Oenological Properties of an Interspecific *Saccharomyces* Hybrid

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An interspecific hybrid between a thermotolerant *Saccharomyces cerevisiae* strain and a cold-fermenting *Saccharomyces uvarum* strain was characterised oenologically. This hybrid was shown to possess the parental traits in a useful combination for winemaking. The wines obtained by the hybrid were characterised by a low titratable acidity. This was due to the ability of the hybrid to degrade malic acid with the same intensity as the thermotolerant *S. cerevisiae* parental strain. The hybrid produced wines with a volatile acidity that was as low as that obtained using the cold-fermenting *S. uvarum* parental strain; a high glycerol concentration (obtained from both parental strains) and it fermented as vigorously as most of the *S. cerevisiae* wine strains at temperatures of oenological interest. This hybrid was successfully used for white wine production and helped to reduce excessive concentrations of malic acid. It was also used for red wine production. In this case the high pH values of the fermented must created an ideal situation for the starting of the malo-lactic fermentation and allowed the production of high quality full-bodied wines.

The *Saccharomyces sensu stricto* group is made up of four species: *Saccharomyces baynaus, Saccharomyces cerevisiae, Saccharomyces paradoxus* and *Saccharomyces pastorianus* (Vaughan-Martini & Martini, 1998). Wine yeasts most commonly belong to the species *S. cerevisiae*. These strains generally ferment grape must vigorously in the temperature range 12 to 36°C.

There are a few *S. cerevisiae* strains that can ferment well at relatively high temperatures (over 40°C) and for this reason they are often referred to as thermotolerant (Hacking, Taylor & Hanas, 1984; Anderson, McNeil & Watson, 1988; Laluce, Palmieri & Lopes da Cruz, 1991; Rainieri *et al.*, 1996). Some of these strains produce large amounts of glycerol, compared with the commercial *S. cerevisiae* wine strains, and display a high deacidifying action, being intense malo-alcoholic fermenters. In spite of these potentially useful oenological traits, thermotolerant *S. cerevisiae* strains are not suitable for vinification because they are not vigorous fermenters at temperatures of oenological interest and, in addition, they produce large amounts of acetic acid (Rainieri *et al.*, 1998b).

Strains possessing good oenological traits but lacking a complete oenological suitability can be improved by means of intraspecific hybridisation, a description of this technique is provided by Pretorius & Van der Westhuizen (1991) and Barre *et al.* (1993). Intraspecific hybridisation has been successfully used for the improvement of wine yeasts (Thornton, 1982; Romano *et al.*, 1985; Thornton, 1985; Eustace & Thornton, 1987). Nevertheless, the parental strains used both belong to *S. cerevisiae*, so the traits that can be exchanged or introduced are limited to those of this species.

The characteristics of hybrids obtained by crossing strains of different species can be novel but difficult to predict. Nevertheless, interspecific *Saccharomyces* hybrids are sterile, stable, vigorous (Naumov, Naumova & Korhola, 1992) and may possess traits of oenological interest. Several studies report the characteristics of hybrids obtained by crossing *S. cerevisiae* wine strains with strains belonging to *S. bayanus* (*sensu* Vaughan-Martini & Martini, 1998) (Zambonelli *et al.*, 1993; Kishimoto, 1994; Zambonelli *et al.*, 1997; Rainieri *et al.*, 1998a). These strains are sometimes referred to as cold-fermenting or cryotolerant due to their ability to ferment well at low temperatures (6 to 12°C). A few recent physiological and molecular studies (Nguyen & Gaillardin, 1997; Rainieri *et al.*, 1999; Nguyen, Lepingle & Gaillardin, 1999) suggested that these cold-fermenting strains form a homogeneous group distinct from the *S. bayanus* type strain. The use of the names *S. bayanus* of the *uvarum* type (Nguyen & Gaillarden, 1998) of *S. uvarum* (Rainieri *et al.*, 1999) have also been proposed. This group of strains possesses interesting characteristics for winemaking, such as the ability to produce wines with low volatile acidity and a high concentration of malic acid. Interspecific hybrids obtained by crossing *S. cerevisiae* and cold-fermenting *S. uvarum* wine strains (i.e. *S. uvarum* according to Rainieri *et al.*, 1999) produce wines with fermentation by-products at concentrations that are midway between those obtained using the parental strains. The basic traits of the

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S. uvarum strains, such as the ability to synthesise malic acid, to produce high amounts of succinic acid, glycerol and 2-phenylethanol, and low amounts of acetic acid, remain present in the hybrids, but to a lesser degree. These hybrid strains have been successfully used to replace *S. uvarum* strains that produced wines with excessive acidity or unacceptably high concentrations of glycerol and 2-phenylethanol (Zambonelli *et al.*, 1997; Caridi *et al.*, 1997).

A recent study showed that the thermotolerant *S. cerevisiae* strains can mate with *S. uvarum* strains to produce sterile interspecific hybrids (Rainieri *et al.*, 1998a). In synthetic must the thermotolerant *S. cerevisiae* X *S. uvarum* hybrid maintained the most useful trait of the *S. cerevisiae* parental strain; the considerable capacity for reducing malic acid (Rainieri *et al.*, 1998a).

The present study was carried out to characterise an interspecific hybrid between a thermotolerant *S. cerevisiae* strain and a *S. uvarum* strain and determine its oenological potential.

MATERIALS AND METHODS

Yeast strains: The *Saccharomyces* hybrid 35G2 X 12233 1A was obtained and described previously (Rainieri *et al.*, 1998a). This hybrid was compared with its parental strains: the thermotolerant *S. cerevisiae* strain 35G2 (Rainieri *et al.*, 1998b) and the cold-fermenting *S. uvarum* strain 12233 1A that is a single spore culture of strain 12233. This strain was isolated and described by Castellari *et al.* (1992) and oenologically characterised by Castellari *et al.* (1994), and was initially classified as *S. cerevisiae* physiological race *uvarum* (according to Yarrow, 1984). According to a later classification the same strain was referred to as *S. bayanus* (Vaughan-Martini & Martini, 1998). More recent studies indicate this strain would be more appropriately referred to as *S. uvarum* (Nguyen & Gaillardin, 1998; Rainieri *et al.*, 1999; Nguyen, *et al.*, 1999). The hybrid strain 35G2 X 12233 1A was also compared with *S. cerevisiae* strain 6167, that is known to be a vigorous strain that produces well-balanced wines. All the strains used in this study were obtained from the Dipartimento di Protezione e Valorizzazione Agroalimentare, University of Bologna, Reggio Emilia, Italy (DIPROVAL) culture collection.

Fermentation: The fermentation experiments were performed at the Centro Assistenza Tecnologica in Enologia e Viticultura, Tebano, Faenza, Italy (C.A.T.E.V.) experimental winery using the 1997 vintage. Fermentations were performed using two different methods for white and red vinification.

White vinification: Fermentations were carried out in triplicate on must from *Vitis vinifera* cv. Chardonnay, using the procedure of Eustace & Thornton (1987). Erlenmeyer flasks (125 mL) containing 70 mL sterilised grape juice were inoculated with 10 mL of a 24 h culture incubated in yeast peptone dextrose (YPD) broth (yeast extract 0,5%, w/v; peptone 0,5%, w/v; glucose 2%, w/v) at 30°C. The preculture obtained was then incubated at 30°C. Pasteurised and sulphured (70 mg SO_2/L) grape juice (800 mL), contained in 1L Erlenmeyer flasks, was inoculated with 80 mL of the preculture (10^5 cells/mL) and incubated at 12, 20, 28 and 36°C in fermentation traps without shaking.

Red vinification: Fermentations were carried out in triplicate on must from *Vitis vinifera* cv. Sangiovese. Crushed berries (3 kg) were placed into 5L wide-necked jars. The berries were then pasteurised, sulphured (70 mg SO_2/kg) and inoculated with a 300 mL preculture. The preculture was obtained as described for white vinification. Samples were then incubated at 28°C and agitated twice a day to facilitate colour extraction. After 5 d the fermenting juice was separated from the skins using a laboratory press. Approximately 2,5L of juice was recovered from each sample. The juice was then collected in sterilised 3L Erlenmeyer flasks that were incubated at 28°C. Fermentation continued until all fermentable sugars were exhausted.

In both white and red vinification the fermentation progress was monitored by determining the weight loss caused by CO_2 release. Sugar exhaustion was determined using the Clinitest kit (Miles Ltd., Slough, UK). Once the fermentation was completed, the wines obtained were cold-stabilised at 4°C, filtered and analysed.

Fermentation product analysis: Acetic acid, glycerol, malic acid and succinic acid were assayed using enzymatic kits (Boehringer Mannheim, Mannheim, Germany) as advised by the manufacturer. Differences in the concentrations of these compounds between hybrid and parental strains were determined by carrying out a one way analysis of variance (Sheffe's test) using the Statistical Analysis System Software System for Windows version 6.11 (SAS Institute Inc., Cary, NC, USA).

The sugar and ethanol concenstrations were determined using standard methods for wine analysis (Ough & Amerine, 1988).

Sensory evaluation: Sensory evaluation was performed on the red wine obtained from the Sangiovese must. Wines obtained using the hybrid and the parental yeast strains were compared to assess whether they could be differentiated on the basis of their overall sensorial characteristics. A panel of 16 people who were trained in sensorial analysis (staff members of C.A.T.E.V.) was emplyed to carry out a triangular test. Each member of the panel was given a set of three wines to taste, two of which were the same. These wines were produced using either the hybrid or a parental strain. Each member of the panel was asked to establish which of the three wines was the different one. A probability table formulated by Rossler *et al.* (1978) was used to estimate whether the wines obtained using the hybrid and the parental strains were significantly different. This table indicates the number of correct judgements required to consider the wines significantly different at various probability levels for a triangular test.

The same panel of judges then carried out a sensory profile of the wines obtained using the hybrid and the parental strains. The following seven attributes were chosen: ruby redness, aroma intensity, aroma delicacy, Sangiovese aroma typicity, acidity, body, astringency and overall quality. Judges were asked to rate the intensity of each attribute for each wine using a seven point scale, where one was just detectable and seven was of high intensity. Ratings were then analysed by analysis of variance (ANOVA).

RESULTS AND DISCUSSION

Fermentation performance: The hybrid, the parental strains and the *S. cerevisiae* wine strain 6167 were used for producing

white wine at different temperatures. The rate of fermentation is indicated in Fig. 1. At both 12 (Fig. 1a) and 20°C (Fig. 1b) the thermotolerant parental strain fermented slowly and left high amounts of residual sugars (Table 1). By contrast, the *S. cerevisiae* wine strain 6167 and the *S. uvarum* parental strain fermented vigorously and left a minimum amount of residual sugars (Table 1). At 28°C the thermotolerant parental strains and strain 6167 fermented vigorously almost all the sugars available. The *S. uvarum* strain did not ferment as vigorously as the other strains (Fig. 1c) and left a high amount of residual sugars (Table 1). At 36°C the thermotolerant *S. cerevisiae* strain was, as expected, more vigorous than the other strains tested (Fig. 1d). The hybrid strain was always as vigorous as the most rapidly fermenting parental strain at 12, 20, and 28°C (Figs. 1a to c). At 36°C, however, the hybrid was slightly less vigorous than the thermotolerant parental strain (Fig. 1d). As reported for other interspecific hybrid cultures (Zambonelli *et al.*, 1993; Kishimoto, 1994; Zambonelli *et al.*, 1997), hybrid 35G2 X 12233 1A was stable, ethanol-tolerant, and exhibited a fermentation vigour similar to that shown by *S. cerevisiae* wine strains. While both parental strains were affected by the conditions under which the fermentation was carried out, the hybrid strain displayed rapid and complete fermentations following white vinification processes over a wide temperature range that included temperatures of oenological interest.

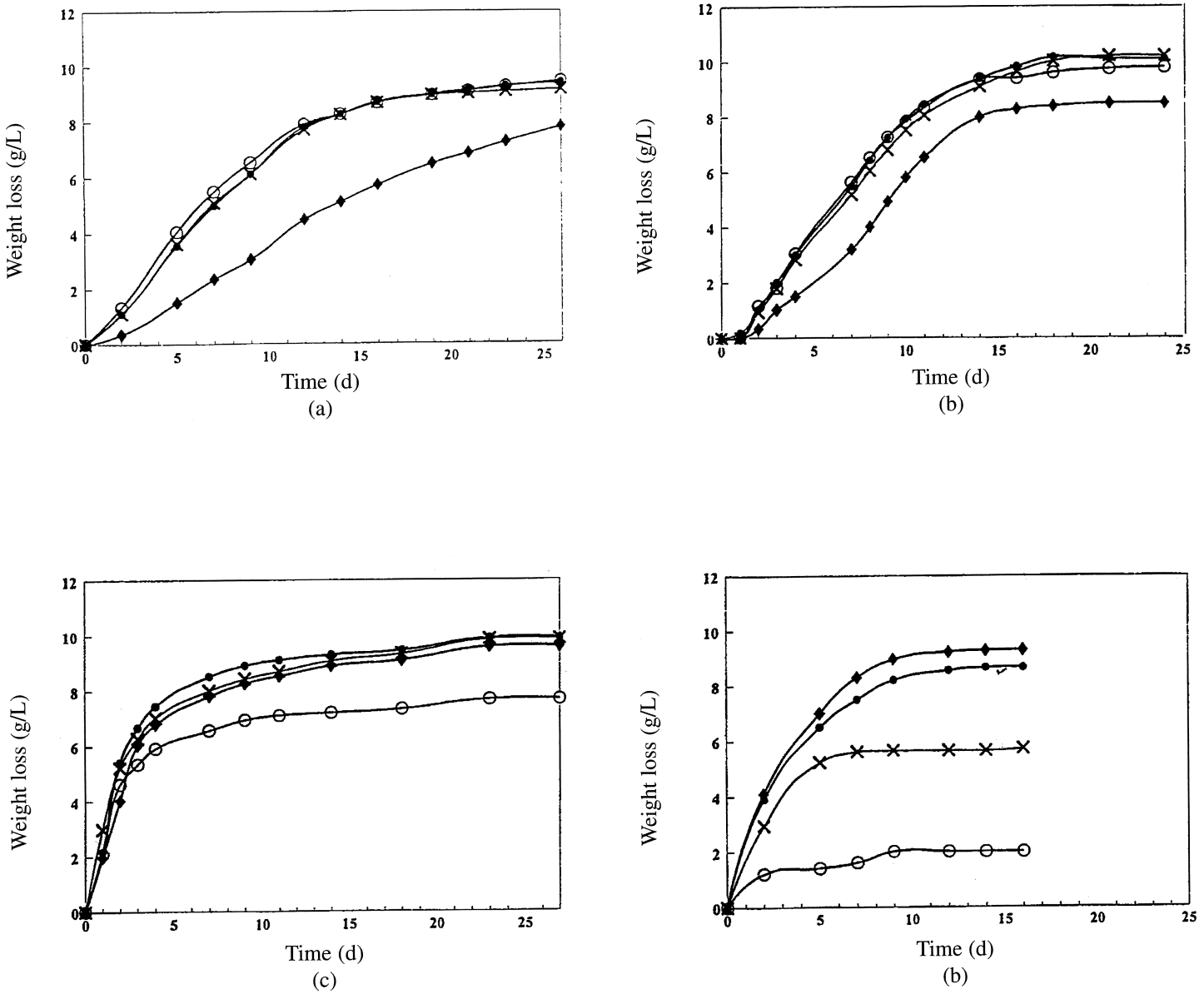

FIGURE 1

Rate of fermentation for thermotolerant *S. cerevisiae* parental strain 35G2 (◆); *S. uvarum* parental strain 12233 1A (O); hybrid (●) and *S. cerevisiae* wine strain 6167 (X) in Chardonnay wine. Fermentation tests were carried out at 12°C (a); 20°C (b); 28°C (c) and 36°C (d). Each curve was obtained using the mean values of three independent replications.

TABLE 1
Composition of white wine (Chardonnay) produced by the hybrid and parental yeast strains at 20 and 28°C. The results are mean values of three independent replications*.

Strain	pH	Total acidity (g/L)**	Ethanol (vol%)	Sugar (g/L)	Acetic acid (g/L)	Malic acid (g/L)	Malic acid variation	Succinic acid (g/L)	Glycerol (g/L)
Fermentation at 20°C									
S. cerevisiae 35G2	3,36	6,52	8,51	22,20	0,38a	3,40a	-38,2%	0,75a	7,74a
Hybrid	3,31	6,45	10,01	1,18	0,08b	3,20a	-41,9%	1,10b	7,70a
S. uvarum 12233 1A	3,15	9,10	9,80	1,40	0,11b	5,50b	0	1,20b	7,55a
S. cerevisiae wine strain 6167	3,22	7,86	10,20	1,61	0,21ab	4,73ab	-14,0%	0,72a	5,71b
Fermentation at 28°C									
S. cerevisiae 35G2	3,25	7,05	9,58	10,35	0,41a	3,24a	-41,1%	0,97a	7,55a
Hybrid	3,24	7,65	9,90	2,30	0,10b	3,48a	-37,0%	1,09a	8,96b
S. uvarum 12233 1A	3,10	9,35	7,68	39,92	0,11b	5,50b	0	0,81ab	5,62ab
S. cerevisiae wine strain 6167	3,20	8,08	10,25	1,56	0,15b	4,30ab	-21,0%	0,92a	4,57c
Must	3,03	7,20		171,60		5,50			

*Within each column, means followed by the same letter are not significantly different (Sheffe's test, $p< 0,05$).
**In g/L as tartaric acid.

The hybrid and the parental strains and the *S. cerevisiae* wine strain 6167 were also used for producing red wine. Fermentation tests were carried out at 28°C. At this temperature all the strains completed the fermentation process, leaving a minimal amount of residual sugar (Table 2). The hybrid displayed a fermentation rate that was very close to that of the *S. cerevisiae* strain 6167 (Fig. 2).

Wine composition: Both the red and white wines obtained from the hybrid culture exhibited a total acidity which was as low as that of the thermotolerant *S. cerevisiae* parental strain (Tables 1 and 2). This seems to be mainly due to malic acid degradation. In fact, in both cases, the initial concentration of malic acid was reduced by approximately 40% in white wines and 50% in red wines. The amount of acetic acid produced by the hybrid strain was as low as that of the *S. uvarum* parental strain thus eliminating one of the undesirable traits of the thermotolerant *S. cerevisiae* parental strain (see Tables 1 and 2).

A comparison between wines obtained by the hybrid and by *S. cerevisiae* wine strain 6167 shows that the former had a higher glycerol yield compared to the latter (Tables 1 and 2). The ethanol yield in the wine produced by the hybrid strain was lower compared to strain 6167. Decreases in the ethanol yields of strains producing high amounts of glycerol have been reported in several studies (see references in Scanes, Hohmann & Prior, 1998).

The deacidification capacity of the hybrid strain through intense malo-alcoholic fermentation and the ability to produce large amounts of glycerol are useful oenological traits. *S. cerevisiae* strains producing large amounts of glycerol were described by Radler & Schütz (1982). Strains resulting in good malo-alcoholic fermentations have been isolated and described by a few authors (Peynaud, 1966; Rankine, 1966; Fuck & Radler, 1972;

TABLE 2
Composition of red wine (Sangiovese) produced by the hybrid and parental yeast strains. The results are mean values of three independent replications*.

Strain	pH	Total Acidity (g/L)**	Ethanol (vol%)	Sugar (g/L)	Acetic acid (g/L)	Malic acid (g/L)	Malic acid variation	Succinic acid (g/L)	Glycerol (g/L)
S. cerevisiae 35G2	3,46	6,80	12,28	3,35	0,77a	1,00a	-56%	1,20a	10,05a
Hybrid	3,42	6,61	13,05	1,23	0,13b	1,15a	-50%	1,39a	10,00a
S. uvarum 12233	3,30	10,35	12,12	4,35	0,11b	3,99c	+75%	1,83b	10,32a
S. cerevisiae wine strain 6167	3,37	7,63	13,31	1,13	0,21c	2,09b	-8%	1,32a	7,23b
Must	3,33	6,67		223,50		2,27			

*Within each column, means followed by the same letter are not significantly different (Sheffe's test, p<0,05).
**In g/L as tartaric acid.

Tortia, Gerbi & Gandini, 1993). However, the combination of both traits is not commonly found in natural isolates.

Sensory evaluation: When the wine obtained using the hybrid was compared with the wine obtained using the thermotolerant *S. cerevisiae* parental strain using the triangular test, nine members of the panel out of 16 could assess the difference and identified the respective wines (p=0,05). When the wine obtained using the hybrid was compared with the wine obtained using the cold-fermenting *S. uvarum* strain 12 testers out of 16 could differentiate between wines (p=0,001). These significance values (p=0,05 and p=0,001) indicate that the wine obtained using the hybrid and the wines obtained using the parental strains can be differentiated, especially in the case of the *S. uvarum* parental strain.

The aroma profiles of the wines obtained using the hybrid and the parental strains are shown in Fig. 3. Colour and aroma intensity were not significantly different in the wines produced by the hybrid and parental strains (p<0,05). Aroma delicacy, body and the characteristic Sangiovese aroma were found to be different in

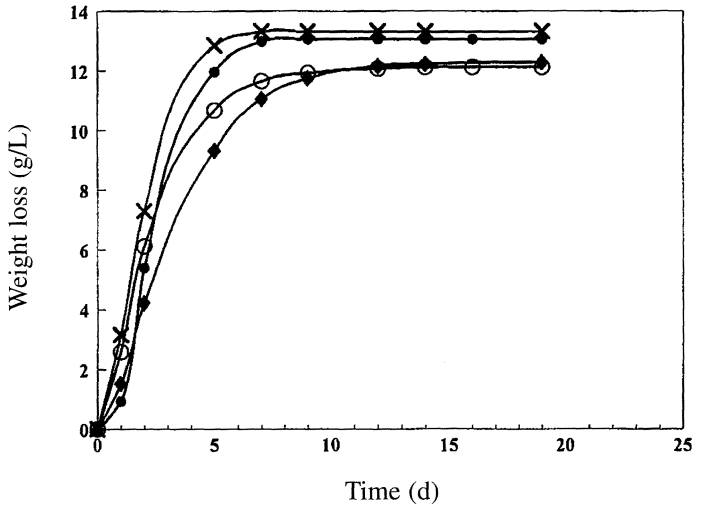

FIGURE 2
Rate of fermentation for thermotolerant *S. cerevisiae* parental strain 35G2 (◆); *S. uvarum* parental strain 12233 1A (O); hybrid (●) and *S. cerevisiae* wine strain 6167 (X) in Sangiovese wine. Fermentation tests were carried out at 28°C. Each curve was obtained using the mean values of three independent replications.

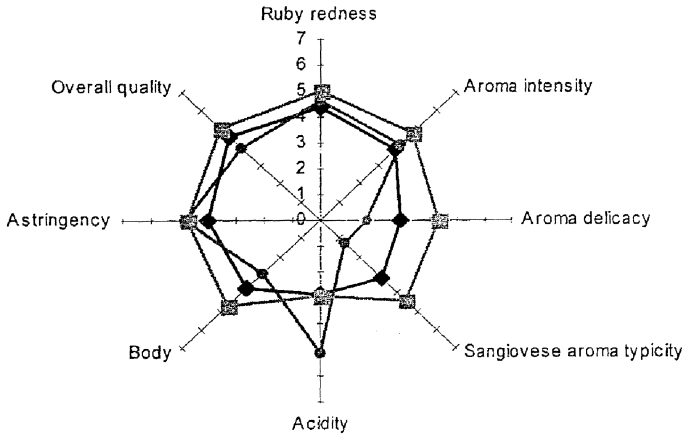

FIGURE 3
Sensory profile of wines obtained from the hybrid and parental yeast strains in Sangiovese wine. Thermotolerant *S. cerevisiae* parental strain 35G2 (◆); *S. uvarum* parental strain 12233 1A (●) and hybrid (■).

the three cases ($p<0,05$). The acidity of the wine produced using the hybrid resulted different from the acidity of the wine obtained using the *S. uvarum* strain ($p<0,05$).

CONCLUSIONS

Thermotolerant *S. cerevisiae* and cold-fermenting *S. uvarum* strains possess oenological useful characteristics, nevertheless they display some traits that make them unsuitable for winemaking. The improvement of yeasts can be achieved by mating strains with different characteristics to obtain a combination of traits that may be difficult to find in natural isolates (Romano *et al.*, 1985; Eustace & Thornton, 1987; Zambonelli *et al.*, 1993; Zambonelli *et al.*, 1997).

The mating of the thermotolerant *S. cerevisiae* strain 35G2 and of the cold-fermenting *S. uvarum* strain 12233 1A resulted in a hybrid that possesses a combination of all the desirable oenological characteristics coming from both parental strains. Furthermore, all the traits that made the parental strains unsuitable for winemaking were apparently absent in the hybrid.

This hybrid is particularly suited for the production of red wines designed for ageing due to its high glycerol yield and the ability to degrade malate. In these wines a high level of glycerol is regarded as a very positive trait. Glycerol improves the sensory qualities and contributes to the smoothness of wine (Eustace & Thornton, 1987; Pretorius & Van der Westhuizen, 1991; Scanes, Hohmann & Prior, 1998). Partial wine deacidification is also advantageous because the resulting higher pH encourages malolactic fermentation that enhances the quality of red wine (Henick-Kling, 1993).

The hybrid can also be successfully employed in white wine production whenever the must shows an excessive amount of malic acid. Musts with high acidity are typical of temperate climates. Biological deacidification through the use of specific yeast strains with desirable traits, is preferable to the use of chemical additives.

LITERATURE CITED

ANDERSON, P.J., McNEIL, K.E. & WATSON, K., 1988. Isolation and identification of thermotolerant yeasts from Australian sugar cane mills. *J. Gen. Microbiol.* **134**, 1691-1698.

BARRE, P., VEZINHET, F., DEQUIN, S. & GLONDIN, B., 1993. Genetic improvement of wine yeasts. In: FLEET, G.H. (ed). Wine microbiology and biotechnology. Harwood Academic Publ., Switzerland. pp. 265-278.

CARIDI, A., RAINIERI, S., PASSARELLI, P. & ZAMBONELLI, C., 1997. Effects of hybrids between cryo and non-cryotolerant *Saccharomyces* strains on the composition of wines from southern Italy. *Vignevini* **24**, 27-30.

CASTELLARI, L., FERRUZZI, M., MAGRINI, A., GIUDICI, P., PASSARELLI, P. & ZAMBONELLI, C., 1994. Unbalanced wine fermentation by cryotolerant *vs* non-cryotolerant *Saccharomyces* strains. Vitis **33**, 49-52.

CASTELLARI, L., PACCHIOLI, G., ZAMBONELLI, C., TINI, V. & GRAZIA, L., 1992. Isolation and initial characterisation of cryotolerant *Saccharomyces* strains. *Ital. J. Food Sci.* **3**, 179-186.

EUSTACE, R. & THORNTON, R.J., 1987. Selective hybridization of wine yeasts for higher yields of glycerol. *Can. J. Microbiol.* **33**, 112-117.

FUCK, E. & RADLER, F., 1972. Äpfelsäurestoffwechsel bei *Saccharomyces*. 1 Der anaerobe Äpfelsäureabbau bei *Saccharomyces cerevisiae*. *Arch. Mikrobiol.* **87**, 149-164.

HACKING, A.J., TAYLOR, I.W.F. & HANAS, C.M., 1984. Selection of yeasts able to produce ethanol from glucose at 40°C. *Appl. Microbiol. Biotechnol.* **19**, 361-363.

HENICK-KLING, T., 1993. Malolactic fermentation. In: FLEET, G.H. (ed). Wine microbiology and biotechnology. Harwood Academic Publ., Switzerland. pp. 289-326.

KISHIMOTO, M., 1994. Fermentation characteristics of hybrids between the cryophilic wine yeast *Saccahromyces bayanus* and the mesophilic wine yeast *Saccharomyces cerevisiae*. *J. Ferment. Bioeng.* **77,** 432-435.

LALUCE, C., PALMIERI, M.C. & LOPES DA CRUZ, R.C., 1991. Growth and fermentation characteristics of new selected strains of *Saccharomyces* at high temperatures and high cell densities. *Biotechnol. Bioeng.* **37**, 157-159.

NAUMOV, G.I., NAUMOVA, E. & KORHOLA, M., 1992. Genetic identification of natural *Saccharomyces sensu stricto* yeasts from Finland, Holland and Slovakia. *Antonie van Leeuwenhoek* **61**, 237-243.

NGUYEN, H-V. & GAILLARDIN, C., 1997. Two subgroups within the *Saccahromyces bayanus* species evidenced by PCR amplification and restriction polymorphism of the non-transcribed spacer 2 in the ribosomal DNA unit. *System. Appl. Microbiol.* **20,** 286-294.

NGUYEN, H-V., LEPINGLE, A. & GAILLARDIN, C., 1999. Molecular typing demonstrate homogeneity of *Saccharomyces uvarum* strains and reveals the existence of hybrids between *S. uvarum* and *Saccharomyces cerevisiae*, including the *Saccharomyces bayanus* type strain CBS 380. *System. Appl. Microbiol.* (Accepted for publication).

OUGH, C.S. & AMERINE, M.A., 1988. Methods for analysis of musts and wines. JOHN WILEY AND SONS (eds). New York, USA.

PEYNAUD, E., 1966. L'acide malique dans les môuts et les vins de Bordeaux. *Ann. Falsif. Frandes.* **31**, 332-347.

PRETORIUS, I.S. & VAN DER WESTHUIZEN, T.J. 1991. The impact of yeast genetics and recombinant DNA technology on the wine industry – A review. *S. Afr. J. Enol. Vitic.* **12**, 3-31.

RADLER, F. & SHCÜTZ, H., 1982. Glycerol production of various strains of *Saccharomyces*. *Am. J. Enol. Vitic.* **22**, 6-12.

RAINIERI, S., ZAMBONELLI, C., GIUDICI, P. & CASTELLARI, L., 1998a. Characterisation of thermotolerant *Saccharomyces cerevisiae* hybrids. *Biotechnol. Lett.* **20**, 543-547.

RAINIERI, S., ZAMBONELLI, C., HALLSWORTH, J.E., PULVIRENTI, A. & GIUDICI, P., 1999. *Saccharomyces uvarum*, a distinct group within *Saccharomyces sensu stricto*. *FEMS Microbiol. Lett.* **177**, 177-185.

RAINIERI, S., ZAMBONELLI, C., PASSARELLI, P., CASTELLARI, L. & FRANZONI, S., 1996. Prima caratterizzazione di ceppi termotolleranti di *Saccharomyces cerevisiae*. *Ind. Bevande* **25**, 565-570.

RAINIERI, S., ZAMBONELLI, C., TINI, V., CASTELLARI, L. & GIUDICI, P., 1998b. The enological traits of *Saccharomyces strains*. *Am. J. Enol. Vitic.* **49**, 319-324.

RANKINE, B.C., 1966. Decomposition of L-malic acid by wine yeasts. *J. Sci. Food Agric.* **17,** 312-316.

ROESSLER, E.B., PANGBORN, R.M., SIDEL, J.L. & STONE, H., 1978. Expanded statistical tables for estimating significance in Paired-Preference, Paired-Difference, Duo-Trio and Triangle tests. *J. Food Sci.* **43**, 940-944.

ROMANO, P., SOLI, M.G., GRAZIA, L., SUZZI, G. & ZAMBONELLI, C., 1985. Improvement of a wine yeast strain by a breeding program. *Appl. Environ. Microbiol.* **50**, 1064-1067.

SCANES, K.T., HOHMANN, S. & PRIOR, B.A., 1998. Glycerol production by the yeast *Saccharomyces cerevisiae* and its relevance to wine: a review. *S. Afr. J. Enol. Vitic.* **19**, 17-24.

THORNTON, R.J., 1982. Selective hybridisation of pure culture wine yeasts. Improvement of fermentation efficiency and inheritance of SO_2 tolerance. *Eur. J. Appl. Microbiol.* **14**, 159-164.

THORNTON, R.J., 1985. The introduction of flocculation into a homotallic wine yeast. A practical example of the modification of winemaking properties by the use of genetic techniques. *Amer. J. Enol. Vitic.* **36,** 47-49.

TORTIA, C., GERBI, V. & GANDINI, A., 1993. Impiego di *Saccharomyces cerevisiae* malo-alcolici in vinificazione. *Vignevini* **20**, 15-20.

VAUGHAN-MARTINI, A. & MARTINI, A., 1998. *Saccharomyces* Meyen ex Ress. In: KURTZMAN, C.P. & FELL, J.W. (eds). The yeasts, a taxonomic study. 4th Ed. Elsevier, Amsterdam. pp. 358-371.

YARROW, D., 1984. Genus *Saccharomyces* Meyen Ex Rees. In: KREGER-VAN RIJ, N.J.W. (ed). The yeasts. A taxonomic study. 3rd Ed. Elsevier, Amsterdam. pp. 379-396.

ZAMBONELLI, C., PASSARELLI, P., RAINIERI, S., BERTOLINI, L., GIUDICI, P. & CASTELLARI, L., 1997. Technological properties and temperature response of interspecific *Saccharomyces* hybrids. *J. Sci. Food Agric.* **74,** 7-12.

ZAMBONELLI, C., PASSARELLI, P., RAINIERI, S. & GIUDICI, P., 1993. Taxonomic and technological implications of sterility in hybrids from cryotolerant and non cryotolerant *Saccharomyces* strains. *Ann. Microbiol. Enzimol.* **43**, 217-223.