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SACCHAROMYCES HYBRIDS AS A TOOL FOR IMPROVING THE QUALITY OF MOSCATO DI SIRACUSA DOC WINE

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ABSTRACT

The study aimed to evaluate four *Saccharomyces cerevisiae* strains, one intraspecific *S. cerevisiae* hybrid, and five interspecific *S. cerevisiae*×*S. uvarum* hybrids with respect to the quality of Moscato di Siracusa DOC wine by comparing them with a commercial *Saccharomyces cerevisiae* strain.

Most of the interspecific hybrids maintained volatile acidity (VA) at very low levels, produced high concentrations of glycerol, malic and succinic acid, and yielded the highest concentration of positive sensory attributes.

On the basis of the results of these experimental fermentation trials, a real opportunity to produce special wines employing *S. cerevisiae*×*S. uvarum* hybrids is proposed.

- Key words: acidic components, intraspecific and interspecific *Saccharomyces* hybrids, Moscato di Siracusa DOC wine, sensory analysis -

INTRODUCTION

The fermentation of high-sugar grape musts, such as in the production of iced or dried grape wines, can give rise to stuck or sluggish fermentations due to the high osmotic pressure and toxicity of ethanol to yeast cells (BISSON, 1999; BISSON and BUTZKE, 2000). When the fermentation of these musts stops prematurely, wines of low quality and stability are produced due to high volatile acidity (VA) from the growth of acetic acid bacteria, heterofermentative lactic acid bacteria, non-*Saccharomyces* yeasts (CARIDI *et al.*, 1999; FLEET and HEARD, 1993), and/or *Saccharomyces cerevisiae* wine yeasts used as starter cultures (MURATORE *et al.*, 2007). The latter seems to be a major contributor of VA in high °Brix musts because previous studies demonstrated that sugar stress regulates the expression of structural genes involved in the synthesis of acetic acid from acetaldehyde (SHIMAZU and WATANABE, 1981; ATTFIELD, 1997; BAUER and PRETORIUS, 2000; ERASMUS *et al.*, 2003), and the production of VA is inversely correlated with the maximum cell concentration and the assimilable nitrogen concentration (BELY *et al.*, 2003). Concerning this problem, MURATORE *et al.* (2007) demonstrated the successful use of a *S. uvarum* strain as a starter culture in the production of Malvasia delle Lipari wine; '*uvarum*-type' strains found in the fermenting yeast biota of sweet wines, which are usually osmotolerant and psychrotolerant, are frequently able to overgrow *Saccharomyces cerevisiae* by the end of the fermentation (SIPICZKI *et al.*, 2001; NAUMOV *et al.*, 2002). As an alternative approach, a recent study of BELY *et al.* (2008) suggested the use of a mixed culture of *Torulaspora delbrueckii* and *S. cerevisiae* as the best combination for improving the analytical profile of sweet wine, particularly volatile acidity and acetaldehyde production.

However, the degree of acetic acid formation is yeast-strain dependent (REMIZE *et al.*, 1999) because different yeast strains react to the same osmotic pressure by producing different concentrations of acetic acid and glycerol. For this reason, the selection and genetic improvement of the starter yeast should consider other more specific properties in addition to the basic oenological traits for the type of wine desired.

At present, wine yeast selection is based mainly on screening wild yeast populations. However, the likelihood of identifying a strain expressing all of the optimal properties for winemaking is very low. An alternative approach to obtaining a strain with numerous oenological properties without using recombinant DNA technology is breeding. Crossing *Saccharomyces* species is considered to be a useful tool for obtaining improved wine yeast strains combining fermentative features of both parents (ROMANO *et al.*, 1985; ZAMBONELLI *et al.*, 1997; RAINIERI *et al.*, 1998; MARULLO *et al.*, 2004; 2006; GIUDICI *et al.*, 2005). Interspecific hybrids between cryotolerant *S. uvarum* and non-cryotolerant *S. cerevisiae* strains have been successfully employed in oenology because they possess higher fermentation competitiveness than the parental strains as well as characteristics of the parents in new and interesting combinations (CASTELLARI *et al.*, 1994; ZAMBONELLI *et al.*, 1997). However, a noteworthy study by SOLIERI *et al.* (2008) demonstrated that the type of mtDNA is an important trait for constructing new improved hybrids for winemaking. Hybrids with *S. uvarum* mtDNA have a higher tendency to ferment and a lower tendency to respire than those with *S. cerevisiae* mtDNA, suggesting that mtDNA type and fermentative:respiratory performance are correlated in *S. cerevisiae*×*S. uvarum* hybrids.

The aim of this study was to compare four *S. cerevisiae* yeast strains, one intraspecific *S. cerevisiae* hybrid, and five interspecific *S. cerevisiae*×*S. uvarum* hybrids with respect to the acidic component, glycerol formation, ethanol tolerance, and sensory characteristics in a very high sugar content must for the production of Moscato di Siracusa from sun-dried grapes, which is one of the most ancient wines produced in Italy. In such a high osmotic must, the practice of inoculating a *Saccharomyces* strain to carry out the vinification requires the previous selection of a suitable wine yeast strain, both for maintaining volatile acidity below the legal limit fixed by COUNCIL REGULATION (EC) No. 479/2008 and for improving overall quality (2008).

MATERIALS AND METHODS

Yeast strains and fermentation trials

Four strains of *S. cerevisiae*, one *S. cerevisiae*×*S. cerevisiae* hybrid, and five *S. cerevisiae*×*S. uvarum* hybrids bearing *S. cerevisiae* mtDNA (SOLIERI *et al.*, 2008) were used. The list of the strains and origins is reported in Table 1. The strains were cultured for 48 h at 25°C on fresh YPD medium (w/v: yeast extract 1%, peptone 2%, glucose 2%, agar 2%) before their use in fermentation experiments.

The strains' ability to perform alcoholic fermentation of special wines was tested in Muscat grape must. Moscato di Siracusa wine is produced from dried grapes in Sicily in the Siracusa area, and its production is regulated by the controlled origin wine appellation system, known in Italy as DOC (Denominazione di Origine Controllata), which was instituted in 1973 (DPR, OFFICIAL GAZETTE OF ITALIAN REPUBLIC, 1973).

Lightly dried Muscat Blanc grapes produced on sandy soil and siliceous clay in the Siracusa area and surroundings (Sicily, Italy), undamaged and without *Botrytis cinerea*, were used.

Table 1 - *Saccharomyces* strains used in this study.

Strain	Species	Origin	Characteristics
LS3	*S. cerevisiae*×*S. uvarum*	Dipartimento di Scienze Agrarie e degli Alimenti (University of Modena and Reggio Emilia, Italy)	*S. cerevisiae* mtDNA
LS6 (4003-1A×7877-10A)	*S. cerevisiae*×*S. uvarum*	"	*S. cerevisiae* mtDNA
LS7 (4003-1B×7877-10B)	*S. cerevisiae*×*S. uvarum*	"	*S. cerevisiae* mtDNA
LS8 (6167-3A×7877-9B)	*S. cerevisiae*×*S. uvarum*	"	*S. cerevisiae* mtDNA
LS9 (6167-8C×7877-6C)	*S. cerevisiae*×*S. uvarum*	"	*S. cerevisiae* mtDNA
FRENCH	*S. cerevisiae*	"	Parental A3B
16003	*S. cerevisiae*	"	Parental A3B
AL41	*S. cerevisiae*	DOFATA (University of Catania, Italy)	β-glucosidase positive strain (RESTUCCIA *et al.*, 2002)
522	*S. cerevisiae*	Davis (California)	Commercial reference strain
A3B	*S. cerevisiae*×*S. cerevisiae*	Dipartimento di Scienze Agrarie e degli Alimenti (University of Modena and Reggio Emilia, Italy)	High fermentation power

The grape berries were crushed and clarified by pressurised filtration through a sack-filter (Spagni s.n.c., Reggio Emilia, Italy); then, 1.5 L was poured into 2-L glass fermentors and inoculated in triplicate with a 48-h yeast pre-culture (5% of volume) that had been prepared in the same sterilised must. Pre-cultures were inoculated independently with the different yeasts to reach an initial population of 7 log CFU mL^{-1}; yeast population densities were estimated by direct count using a haemocytometer.

Fermentation was carried out in a conditioned room where the temperature was maintained at 18°±1°C. The fermentation was monitored by daily measuring of the weight loss associated with the liberation of carbon dioxide. Fermentation was considered complete when the weight loss was negligible.

At the end of fermentation, the wine was filtered, poured into 0.375-L glass bottles, corked, and stored at 4°C.

Analytical determinations

Ethanol content, total acidity, volatile acidity, reducing sugars, and pH were determined for the musts and wines according to the official methods of the Office International de Vigne et du Vin (OIV, 1990).

Wine colour was determined using a spectrophotometer set at 420 nm.

L-malic acid, succinic acid, and glycerol were quantified using enzymatic assay kits (K-LMALR, K-SUCC and K-GCROL, Megazyme International Ireland Ltd, Bray, Co. Wicklow, Ireland).

All analyses were performed in triplicate. The data shown are the average of all repetitions with standard deviations.

Principal component analyses (PCA) and analysis of variance (ANOVA) were performed by STATGRAPHICS® Plus version 4.0 (Manugistics, Scottsdale, AZ, USA) on chemical and physical data to ascertain significant differences among mean values.

Sensory analysis

To define the attributes of the products and to investigate the differences among the samples, the sensory profile method (ISO 13299, 2003) was used. The wines were evaluated by a panel of ten (six female and four male) trained judges experienced in wine sensory analysis and ranging in age between 20 and 30 years selected from among the Food Science and Technology Department staff members of the University of Catania.

A preliminary session was performed using several commercial Moscato di Siracusa wines to develop a common vocabulary, and this allowed the assessors to use the same terms for describing their perceptions. Descriptors with at least a 70% frequency of citation were chosen. The nineteen descriptors used included: two for appearance (yellow intensity and golden reflection), seven for aroma (fruity, exotic fruit, raisins, wood, honey, sourdough, alcohol), two for taste (sour and sweet), one for mouth feel (sharp), one for rheological properties (viscosity), and six for flavour (fruity, exotic fruit, raisins, wood, honey, sourdough). All evaluations were conducted in individual testing booths at the sensory laboratory (ISO 8589, 1988) at 20°C, asking the judges to quantify the intensity of each attribute by assigning a score between 1 (absence of perception) and 9 (extremely intense). A data collection program was used (FIZZ Software® solutions for sensory Analysis and consumer Tests, Ver.2, Biosystemes, Couternon, France). Samples were evaluated in five sessions (two by two) using 20

mL of wine in approved wine glasses (ISO 3591, 1977) labelled using a 3-digit code and covered with a plastic lid to minimise the loss of volatile compounds.

Data were statistically processed using STATGRAPHICS® Plus version 4.0 (Manugistics, Scottsdale, AZ, USA). Each attribute was analysed by one way analysis of variance (ANOVA) to verify significant differences among the samples. The significance was evaluated by means of the F test; the mean values were subjected to the multiple comparison test using the LSD procedure (least significant difference), which allows the attributes differentiating the samples to be determined. Principal component analysis was applied to the sensory data.

RESULTS AND DISCUSSION

Different yeast strains were screened for their vinification properties in must from partially dried Muscat Blanc grapes to select the most appropriate strain for this kind of product. Fermentations of high-sugar grape musts are often sluggish, taking months to reach the desired ethanol level, and they usually have high levels of volatile acidity.

The sugar content of freshly squeezed Muscat must was 319 g L^{-1}, comparable with those reported by NICOLOSI ASMUNDO *et al.* (1990) for similar grape musts (280-340 g L^{-1}). Total acidity was 7.0 g L^{-1} of tartaric acid; malic acid content was estimated at 0.5 g L^{-1}, and the pH was 3.34.

Fermentation with the different yeast strains resulted in widely variable residual sugar content. The sample inoculated with strain LS9 presented the highest sugar content (98.3 g L^{-1}), while the strains LS7, 522, and AL41 showed a strong ability to reduce the initial amount, and therefore to perform alcoholic fermentation, in musts with high sugar content (Table 2).

Except for the LS9 strain, which probably was influenced by both ethanol and sugar stressors (TROLLMO *et al.*, 1988; PIPER, 1995), ethanol content (Table 2) was about 15% vol. for all the hybrid strains, as required by the disciplinary regulations of production; among them, LS7 yielded the highest value (16.2% vol.).

In spite of the very low levels of reducing sugars at the end of the alcoholic fermentation, strains AL41 and 522 exhibited lower ethanol levels (14.3 and 14.6% vol., respectively) than the interspecific hybrids, which could be explained by the diversion of sugar metabolism into by-products other than ethanol.

Except for LS9 and LS8, which produced the highest total acidity values, the total acidity of the wine samples at the end of fermentation was largely unaffected by the identity of the yeast strain (Table 2). The ability to increase the acidic component is considered particularly favourable, especially for wines produced in warm climates such as southern Italy, because advanced ripening leads to products with very low acidity that lack the sensory characters of freshness and vivacity. In addition, acidity contributes resistance to oxidative and microbial spoilage.

VA content was below 1 g L^{-1} for most of the wine samples, except for LS9 and 16003, which produced the highest VA levels (2.06 and 1.14 g L^{-1} respectively). In particular, strains LS3, LS6, and LS7 showed a marked ability to maintain volatile acidity at very low levels (Table 2). VA has a negative effect on the quality of wines and is considered one of the principal problems for the marketability of wines produced from dried grapes. The results of the present study are particularly interesting if compared with those found by MALACRINÒ *et al.* (2005) in wines from partially dried Corvina grapes fermented with selected *S. cerevisiae* (0.81-1.24 g L^{-1}), and with those reported by MURATORE *et al.* (2007) in Malvasia delle Lipari wines fermented with *S. uwarum* strains during two consecutive vintages (0.64-0.85 g L^{-1}).

The malic acid content of the grape must was very low due to the over-ripening of the grapes. The production of malic acid during the fermentation process was variable among the *S. cerevisiae* strains, with a minimum content of 0.89 g L^{-1} and a maximum of 1.46 g L^{-1}. On the other hand, the interspecific hybrids produced higher amounts of malic acid, with LS9 producing the highest level of 2.00 g L^{-1} (Table 2).

Succinic acid is not usually present in grape must, and its origin is related to the yeast strain employed. *S. cerevisiae* strains produced the lowest concentrations, with a minimum of 0.23 g L^{-1}. The interspecific hybrids, except for LS9, showed a good ability to synthesise succinic acid as reported in the literature (ZAMBONELLI *et al.*, 1997; RAINIERI *et al.*, 1998), with a peak content of 1.20 g L^{-1} for LS8 (Table 2). This was probably due to stimulation of succinic acid production by sugar stress, as the transcription of all genes involved in the production of succinic acid is enhanced under these conditions (ERASMUS *et al.*, 2003).

Enzymatic assessment of glycerol levels showed wide variability among the strains. The lowest values were synthesised by the *S. cerevisiae* strains and the intraspecific hybrid A3B. The highest concentrations of glycerol were produced by the interspecific hybrids, as demonstrated by EUSTACE and THORTON (1987), with a maximum value of 11.63 g L^{-1} for LS8 (Table 2). This is a considerable amount if compared with what is usually formed by *S. cerevisiae* in wine, which is in the range of 4-9 g L^{-1} (RIBEREAU-GAYON *et al.*, 1972; GRAZIA *et al.*, 1995). This finding confirms a previous study that demonstrated a direct correlation between succinic acid and glycerol production (GIUDICI *et al.*, 1995). Glycerol is a non-volatile compound with no aromatic properties, but it significantly contributes to wine quality by providing sweetness and fullness

Table 2 - Chemical and physical parameters of the Moscato di Siracusa DOC wine samples.

	16003	522	A3B	AL41	FRENCH	LS3	LS6	LS7	LS8	LS9
Sugar (g L^{-1})	46.00^{c}±5.00	2.00^{e}±0.00	18.58^{d}±4.00	2.90^{e}±0.25	43.40^{c}±3.00	19.65^{d}±2.00	48.40^{c}±6.00	3.20^{e}±0.50	60.40^{b}±8.00	98.30^{a}±4.00
Ethanol (% vol.)	14.80^{ab}±0.00	14.55^{bc}±0.95	14.01^{c}±0.81	14.30^{c}±0.20	14.12^{c}±0.92	15.20^{ab}±0.50	15.85^{ab}±0.25	16.20^{a}±0.00	14.58^{bc}±0.99	12.50^{d}±0.30
Total acidity	8.50^{bc}±0.00	6.45±0.05^{e}	7.23±0.53^{de}	6.70±0.00^{e}	8.08±0.40^{c}	6.85±0.75^{e}	7.80±0.20^{cd}	7.90±0.00^{cd}	9.25±0.45^{b}	10.90±0.00^{a}
Volatile acidity (g L^{-1})	1.14^{b}±0.02	0.51^{cd}±0.13	0.60^{cd}±0.18	0.65^{cd}±0.01	0.81^{bc}±0.12	0.38^{ef}±0.15	0.44^{de}±0.06	0.25^{f}±0.02	0.88^{bc}±0.29	2.06^{a}±0.26
Malic acid (g L^{-1})	1.05^{ef}±0.04	0.89±0.01^{fg}	0.72±0.05^{g}	1.29±0.06^{cd}	1.46±0.13^{c}	1.21^{de}±0.16	1.39^{cd}±0.09	1.72^{b}±0.06	1.74^{b}±0.14	2.00^{a}±0.12
Succinic acid (g L^{-1})	0.23^{d}±0.08	0.50^{c}±0.09	0.51^{c}±0.02	0.49^{c}±0.03	0.46^{c}±0.09	0.54^{c}±0.09	0.74^{b}±0.06	1.15^{a}±0.10	1.20^{a}±0.09	0.45^{c}±0.11
Glycerol (g L^{-1})	6.71^{de}±0.70	6.61^{de}±0.61	6.37^{de}±0.93	5.71^{e}±0.65	7.90^{bc}±1.00	7.53^{cd}±0.19	9.60^{ab}±0.35	9.24^{ab}±1.05	10.39^{a}±1.05	9.47^{ab}±0.71
Colour intensity (Abs)	0.37^{c}±0.04	0.53^{b}±0.03	0.23^{d}±0.04	0.21^{d}±0.05	0.26^{d}±0.03	0.23^{d}±0.06	0.37^{c}±0.06	0.19^{d}±0.01	0.67^{a}±0.04	0.47^{b}±0.05

Means in rows followed by the same letter are not significantly different (p≤0.05).

Table 3 - Analysis of variance of sensory attributes (F values). Mean scores of the nineteen sensory attributes for the ten samples.

		LSD Samples									
Attribute	**F value**	**16003**	**522**	**A3B**	**AL41**	**FRENCH**	**LS3**	**LS6**	**LS7**	**LS8**	**LS9**
Yellow colour	10.19***	7.0b	4.9^{a}	6.9b	4.9^{a}	6.8b	4.1^{a}	6.8b	4.8^{a}	7.6b	7.7b
Golden reflection	2.47*	4.2^{abcd}	3.1^{a}	5.3^{cde}	4.2^{abcd}	4.3^{abcde}	3.3^{ab}	5.2^{bcde}	3.7^{abc}	6.1^{de}	6.2^{e}
Fruity aroma	1.11 n.s	5.1	4.7	5.4	5.0	5.2	6.0	4.3	3.4	5.1	4.7
Exotic fruit aroma	1.79 n.s.	4.5	4.1	4.6	4.8	4.8	5.8	4.6	2.7	5.5	4.4
Raisin aroma	3.36**	5.2^{b}	4.8^{b}	6.0^{bc}	4.4^{ab}	5.2^{b}	4.3^{ab}	5.3^{b}	2.8^{a}	5.7^{bc}	7.4^{c}
Wood aroma	1.25 n.s.	3.7	3.2	3.5	3.1	4.5	3.7	5.0	4.7	5.1	5.0
Honey aroma	2.20*	4.7^{bc}	3.7^{ab}	4.4^{bc}	3.9^{bc}	4.2^{bc}	3.4^{ab}	4.2^{bc}	2.2^{a}	4.4^{bc}	5.3^{c}
Sourdough aroma	2.18*	2.9^{a}	3.2^{a}	2.9^{a}	3.0^{a}	2.6^{a}	3.8^{ab}	3.9^{ab}	5.2^{b}	3.4^{a}	2.7^{a}
Alcohol aroma	0.59 n.s.	4.8	5.0	4.9	4.4	4.7	5.0	6.0	5.2	5.3	4.7
Sourness	3.11**	4.8^{abc}	6.0^{cd}	4.4^{abc}	5.4^{bcd}	4.1^{ab}	6.0^{cd}	4.5^{abc}	6.6^{d}	4.5^{abc}	3.3^{a}
Sweetness	13.03***	4.5^{b}	2.1^{a}	4.9^{bc}	2.3^{a}	5.5^{bc}	2.7^{a}	5.3^{bc}	2.4^{a}	5.9^{cd}	7.0^{d}
Sharpness	1.70 n.s.	4.6	4.9	3.8	3.8	5.1	5.3	4.0	5.4	3.7	2.7
Viscosity	3.38**	4.0^{abcd}	3.4^{ab}	3.9^{abc}	2.7^{a}	4.1^{abcd}	3.3^{a}	4.8^{bcd}	2.9^{a}	5.2^{cd}	5.4^{d}
Fruity flavour	2.22*	4.0^{bcd}	3.5^{ab}	3.7^{abc}	4.0^{bcd}	5.2^{d}	4.0^{bcd}	4.3^{bcd}	2.5^{a}	5.1^{cd}	4.6^{bcd}
Exotic fruit flavour	1.23 n.s.	4.1	3.4	3.2	4.0	4.5	3.6	4.3	2.9	4.9	3.6
Raisin flavour	4.32***	5.3^{cde}	4.3^{abcd}	4.4^{bcd}	3.4^{ab}	5.4^{cde}	3.8^{abc}	5.9^{de}	2.6^{a}	6.0^{de}	6.8^{e}
Wood flavour	1.10 n.s.	3.5	3.5	4.4	2.6	4.1	3.7	4.8	4.4	3.9	5.1
Honey flavour	5.09***	4.0^{cde}	2.9^{abc}	3.8^{cde}	3.5^{bcd}	5.0^{ef}	2.4^{ab}	4.4^{def}	2.0^{a}	4.7^{def}	5.4^{f}
Sourdough flavour	0.69 n.s.	4.1	3.1	2.9	3.2	2.9	3.6	3.2	4.4	3.2	3.0

Values for each parameter followed by different lower-case letters indicate differences according to the Student-Newman-Keuls test (p≤0.05).
*p ≤ 0.05; **p ≤ 0.01; ***p ≤ 0.001; n.s. not significant.

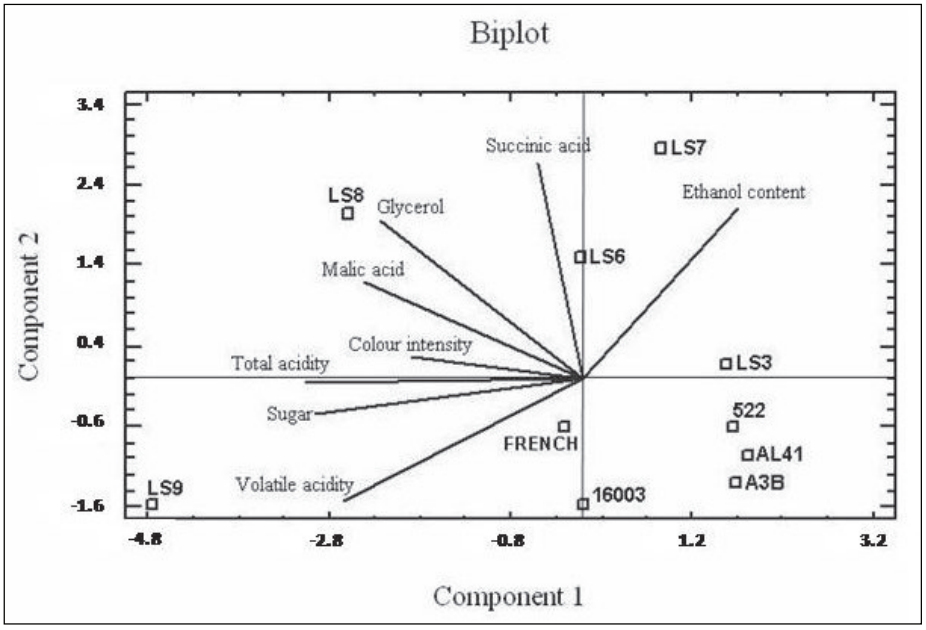

Fig. 1 - Principal component analysis of mean chemical data from the Moscato di Siracusa DOC wine samples.

(MALACRINÒ *et al.*, 2005; RIBEREAU-GAYON *et al.*, 1972). Wine yeasts generally adapt to increased osmotic stress by enhanced production of intracellular glycerol, which is the main compatible solute that counter-balances the osmotic pressure (NEVOIGT and STAHL, 1997). Due to the favourable impact of glycerol on wine quality, the benefits of increasing glycerol production to improve the sensory characteristics of wines lacking in body have been emphasised (PRETORIUS and VAN DER WESTHUIZEN, 1991; BARRE *et al.*, 1993; DEGRE, 1993; BISSON, 1996).

The absorbance values at 420 nm, indicating the influence of the yeast strains on the yellowness of the wines, are reported in Table 2. The ability of the yeast strains to delay browning has recently been investigated by several authors such as LOPEZ-TOLEDANO *et al.* (2006), who ascribed this characteristic to an inhibitory effect on the formation of coloured compounds. Spectrophotometric analysis revealed a different yellow intensity for each sample. The results of the experimental trials indicated no correlation between browning prevention and the type of yeast strain, as strains LS7, AL41, and A3B, which all showed a good ability to maintain the pale yellow colour of Moscato di Siracusa wine, are (respectively) an interspecific hybrid, a *Saccharomyces cerevisiae*, and an intraspecific hybrid.

The relationships among the chemical parameters are shown in Fig. 1, where the first two principal components explained 82.76% of the variance. Sugar, total acidity, and volatile acidity had the highest negative loading on PC1 (explained variance 53.48%), and the sample LS9 had the highest values for these parameters. Ethanol content, succinic acid, and glycerol had the highest positive loading on PC2 (explained variance 29.28%); in particular, the sample produced by the strain LS7 (unlike the other samples) was characterised by a high level of ethanol and low sugar content. Except for the interspecific hybrid LS3, all the *S. cerevisiae* strains and the intraspecific hybrid were grouped as a separate cluster, which was characterised by lower levels of acid and glycerol.

Among the 19 sensory attributes considered, only 11 (Table 3) significantly contributed to the character of the wines: yellow intensity ($p \leq 0.001$), golden reflection ($p \leq 0.05$), raisin aroma ($p \leq 0.01$), honey aroma, sourdough aroma ($p \leq 0.05$), sour aroma ($p \leq 0.01$), sweetness ($p \leq 0.001$), viscosity ($p \leq 0.01$), fruity flavour, ($p \leq 0.05$), raisin flavour, and honey flavour ($p \leq 0.001$).

The relationships among the eleven significant attributes were explicated by PCA analysis; the first two principal components explained 90.43% of the variance. As can be observed from the PCA plot (Fig. 2), the wines were distinct in terms of sensory attributes. Moving from left to right along the first component (explained variance 80.72%), the samples produced with LS3, 522, and AL41 are distinct from the others. The second component (explained variance 9.71%) distinguishes the LS7 sample (in the upper left corner) from the other wines. Raisin aroma, sweetness, raisin flavour, and honey flavour (positive loading), as well as acid (negative loading) are on PC1; sourdough aroma and golden reflection had the highest positive loading on PC2, while the attribute viscosity was equally positively loaded on PC1 and PC2.

The sample fermented with LS7 was characterised by a more intense sourdough aroma, while LS6, LS8, and LS9 provided wines with intense yellow colour and golden reflection, high

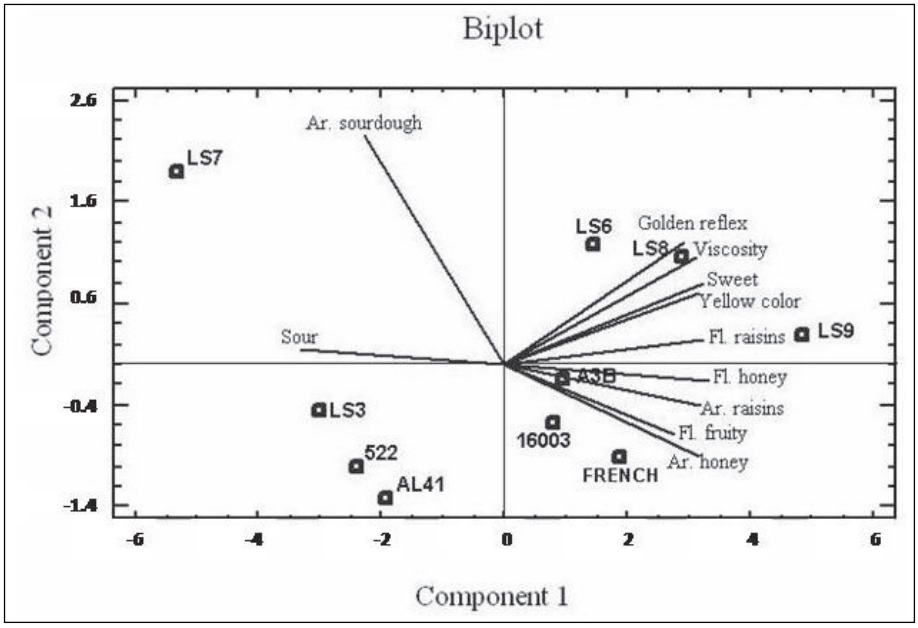

Fig. 2 - Principal component analysis of mean significant sensory attributes of the Moscato di Siracusa DOC wine samples.

viscosity and sweetness, raisin and honey flavours, and a negative correlation with undesirable attributes.

The sensory profiles of six of the wine samples selected on the basis of the previous PCA (those fermented with 522, 16003, A3B, FRENCH, LS6, and LS8) were defined by the mean values of the eleven significant attributes. Except for the sour attribute, the wine fermented with the commercial 522 strain had the least intense sensory attributes in comparison with the other five samples, while strains LS8 and LS6 showed a richness of aroma and flavour compounds in addition to intense yellow colour, golden reflection, and high viscosity. An intermediate sensory profile was found for the A3B sample.

CONCLUSIONS

To make wines of high quality, starter cultures are usually used to induce effective and rapid fermentation. Thus, strains of *S. cerevisiae* characterised with respect to their principal technological traits are normally used. However, in some cases, such as in the production of special wines from dried grapes, strains that match the specific characteristics of the particular wine should be selected. The results of the present study demonstrate that hybrids between cryo- and non-cryotolerant *Saccharomyces* strains may be technologically promising for the production of Moscato di Siracusa wine.

Their ability to perform alcoholic fermentation in grape must with high sugar content was comparable (LS8) or higher (LS3, LS6, LS7) than that of a *Saccharomyces cerevisiae* reference strain. In addition, they were able to increase total acidity, malic acid, succinic acid, and glycerol, and they strongly reduced VA.

The sensory analysis performed on the wine samples by experienced judges revealed (especially for the interspecific hybrids LS6 and LS8) a greater richness of aroma and fruity, raisin, and honey flavour compounds relative to the commercial yeast strain, in addition to intense yellow colour, golden reflection, and high viscosity; most of these attributes are reported in the production disciplinary of Moscato di Siracusa DOC as characterising this precious wine.

The PCA plot of chemical and sensory parameters revealed that the *Saccharomyces* strains employed in this study strongly differ in their chemical and sensory properties.

Among the interspecific hybrids, LS9 produced the highest VA content, while LS7 and LS3 yielded a sourdough aroma and a sour taste, respectively, that were considered to be negative traits. Thus, LS6 and LS8 provided the best fit and might be of use for the production of this quality wine.

This study proved and clearly confirmed the contribution that newly selected yeast strains can make to improve the quality of wine produced according to the most ancient traditions.

REFERENCES

Attfield P.V. 1997. Stress tolerance: the key to effective strains of industrial baker's yeast, Nat. Biotechnol. 15: 1351.

Barre P., Vezinhet F., Dequin S. and Blondin B. 1993. Genetic improvement of wine yeasts. Ch. 9. In: "Wine Microbiology and Biotechnology". G.H. Fleet (Ed.), p. 265. Harwood Academic, Chur, Switzerland.

Bauer F.F. and Pretorius I.S. 2000. Yeast stress response

and fermentation efficiency: how to survive the making of wine – a review. S. Afr. J. Enol. Vitic. 21: 27.

Bely M., Rinaldi A. and Dubourdieu D. 2003. Influence of assimilable nitrogen on volatile acidity production by *Saccharomyces cerevisiae* during high sugar fermentation. J. Biosci. Bioeng. 96: 507.

Bely M., Stoeckle P., Masneuf-Pomarède I. and Dubourdieu D. 2008. Impact of mixed *Torulaspora delbrueckii-Saccharomyces cerevisiae* culture on high-sugar fermentation. Int. J. Food Microbiol. 122: 312.

Bisson L. 1999. Stuck and sluggish fermentations, Am. J. Enol. Viticult. 50: 107.

Bisson L. and Butzke C.E. 2000. Diagnosis and rectification of stuck and sluggish fermentations. Am. J. Enol. Viticult. 51: 168.

Bisson L.F. 1996. Genetic engineering of yeast for wine production. Agro Food Industry Hi-Tech: 26.

Bonilla F., Mayen M., Merida J. and Medina M. 2001. Yeast used as fining treatment to correct browning in white wines. J. Agric. Food Chem. 49: 1928.

Caridi A., Crucitti P. and Ramondino D. 1999. Winemaking of must at high osmotic strength by thermotolerant yeast. Biotechnol. Lett. 21: 617.

Castellari L., Ferruzzi A., Magrini A., Giudici P., Passarelli P. and Zambonelli C. 1994. Unbalanced wine fermentation of cryotolerant *vs* non-cryotolerant *Saccharomyces* strains. Vitis 33: 49.

Council Regulation (EC) No 479/2008 of 29 April 2008 on the common organisation of the market in wine, amending Regulations (EC) No 1493/1999, (EC) No 1782/2003, (EC) No 1290/2005, (EC) No 3/2008 and repealing Regulations (EEC) No 2392/86 and (EC) No 1493/1999. Official Journal of the European Communities, L148/1 (2008).

Degre R. 1993. Selection and commercial cultivation of wine yeast and bacteria. Ch. 15. In: "Wine Microbiology and Biotechnology". G.H. Fleet (Ed.), p. 421. Harwood Academic, Chur, Switzerland.

DPR, Decree of the President of the Republic of 26 June 1973. Published on "Official gazette of Italian Republic of 06 December 1973, No 315.

Erasmus D.J., Van der Merwe G.K. and Van Vuuren H.J.J. 2003. Genome-wide expression analyses: metabolic adaptation of *Saccharomyces cerevisiae* to high sugar stress. FEMS Yeast Res. 2: 375.

Eustace R. and Thornton R.J. 1987. Selective hybridization of wine yeasts for higher yields of glycerol. Can. J. Microbiol. 33: 112.

Fleet G.H. and Heard G.M. 1993. Yeasts-growth during fermentation. Ch. 2. In: "Wine Microbiology and Biotechnology". G.H. Fleet (Ed.), p. 42. Harwood Academic, Chur, Switzerland.

Giudici P., Solieri L., Pulvirenti A.M. and Cassanelli S. 2005. Strategies and perspectives for genetic improvement of wine yeasts. Appl. Microbiol. Biot. 66: 622.

Giudici P., Zambonelli C., Passarelli P. and Castellari L. 1995. Improvement of wine composition with cryotolerant *Saccharomyces* strains. Am. J. Enol. Viticult. 46: 143.

Grazia L., Iorizzo M., Venditti M. and Sorrentino A. 1995. The yeasts during the ripening of the grapes. Industrie delle Bevande 24: 589.

ISO 13299 (2003). Sensory analysis - Methodology - General guidance for establishing a sensory profile. International Organization for Standardization, Geneva.

ISO 3591 (1977). Sensory analysis – Apparatus – Wine – Tasting glass. International Organization for Standardization, Geneva.

ISO 8589 (1988). Sensory analysis – general guidance for the design of test rooms. International Organization for Standardization, Geneva.

Lopez-Toledano A., Mayen M., Merida J. and Medina M. 2006. Yeasts used to delay browning in white wines. Food Chem. 97: 498.

Malacrinò P., Tosi E., Caramia G., Prisco R. and Zapparoli G. 2005. The vinification of partially dried grapes: a comparative fermentation study of *Saccharomyces cerevisiae* strains under sugar stress. Lett. Appl. Microbiol. 40: 466.

Marullo P., Bely M., Masneuf-Pomarede I., Aigle M. and Dubourdieu D. 2004. Inheritable nature of enological quantitative traits is demonstrated by meiotic segregation of industrial wine yeast strains. FEMS Yeast Res. 4: 711.

Marullo P., Bely M., Masneuf-Pomarede I., Pons M., Aigle M. and Dubourdieu D. 2006. Breeding strategies for combining fermentative qualities and reducing off-flavor production in a wine yeast model. FEMS Yeast Res. 6: 268.

Muratore G., Nicolosi Asmundo C., Lanza C.M., Caggia C., Licciardello F. and Restuccia C. 2007. Influence of *Saccharomyces uvarum* on volatile acidity, aromatic and sensory profile of Malvasia delle Lipari wine. Food Technol. Biotech. 1: 101.

Naumov G.I., Naumova E.S., Antunovics Z. and Sipiczki M. 2002. *Saccharomyces bayanus* var. *uvarum* in Tokaj wine-making of Slovakia and Hungary. Appl. Microbiol. Biot. 59: 727.

Nevoigt E. and Stahl U. 1997. Osmoregulation and glycerol metabolism in the yeast *Saccharomyces cerevisiae*. FEMS Microbiol. Rev. 21: 231.

Nicolosi Asmundo C., Cataldi Lupo M.C., Campisi S. and Russo C. 1990. La Malvasia delle Lipari: influenza delle tecnologie di produzione sui componenti volatili dell'aroma. Vignevini 4: 33.

Office International de la Vigne et du Vin (OIV). Récueil des méthodes internationales d'analyse des vins et des moûts (Collection of the international methods of analysis of wines and musts), Paris (1990).

Piper P.W. 1995. The heat shock and ethanol stress responses of yeast exhibit extensive similarity and functional overlap. FEMS Microbiol. Lett. 134: 121.

Pretorius I.S. and 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.

Rainieri S., Zambonelli C., Tini V., Castellari L. and Giudici P. 1998. The enological traits of thermotolerant *Saccharomyces* strains. Am. J. Enol. Viticult. 49: 319.

Remize F., Roustan J.L., Sablayrolles J.M., Barre P. and Dequin S. 1999. Glycerol overproduction by engineered *Saccharomyces cerevisiae* wine yeast strains leads to substantial changes in byproduct formation and to a stimulation of fermentation rate in stationary phase. Appl. Environ. Microb. 65: 143.

Restuccia C., Pulvirenti A., Caggia C. and Giudici P. 2002. A β-glucosidase positive strain of *Saccharomyces cerevisiae* isolated from grape must. Ann. Microbiol. 52: 47.

Ribereau-Gayon J., Peynaud E., Sudraud P. and Ribereau-Gayon P. 1972. In: "Traité D'oenologie. Sciences et Techniques du Vin", p. 353. Dunod, Paris, France.

Romano P., Soli G., Suzzi G., Grazia L. and Zambonelli C. 1985. Improvement of a wine *Saccharomyces cerevisiae* strain by a breeding program. Appl. Environ. Microb. 50: 1064.

Shimazu Y. and Watanabe M. 1981. Effects of yeast strains and environmental conditions on formation of organic acids in must during fermentation. J. Ferment. Technol. 59: 27.

Sipiczki M., Romano P., Lipani G., Miklos I. and Antunovics Z. 2001. Analysis of yeasts derived from natural fermentation in a Tokaj winery. Anton. Leeuw. Int. J. G. 79: 97.

Solieri L., Antúnez O., Pérez-Ortín J.E., Barrio E. and Giudici P. 2008. Mitochondrial inheritance and fermentative: oxidative balance in hybrids between *Saccharomycescerevisiae* and *Saccharomyces uvarum*. Yeast 25: 485.

Trollmo C., André L., Blomberg A. and Adler L. 1988. Physiological overlap between osmotolerance and thermotolerance in *Saccharomyces cerevisiae*. FEMS Microbiol. Lett. 56: 321.

Zambonelli C., Passarelli P., Rainieri S. and Bertolini L. 1997. Technological properties and temperature response of interspecific *Saccharomyces* hybrids. J. Sci. Food Agr. 74: 7.

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