apparent grouping was observed with the higher alcohols and volatile acids. When PCA was applied to the esters, strains FC, NT116, and DY502 were clearly separated from the other strains. Of all the volatile compounds, only FC was separated from the other strains (**Figure 4**). No apparent trend of yeast strains differentiated 1999 and 2000. More work needs to be done over a longer period of time to establish any correlation between climatic conditions and yeast performance. It is therefore very important to analyze the fruit extensively at the beginning of each season and choose the yeast strain accordingly.

Sensory Analysis. The perceived quality of the individual marula base wines and distillates as assessed by the tasters is depicted by Duncan's rankings (SAS program). A sensory analysis was done on all of the wines and distillates using a panel of 10 judges. The Kolmogorov−Smirnov test showed that distribution from the normal did not differ significantly in any of the judges' rankings.

For differences in flavor intensity and acceptability, differences for the yeast strains were obtained according to ANOVA. As shown in **Table 7**, the tasters differed in the way they judged the acceptability and flavor intensity of the wines, but there was no significant difference among the wines produced by the different strains. However, there was a significant difference among wines after distillation ($p < 0.00003$). The mean values of the flavor intensity and acceptability sensory analysis were grouped according to Duncan's grouping (**Tables 8** and **9**) for wines and distillates, respectively. The wines formed into two groups, with wine fermented by the strain DY502 performing

Table 7. Two-Way ANOVA of Flavor Intensity and Acceptability for the Marula Base Wines and Their Distillate Assessed by a Panel

	marula wine			final distillates		
effect	df	*F* value	*p* value	df	*F* value	*p* value
taster	9	2.02	0.046	9	2.8687	0.0051
strain	19	1.49	0.1546	10	4.5464	0.00003

Table 8. Sensory Flavor Intensity and Acceptability Ratings for Base Wines

Duncan grouping		mean	*N*	strain
	A	50.60	10	N96
	A	45.30	10	WE14
B	A	43.70	10	DY10
B	A	40.80	10	VIN13
B	A	40.00	10	WE372
B	A	39.20	10	NT116
B	A	34.20	10	WE228
B	A	32.30	10	VIN7
B	A	18.30	10	SPONT
B	A	10.10	10	FC
B	A	2.30	10	DY502

Table 9. Sensory Flavor Intensity and Acceptability Score Rating for Final Distillates

Duncan grouping		mean	*N*	strain
	A	56.40	10	DY10
	A	55.60	10	NT116
	A	55.10	10	VIN13
	A	54.70	10	WE14
	A	54.40	10	VIN7
	A	53.20	10	SPONT
	A	45.20	10	WE22
	A	44.70	10	N96
B	A	27.90	10	DY502
B	C	12.67	9	WE372
	C	−1.80	10	FC

the worst. The wines fermented with strains N96 and WE14 were judged to possess the authentic marula flavor complex. The sensory evaluation performed on the resultant distillates, on the other hand, gave three groups, with strains DY502, WE372, and FC performing the worst. It is interesting to note that strains DY10, NT116, and VIN13 were ranked highly; these strains have similar volatile profiles, as shown in **Figure 2**. The clustering of WE372 with strains VIN13, DY10, and NT116 could not be explained.

Correlation between Sensory Evaluation Results and Volatile Compound Composition. The marula base wines fermented with the yeast strains N96 and WE14 were ranked as the best, whereas the wines fermented with strains FC and DY502 were rated low. Comparison of the volatile compounds of these strains showed that despite the very high ester levels in DY502 (almost double those of N96), DY502 had a very high HA concentration. In this case the VA levels, 3-fold those in N96, clearly might have contributed to the overall performance of the strain. After DY502, strain FC performed the worst, as shown in **Table 8**. In this case the wine showed low VA with very high levels of esters and relatively high total HAs. It is important to note that FC and DY10 had the highest concentrations of methanol compared to the rest of the strains. Methanol gives a cooked cabbage odor, with a threshold of 1.20 g/L (*21*). This could have contributed to the disagreeable olfactory flaws in the wines. On the other hand, the distillate of strain DY10 was judged to be best despite having intermediate total ester concentrations of 27.69 mg/L. This distillate had low propanol levels, whereas the isoamyl alcohol levels were very high in the wines. In contrast, the distillations of FC and WE372 were rated the poorest performers. They both had relatively high ester levels, FC with the highest concentration at 101.90 mg/L. It is interesting to note that these two had low total HA concentrations and the total VA was also low. Excessive propanol levels seemed to mask all of the other positive notes in the distillates, producing what the panel described as a solvent note. Given the fact that strains with high concentrations of esters did not necessarily perform best in both the wines and the distillates, one is tempted to speculate on the effect of too much esters in the marula fruit. It has been reported that marula fruit that is too ripe exhibits a repulsive odor (*22*).

From the study, it is apparent that fermentation temperature had a significant influence on the production of an acceptable fermentation volatile flavor balance in both the marula wines and the distillates. It can thus be recommended that marula fermentations be carried out at low fermenting temperatures. However, more work needs to be done to determine the effect of temperatures between 15 and 30 °C. The different yeast strains produced different volatile compound concentrations. It is thus clear that depending on the end product produced, the choice of yeast strain should be based on the quality of either the wine or distillate.

The volatile profile ratios of wines change on distillation; therefore, when a yeast strain for the production of a distilled beverage is selected, it is recommended that the performance of the strain be judged after distillation.

The performance of the spontaneous fermentations in this study highlights the importance of tapping the hidden wealth of indigenous yeast species present on the marula and the selection and genetic development of yeast starter cultures with improved flavor profiles.

At this point we can recommend the strains that performed well in this study; however, analysis should in future be taken a step further by increasing the size of the fermentations and distillations to get a representative picture of their performance on a larger scale.

ACKNOWLEDGMENT

We thank Prof. D. Ward for assistance with the statistical analysis.

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Received for review August 28, 2001. Revised manuscript received December 26, 2001. Accepted December 28, 2001. We thank WINETECH and the Scientific and Industrial Research and Development Centre (SIRDC) for financial support.

JF0111514