The Use of *Candida pulcherrima* in Combination with *Saccharomyces cerevisiae* for the Production of Chenin blanc Wine

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Wine fermentations are conducted by naturally occurring or selected industrial wine yeast strains of *Saccharomyces cerevisiae.* However, *non-Saccharomyces* yeasts also occur naturally in fermenting grape musts, especially in the initial stages of the fermentation. It has been speculated that these yeasts can contribute to the overall characteristics of the wine. Generally, it is accepted that *Kloeckera apiculata* is the predominant non-*Saccharomyces* yeast species in grape must. However, it was shown previously that *Candida pulcherrima* was the predominant *non-Saccharomyces* yeast species in a grape must after sedimentation and prior to inoculation with commercial wine yeast. Subsequently, this yeast was investigated in laboratory and small-scale wine fermentations of Chenin blanc wine. As it could not ferment grape juice to dryness on its own, it was used in combination with a *S. cerevisiae* wine yeast strain. The effect of SO2, di-ammonium phosphate (DAP), pH and temperature on the growth of *C. pulcherrima* was also investigated. In combined fermentations, no change in overall fermentation rate or standard wine chemical analyses could be observed in comparison to a control *S. cerevisiae* fermentation. However, wine production in three consecutive years showed that the wine produced by the combined fermentation was of higher quality than that produced by the *S. cerevisiae* only.

In South Africa the most widely planted white grape variety is Chenin blanc. However, wines produced from this cultivar tend to be neutral in aroma and taste. A number of viticultural (pruning, ripeness at harvest) and winemaking practices (skin contact, post-fermentation lees contact, maturation practices) are currently being investigated to improve wine quality. A further aspect receiving attention is the role of yeast.

Chenin blanc fermentations are normally conducted by selected *Saccharomyces cerevisiae* industrial wine yeast strains. However, *non-Saccharomyces* yeasts also occur naturally in fermenting grape musts, especially in the initial stages of the fermentation (Heard & Fleet, 1985). It has been shown that some *non-Saccharomyces* yeasts can contribute to the overall characteristics of the wine (Romano *et al,* 1997; Soden *et al.,* 2000). These facts were confirmed by Jolly *et al.* (2003a; 2003b). Furthermore, species present in higher numbers could be expected to have a greater effect on the fermentation and resultant wine quality than species present in lower numbers.

Generally, it is accepted that *Kloeckera apiculata* is the predominant *non-Saccharomyces* yeast species found in grape must (Fleet *et al.,* 1984; Querol *et al.,* 1990; Longo *et al,* 1991), but, as this yeast is usually associated with volatile acidity production, the potential for a positive contribution to wine quality is low (Romano *et al.,* 1992; Gil *et al,* 1996). Previously, it was shown that *Candida pulcherrima* can also occur in high numbers in must (Schiitz & Gafner, 1993; Jolly *et al,* 2003a). This **non-***Saccharomyces* yeast is not normally associated with volatile acidity production, but can form relatively high concentrations of esters (Bisson & Kunkee, 1991). These esters, as well as other metabolites could have a positive benefit for a Chenin blanc wine with neutral cultivar characteristics. While *C. pulcherrima* has been used to improve wine quality (Jolly *et al.,* 2003b), it is not known how must pH and different winemaking practices, i.e. fermentation temperature, addition of di-ammonium phosphate (DAP) and SO2, will affect tiiis yeast. Therefore the aim of this study, which forms part of the ongoing research programme documented by Pretorius *et al.* (1999), was to investigate the effect of pH, fermentation temperature, DAP and SO_2 addition on the growth of *C. pulcherrima.* Subsequently, two *C. pulcherrima* isolates were investigated in small-scale wine fermentations over three vintages during the production of Chenin blanc wines.

MATERIALS AND METHODS

Yeast strains

Three yeast strains were used in this investigation, viz. *C. pulcherrima* (strains 825 & Cl-15), previously isolated from vineyards and grape must from the Western Cape, South Africa (Jolly *et al.,* 2003a) and an industrial *S. cerevisiae* yeast strain (strain

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VIN 13, Anchor Bio-Technologies, South Africa). The first *C. pulcherrima* strain (strain 825) was selected randomly for wine production (Jolly *et al.*, 2003b) and the second (strain Cl-15) was selected after screening 71 C. *pulcherrima* isolates from South African grape musts. The screening criteria were highest growth tempo, equal or lower formation of volatile acidity (in comparison to *S. cerevisiae* reference) and highest ethanol production in a grape must at 15°C. Strain 825 was used for the laboratory-scale fermentation and wine production for all three vintages, while strain Cl-15 was used for wine production in the second and third vintages only.

Laboratory-scale fermentations

Four sets of laboratory-scale fermentations were conducted to determine the individual effects of pH, temperature, DAP and SO2, respectively. A further two sets of fermentations were done to determine combined effects of the aforementioned (Table 1).

Grape must

A previously frozen white grape must (21.5°B sugar, 5.6 g/L total acidity, pH 3.4, 0.50 g/L volatile acidity and 1 mg/L total SO_2) was used for all laboratory-scale fermentations. After thorough mixing, 500 mL aliquots were placed in 750 mL glass bottles and sterilised (121°C for 15 min). The bottles were closed tightly by plastic fermentation caps filled with sterile distilled water. For the relevant experiments the pH was adjusted (3.2 & 3.5), DAP (0.5 g/L) and a 10% solution of sodium meta-bisulphite were added to give 30 and 60 mg/L total SO_2. In the other instances no changes or additions were made to the must.

TABLE 1

The effect of four different winemaking practices on the slope of the logarithmic phase of the fermentation curve of laboratory-scale fermentations with *Candida pulcherrima* (strain 825) and *S. cerevisiae* (strain VIN 13).

Grape must parameter and winemaking practice		Slope[1]	
		C. pulcherrima (strain 825)	*S. cerevisiae* (strain VIN 13) (reference)
Individual effects:			
pH 3.2 (low)		1.08 ±0.14	9.37 ± 0.57
pH 3.5 (high)		1.29 ±0.19	**9.04** ± 0.45
DAP (none)		1.04 ±0.09	9.86 ± 0.23
DAP (0,5g/l added)		1.35 ± 0.07	10.31 ± 0.59
Low temperature	15°C	1.06 ± 0.66	5.17 ± 0.06
Intermediate temperature	20°C	1.64 ±0.21	14.92 ± 0.23
High temperature	28°C	1.82 ±0.21	23.88 ± 0.56
SO_2 (0 mg/1)		0.78 ± 0.08	8.38 ± 0.35
SO_2 (30 mg/1)		0.80 ±0.10	8.53 ±0.18
SO_2 (60 mg/1)		0.63 ± 0.09	8.40 ±0.12
Combined effects:			
28°C; pH 3.5; 0.5g/L DAP; No SO_2		2.62 ±0.16	22.62 ± 0.55
15°C; pH 3.2; no DAP; 60mg/L SO2		0.54 ± 0.13	5.56 ± 0.99

[1] Slope of the logarithmic phase of the fermentation curve (m = y/x-c/x, where m is the slope of the logarithmic growth phase; x and y are the standard co-ordinates; and c is the x-axis intercept). Average value of fermentations in triplicate ± standard deviation.

Yeast inoculum and fermentation procedures

Yeast starter cultures were grown for 24 h in YPD liquid medium (1% yeast extract, 2% peptone, 2% glucose). Total cell counts were carried out in a Neubauer improved bright-lined counting chamber (1 mm depth) and all inoculations were done at 1 x 10^6 cells/mL per yeast strain. The two *C. pulcherrima* strains were inoculated individually and all fermentations were conducted in triplicate. Reference fermentations were conducted by *S. cerevisiae* (strain VIN 13) only. The fermentation vessels were placed on an orbital shaker at 20°C, except for the temperature experiment, where fermentations were conducted at 15, 20 and 28°C. Fermentations were monitored by CO_2 weight loss and allowed to proceed until the reference fermentation was dry (14 days). Completion of fermentation (no further weight loss) was confirmed by use of glucose test strips (Clinistix, Bayer). The progression of CO2 weight loss was used to plot a fermentation curve. The slope of the logarithmic phase of the fermentation curve was calculated and used for comparison within an experiment as an indication of the yeast's ability to ferment.

Small-scale wine production

The ***C.*** *pulcherrima* yeast strains were investigated in combination with the 5. *cerevisiae* for small-scale production of Chenin blanc wine.

Grape must

The Chenin blanc grapes were commercially harvested from the vineyards of the ARC Infruitec-Nietvoorbij Research Institute, Stellenbosch. After crushing and sedimentation (0.5 g/hL Ultrazym, Novazymes, Denmark), the clear juice was racked off the lees and divided into the fermentation containers. The chemical analyses for the musts were: 21.7°B sugar, 6.9 g/L total acidity, pH 3.71; 23.0°B sugar, 7.8 g/L total acidity, pH 3.33; and 24.3°B sugar, 6.8 g/L total acidity and pH 3.55 for the 2000, 200J and 2002 vintages, respectively. Di-ammonium phosphate (0.5 g/L) and 10% sodium meta-bisulphite solution (50 mg/L total SO2) were added before fermentation.

Fermentation procedure

The *C. pulcherrima* yeasts were propagated in the same way as for the laboratory-scale fermentations and inoculated at a concentration of 1 x 10^6 cells/mL into 18 L of the freshly prepared grape musts. This first inoculation was followed one hour later by an active dried *S. cerevisiae* (strain VIN 13) inoculation at a concentration of 0.04 g/L. A reference grape must was inoculated with the *S. cerevisiae* only. Wine production was done in duplicate and continued as described by Jolly *et al.* (2003b).

Sensory evaluation of **small-scale wines**

The wines (small-scale only) were subjected to a sensory evaluation by different panels of seven trained wine tasters. Descriptive sensory analyses were done on all the wines five months after production. A ten-centimetre unstructured line scale was used and the judges were asked to rate 'fruity' and 'guava' aroma intensity (undetectable to prominent) and general quality (unacceptable to excellent). In addition, wines from the first two vintages were evaluated at five and 18 months, while wines from the third vin-

tage were evaluated at five months only according to the multi-wine preferences method (McCloskey *et al.*, 1995). Further wine evaluation protocols were according to Jolly *et al.* (2003b).

Chemical and statistical analyses

The wines (laboratory and small-scale) were analysed for alcohol (infralyser technique - Cape Wine Laboratory, Stellenbosch), glycerol (enzymatic test kits, Boehringer Mannheim, Roche, Germany; and Winescan, Institute for Wine Biotechnology, Stellenbosch University), and for residual sugar (Rebelein), volatile acidity and SO_2 as described by Hand *et al.* (2000). Analyses for esters (volatile component analyses - Research Chemistry, Distell, Stellenbosch) were carried out at the time of the five-month sensory evaluations on the small-scale wines only. Analysis of variance was performed on the ester values and the descriptive sensory analyses values using SAS version 8.2 (SAS, 1999). The Shapiro-Wilk test was done to test for non-normality (Shapiro & Wilk, 1965) and Student's t-Least Significant Difference was calculated at the *5%* confidence level to compare treatment means (Ott, 1998).

RESULTS AND DISCUSSION

As our *C. pulcherrima* isolates could not ferment a grape must to dryness on their own (Fig. 1), they needed to be co-inoculated with a *S. cerevisiae* wine yeast. The *S. cerevisiae* strain chosen for this, viz. VIN 13, is recommended by the manufacturers for the production of aromatic white wines at low temperatures. This made it an ideal choice for Chenin blanc production. It is also a strong fermentor and is not generally implicated in stuck fermentations. Although it has previously been reported that some strains of *C. pulcherrima* have an inhibitory effect against *S. cerevisiae* (Nguyen & Panon, 1998), the two strains used in this investigation did not show this effect against *S. cerevisiae* strain VIN 13 (data not shown). It was, however, expected that the *S. cerevisiae* would be competing for the same nutrients as the *C. pulcherrima,* but there was no reduction in fermentation rate in comparison to the VIN 13 reference fermentation (Fig. 1). Typically, *C. pulcherrima* could be detected until the ninth day of a co-inoculated 14-day fermentation by streaking 200(iL aliquots onto lysine medium and checking for colonies producing the red-brown pigment pulcherrimin (Heard & Fleet, 1986; Miller & Phaff, 1998; Jolly *et al.*, 2003b). The one-hour time lapse between inoculating the *C. pulcherrima* and the *S. cerevisiae* yeast was chosen to allow the *C. pulcherrima* to adapt to the must and start its growth, before being dominated by the *S. cerevisiae.* In addition, the short time lapse before the start of fermentation will minimise any detrimental oxidation of the must. Placement of the fermentation vessels on the orbital shaker copied the natural turbulence found in large fermentations due to the generation of CO_2 (Henschke, 1990). The tightly sealed fermentation caps ensured that no oxygen entered the fermentation vessel.

Laboratory-scale fermentations

The manipulation of winemaking practices (use of DAP and SO2), fermentation temperature and must pH generally had similar effects on the fermentation ability of *C. pulcherrima* and the *S. cerevisiae* reference strain (Table 1). Di-ammonium phosphate addition, higher pH values and increased temperatures all resulted in a slight increase in the fermentation ability of strain 825 as is shown by the increased slope of the logarithmic phase of the fermentation curve. However, the increase in fermentation ability due to increased fermentation temperature was not as dramatic as for the *S. cerevisiae* reference. Higher levels of ethanol were also formed (Table 2), reflecting a more efficient fermentation by strain 825.

Sulphur dioxide in the concentration range normally used in wine fermentation, i.e. 0-30 mg/L, did not affect the fermentation ability

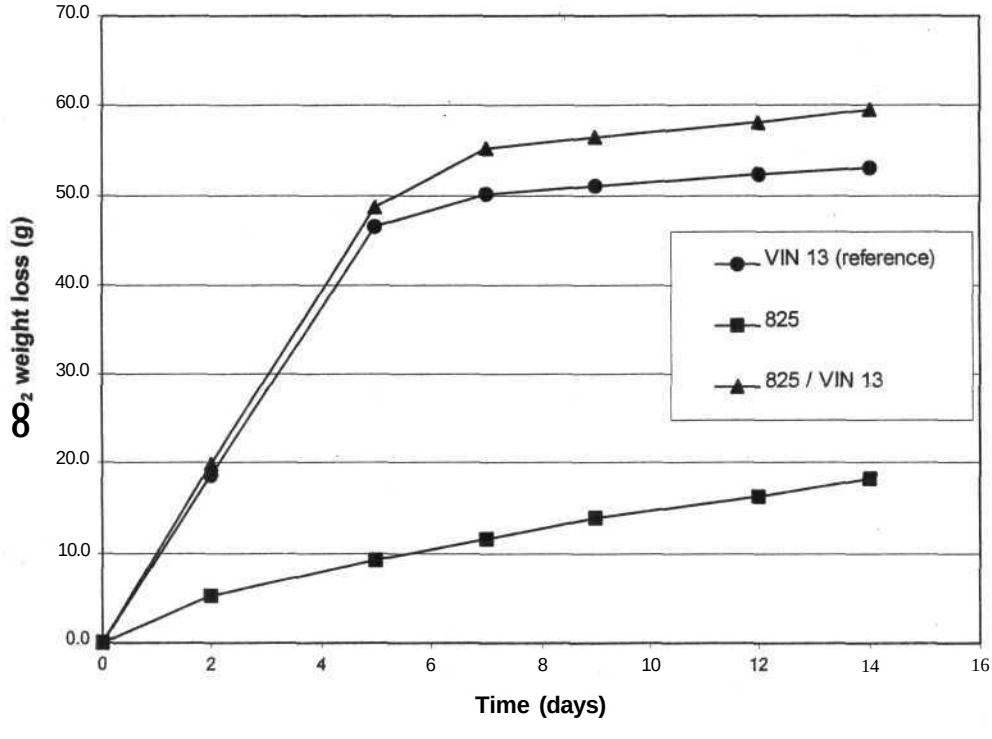

FIGURE 1

Laboratory-scale fermentation curves of *C. pulcherrima* (strain 825) and *S. cerevisiae* (strain VIN 13) compared to a combined *S. cerevisiae/C. pulcherrima* fermentation.

TABLE 2

The effect of four different winemaking practices on chemical analyses of wines produced by laboratory-scale fermentations with *Candida pulcherrima* (strain 825).

Grape must parameter and winemaking practice		Chemical analyses[1]							
		Alcohol (% v/v)		Volatile acidity (g/L)		Total SO_2 (mg/L)		Glycerol (g/L)	
		C. pulcherrima (strain 825)	*S. cerevisiae* (strain VIN 13) (reference)	*C. pulcherrima* (strain 825)	*S. cerevisiae* (strain VIN 13) (reference)	*C. pulcherrima* (strain 825)	*S. cerevisiae* (strain VIN 13) (reference)	*C. pulcherrima* (strain 825)	*S. cerevisiae* (strain VIN 13) (reference)
Individual effects:									
pH 3.2 (low)		3.2 ±0.1	12.8 ±0.1	0.22 ±0.02	0.20 ±0.01	14±1	21±2	5.17 ±0.96	6.15 ±0.16
pH 3.5 (high)		3.6 ± 0.1	12.9 ± 0.1	0.24 ± 0.01	0.18 ± 0.02	14 ± 1	20 ± 1	5.51 ± 0.67	6.60 ±0.10
DAP (none)		3.2 ±0.2	12.8 ± 0.1	0.23 ± 0.01	0.23 ± 0.03	18 ± 2	27 ± 1	6.14 ± 0.52	6.48 ± 0.48
DAP (0,5g/l added)		3.5 ± 0.2	12.8 ± 0.1	0.25 ± 0.02	0.19 ±0.04	18 ± 1	30 ± 1	6.38 ± 0.14	6.16 ± 0.43
Low temperature:	15°C	3.9 ± 0.5	12.5 ± 0.1	0.14 ±0.04	0.18 ±0.04	12 ± 1	24 ± 1	6.88 ± 0.96	5.76 ± 0.35
Intermediate temperature:	20°C	4.4 ± 0.2	12.5 ± 0.1	0.14 ±0.02	0.14 ±0.02	12 ± 2	17 ± 1	6.75 ±1.22	5.92 ±0.11
High temperature:	28°C	3.7 ± 0.2	12.5 ± 0.1	0.14 ±0.01	0.32 ±0.01	15 ±1	17 ± 1	5.36 ±1.51	6.21 ±0.10
SO_2 (0mg/l)		2.7 ± 0.2	12.8 ± 0	0.25 ±0.01	0.35 ±0.01	17 ± 2	22 ±1	7.14 ± 0.07	5.05 ±0.37
SO_2 (30 mg/l)		2.5 ± 0.1	12.8 ± 0	0.23 ±0.01	0.33 ±0.02	25± 2	37± 3	6.06 ± 0.38	5.78 ± 0.57
SO_2 (60 mg/l)		2.2 ±0.1	12.8 ±0.1	0.19 ±0.03	0.35 ±0.02	61± 1	59±4	4.81±0.63	5.40 ±0.12
Combined effects:									
28°C; pH 3.83; 0.5g/L DAP; No SO_2		1.2 ±0.1	13.5 ± 0.1	0.11 ±0.02	0.27 ±0	7 ± 2	11 ± 1	12.17 ±0.57	7.20 ±0.10
15°C; pH 3.2; no DAP; 60mg/L SO_2		0.7 ± 0.5	13.7 ± 0.4	0.15 ± 0	0.21 ±0.01	38 ±2	46 ±1	6.87 ± 1.31	7.43 ± 0.06

[1] Average of triplicate fermentations ± standard deviation.

of 825. This contrasts with the accepted opinion that all non-*Saccharomyces* yeasts are sensitive to wine-related SO_2 levels. Under normal winemaking conditions, the growth of *C. pulcherrima* strain 825 therefore will not be hampered by SO_2. This insensitivity to SO_2 was also noted by Granchi *et al.* (1998), who found that SO_2 in the range of 50-100 mg/L did not succeed in preventing the growth of *non-Saccharomyces* yeasts in Sangiovese wine fermentations. However, at higher SO_2 concentrations, i.e. 60 mg/L, the growth of 825 was slightly retarded, as also reflected in the lower alcohol, volatile acidity and glycerol levels. In comparison, the VEST 13 fermentation ability remained unaffected. Excessive volatile acidity, which is often formed when yeasts grow under stress conditions (lower temperature, pH and nitrogen limitation), was not formed by strain 825 during the laboratory-scale fermentations.

The combined effects of lower temperature, low pH, no DAP and high SO_2 had a limiting effect while, as expected, the combined conditions of higher temperature, higher pH with the addition of DAP and no SO_2 enhanced *C. pulcherrima* growth (Table 1). Furthermore, the chemical analyses (Table 2) show a marked increase in the glycerol concentration. This is supported by a reduction in alcohol of 2% when compared to the previous fermentations (individual effects). This phenomenon was not observed in the previous investigations (Jolly *et al.*, 2003b) and may be due to the high pH and fermentation temperature.

In a warm climate like that of South Africa, where must pH tends to be high and red wine fermentation temperatures can exceed 25°C, these yeasts, which are present naturally (Jolly *et al.*, 2003a), may already be playing a role in wine quality. Their role would be smaller at lower fermentation temperatures in low pH musts.

Screening of *C. pulcherrima* isolates for wine production

As *C. pulcherrima* strain 825 has a low fermentation ability, a stronger fermentor was desired so that the yeast could make a greater impact on the fermentation. Therefore, fermentation ability and alcohol production were chosen as selection criteria. Production of volatile acidity, as potentially detrimental to wine quality, was also checked. A fermentation temperature of 15°C was used, as it is most representative of the production of South African Chenin blanc in a fruity, non-wooded style.

The screening results (data not shown) did not show much variation from strain 825, although strain Cl-15 showed an improvement in fermentation ability, which was also reflected in a marginal improvement in alcohol production (Table 3). The volatile acidity was lower than that of strain 825 and comparable to that of the *S. cerevisiae* reference.

Small-scale wine production

Chemical analyses

The fermentations for all three vintages were completed and there were no marked differences between the standard wine chemical analyses of the *S. cerevisiae* reference wine and the wines produced by the *C. pulcherrimalS. cerevisiae* combinations (Table 4). No yeast counts were done during the fermentations; howev-

er, it was previously shown that *C. pulcherrima* could be detected for approximately two thirds of a combined fermentation (Jolly *et al.*, 2003b) and the same scenario was expected to have occurred here. The combined fermentations did show a tendency towards lower residual sugars than the *S. cerevisiae* monoculture. This shows a more efficient must sugar utilisation by the dual culture and was also noted by Ciani & Ferraro (1998) in their combined fermentations with *C. stellata* and *S. cerevisiae.* No increased glycerol concentration, as was the case in the laboratory-scale fermentation, was noted. However, this may have been due to the fermentation taking place at 15°C and not at 28°C.

It has been reported previously that *C. pulcherrima* yeasts are high ester producers (Bisson & Kunkee, 1991). As mentioned in the introduction, esters can make a positive contribution to a Chenin blanc wine with neutral cultivar characteristics, even though this may be of short duration. Gas chromatographic analyses of total esters, total higher alcohols and total acids showed no significant differences between the *S. cerevisiae* reference wines and the *C. pulcherrima/S. cerevisiae* combinations (Table 5). This may be due to there not being enough *C. pulcherrima* yeast or that a longer period is needed for ester formation to become apparent. The results of the 2001 and 2002 vintages therefore confirmed the findings of Jolly *et al.* (2003b) that *C. pulcherrima* strains 825 and Cl-5 did not make a contribution to total esters in the wine under the conditions used.

Sensory evaluation

Sensory evaluation can be subjective. However, the human nose is capable of detecting aroma, flavour and other sensory nuances that are not measurable by current instrumentation. Furthermore, in the wine industry most decisions regarding wine quality with the subsequent economic implications often rely more on sensory evaluation and less on chemical analyses. Sensory evaluation is also the final criterion for judging any wine-making manipulation.

In this investigation the sensory evaluation of the two wine types by descriptive analyses (Table 6) showed that, for the 2000 vintage wines, there was no significant difference in aroma profile and quality of the five-month old wines. However, the *C. pulcherrima* (strain 825) wine did have a higher aroma note of 'guava'. Wines from the second vintage (2001) were judged to differ more from each other, with the *C. pulcherrima* wines having the highest scores for 'guava'. For the wines produced with strain Cl-15, this was significantly higher than the reference wine *(S. cerevisiae* only). The 'fruity' aroma note and 'general quality' were judged to be similar (no significant difference). Wines from the third vintage (2002) were again judged similar for 'fruity', but the *C. pulcherrima* strain Cl-15 wine again scored the highest for

TABLE 3

Comparison of two *C. pulcherrima* strains and one *S. cerevisiae* strain for three selection criteria during fermentations at 15°C.

Yeast strain	**Selection criteria**[1]		
	Slope[2]	**Alcohol** (% v/v)	**Volatile acidity (g/L)**
S. cerevisiae (reference 1)	5.26(5.13-5.39)	12.6 (12.5-12.6)	0.33 (0.33-0.33)
C. pulcherrima (strain 825) (reference 2)	0.67 (0.65-0.68)	3.3 (3.1-3.4)	0.39 (0.37-0.40)
C. pulcherrima (strain Cl-15)	1.16 (0.77-1.55)	**4.6** (3.4-5.7)	0.35 (0.30-0.40)

[1] Average of duplicate fermentation at 15°C. Range indicated in brackets.

[2] Slope of the logarithmic growth phase of the fermentation curve (m= y/x-c/x, where m is the slope of the logarithmic growth phase; y is the y co-ordinate; x is the x co-ordinate; and c is the x-axis intercept).

TABLE 4

Chemical analyses of Chenin blanc wines fermented by *C. pulcherrima/S. cerevisiae* combinations during small-scale wine production at 15°C.

Yeast strain combination	**Vintage**	**Chemical analyses**[1]				
		Residual sugar (g/1)	**Ethanol** (% v/v)	**Volatile acidity** (8/1)	**Total SO_2 (mg/1)**	**Glycerol (g/1)**
S. cerevisiae (reference)	2000[2]	1.2(1.0-1.4)	13.2(13.1-13.2)	0.19(0.18-0.19)	104 (104-104)	5.6 (5.5-5.6)
C. pulcherrima (strain 825) / *S. cerevisiae*	2000[2]	0.9 (0.2-1.6)	13.0 (12.9-13.0)	0.19(0.18-0.19)	98 (94-101)	5.6 (5.5-5.6)
S. cerevisiae (reference)	2001	1.8 (1.6-2.0)	14.6 (14.6-14.6)	0.29 (0.28-0.29)	95 (90-100)	7.7 (7.5-7.8)
C. pulcherrima (strain 825) / *S. cerevisiae*	2001	1.4(1.2-1.5)	14.6 (14.5-14.6)	0.28 (0.27-0.28)	82 (81-82)	7.9 (7.8-7.9)
C. pulcherrima (strain Cl-15) / *S. cerevisiae*	2001	1.2(1.2-1.2)	14.6 (14.6-14.6)	0.29 (0.28-0.30)	88 (82-93)	7.8 (7.8-7.8)
S. cerevisiae (reference)	2002	1.95 (1.90-2.00)	14.9 (14.7-15.1)	0.25 (0.24-0.26)	115(115-115)	7.9 (7.7-8.0)
C. pulcherrima (strain 825) / *S. cerevisiae*	2002	1.90 (1.90-1.90)	15.0 (14.9-15.0)	0.26 (0.25-0.27)	113(111-115)	7.7 (7.7-7.7)
C. pulcherrima (strain Cl-15) / *S. cerevisiae*	2002	1.90 (1.90-1.90)	15.0 (15.0-15.0)	0.26 (0.25-0.27)	117(115-118)	8.0 (8.0-8.0)

[1] Average values of duplicate fermentations. Range indicated in brackets.

[2] Data for the 2000 vintage obtained from Jolly *et al.* (2003b).

TABLE 5

Gas chromatographic analysis of Chenin blanc wines of the 2000, 2001 and 2002 vintages fermented by *C. pulcherrima / S. cerevisiae* combinations during small-scale wine production.

Yeast Combination	Vintage[1]	Total esters[2] (mg/L)	Total higher alcohols[2] (mg/L)	Total acids[2] (mg/L)	Ethyl-acetate[2] (mg/L)	Total esters -ethyl acetate[2] (mg/L)	Ethyl-lactate[2] (mg/L)	Total esters -ethyl acetate -ethyl lactate[2] (mg/L)
S. *cerevisiae* (reference)	2000	216.06a	321.45a	233.64a	176.35a	39.71a	11.41a	28.30a
C. pulcherrima (strain 825) / *S. cerevisiae*	2000	231.62a	311.33a	195.60a	188.32a	43.31a	13.97a	29.34a
S. cerevisiae (reference)	2001	109.04a	194.40a	21.54a	57.13a	51.91a	15.96a	35.95a
C. pulcherrima (strain 825) / *S. cerevisiae*	2001	109.19a	198.84a	20.90b	56.25a	52.94a	16.78a	36.17a
C. pulcherrima (strain Cl-15) / *S. cerevisiae*	2001	110.50a	195.84a	21.65a	56.76a	53.74a	15.21a	38.53a
S. cerevisiae (reference)	2002	127.18a	208.52a	32.63a	98.68a	28.51a	5.21a	23.3a
C. pulcherrima (strain 825) / *S. cerevisiae*	2002	122.40a	202.90a	30.75a	94.92a	27.48a	4.87a	22.62a
C. pulcherrima (strain Cl-15) / *S. cerevisiae*	2002	117.30a	203.70a	29.95a	90.97a	26.34a	**4.71a**	21.62a

[1] Data for the 2000 vintage obtained from Jolly *et al.* (2003b).

[2] Average value of two wines. Values within columns, for the same vintage, followed by the same letter do not differ significantly ($p<0.05$).

TABLE 6

Descriptive sensory analyses of five-month-old Chenin blanc wines produced by combinations of *C. pulcherrima* and 5. *cerevisiae* yeasts for the 2000, 2001 and 2002 vintages.

Yeast strain	Wine characteristic (%)[1]								
	2000 vintage			2001 vintage			2002 vintage		
	Fruity aroma intensity	Guava aroma intensity	General quality	Fruity aroma intensity	Guava aroma intensity	General quality	Fruity aroma intensity	Guava aroma intensity	General quality
S. *cerevisiae* (reference)	44.79a	16.57a	48.86a	52.92a	25.17b	52.50a	64.00ab	25.14a	49.43b
C. pulcherrima (strain 825) / 5. *cerevisiae*	43.57a	21a	52.36a	59.50a	28.83ab	50.33a	57.86b	23.57a	53.43ab
C. pulcherrima (strain Cl-15) / *S. cerevisiae*	–[2]	–[2]	–[2]	49.25a	42.83a	48.00a	67.86a	30.00a	60.57a

[1] Average value of duplicate wines judged by panels of seven judges. Values within columns for the same vintage, followed by the same letter, do not differ significantly ($p<0.05$).

[2] Not produced during the 2000 vintage.

the 'guava' aroma note (although not statistically significant). The quality of the Cl-15 wine was also significantly better than the reference wine.

The wines were also tasted according to the multi-wine preference method (McCloskey *et al.*, 1995) to obtain a relative score. During this evaluation the judges were asked to judge overall wine quality (inclusive of wine colour, aroma, flavour and body/mouthfeel). From Table 7 can be seen that the combined *C. pulcherrima/S. cerevisiae* wines always scored higher than the reference wine produced by the *S. cerevisiae* only over the three vintages investigated. This, despite the possible subjectivity of the measuring instrument, should be considered significant. Furthermore, in the two vintages for which the selected *C. pulcherrima* strain Cl-15 was used, that wine received the highest rating. This supports the choice of selection criteria used for selecting Cl-15. It was further noted in the 2002 vintage that the wines judged to be better had lower total ester values than the reference wine (Tables 5 & 7), indicating that other metabolites were playing a role in wine quality.

From the data presented it appears that the effect of the *C. pulcherrima* strains in wine fermentation was more complex than could be measured by the chemical techniques used. At this stage of the investigation it is also not yet clear by how much wine quality can be improved. Strain selection criteria could also be

TABLE 7

Relative score of Chenin blanc wines fermented by *C. pulcherrima I S. cerevisiae* combinations during small-scale wine production.

Yeast combination	Vintage / Time of evaluation / Relative score[1]				
	2000 vintage[2]		2001 vintage		2002 vintage
	5 months	18 months	5 months	18 months	5 months
S. cerevisiae (reference)	**-2 (-5, 1)**	**-1 (-5, 3)**	**-2 (-3, -1)**	**-2 (-3, -1)**	**-1** (-3, **1)**
C. pulcherrima (strain 825) / *S. cerevisiae*	2(1,3)	**5 (5, 5)**	**1 (-1, 3)**	**-1 (-5, 3)**	0(-l, 1)
C. pulcherrima (strain Cl-15) / 5. *cerevisiae*	–[3]	–[3]	**4(1,7)**	**3(1,5)**	**1(1,1)**

[1] Average score of two wines evaluated by the multi-wine preference method (McCloskey *et ah,* 1995). Only values within a column are related to each other. Highest score in bold type. Range indicated in brackets.

[2] Data for the 2000 vintage obtained from Jolly *et al.,* 2003b.

[3] Not produced during the 2000 vintage.

sharpened, while combinations with other strains of 5. *cerevisiae* can also be investigated, bearing in mind the possible inhibitory effect that *C. pulcherrima* may have on some 5. *cerevisiae* strains. Scaling up to pilot and commercial scale should also be carried out, while the extent to which the *C. pulcherrima* yeasts survive during the fermentation can also be ascertained, enabling wine-making conditions to be adjusted so that the survival and growth of the *C. pulcherrima* yeast is optimised.

CONCLUSIONS

The effects of pH, SO2, DAP addition and temperature on *C. pulcherrima* follows the same pattern as that on *S. cerevisiae.* However, elevated levels of SO_2 can suppress the growth of *C. pulcherrima,* but these levels are much higher than normally found in practice. The use of a selected strain of C. *pulcherrima* had a positive influence on wine quality not linked to ester levels, which also did not detrimentally affect standard wine chemical analyses. Further isolation from grape musts and strain selection for more vigorous strains will make it possible to optimise the improved quality contribution. Further chemical analyses and methodology development could identify the metabolites responsible for this quality improvement. In the interim, the selected strains could be employed for the improvement of South African Chenin blanc wines.

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