Influence of *Saccharomyces uvarum* on Volatile Acidity, Aromatic and Sensory Profile of Malvasia delle Lipari Wine

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Summary

The present study investigated chemical and sensory properties of Malvasia delle Lipari DOC (Denomination of Controlled Origin) wine fermented with a cryotolerant strain of *Saccharomyces uvarum,* characterized by low levels of acetic acid production. In particular, experimental wine was tested for volatile acidity and for aromatic profile by gas chromatography and the results were compared with the same wine produced with a commercial strain of *Saccharomyces cerevisiae.* Sensory analysis was carried out to assess the identification of experimental wine as Malvasia delle Lipari by defining its sensory profile. Fermentation with *S. uvarum* gave a final product with lower volatile acidity, lower alcohol content and higher total acidity. Moreover, differences in the aroma profile could be ascribed to different characteristics of the yeasts. Concerning sensorial analysis, the panel assigned higher scores in positive attributes to the wine fermented with *S. uvarum.*

Key words: Malvasia delle Lipari DOC wine, *Saccharomyces uvarum*, volatile acidity, wine aroma

Introduction

Malvasia delle Lipari DOC (*1*) wine, produced in the Aeolian Islands, comes in three types, one for consumption with meals, one to accompany desserts and one as a liqueur wine, with minimum developed alcohol levels of 11.5, 18 and 20 degrees, respectively, and is one of the most ancient and aromatic wines of Sicily. This white wine is made with techniques that have changed little over the centuries: the grapes are gathered when they are fully ripe and then put out in the sun for 10 to 15 days on large mats made of bamboo canes, to increase their sugar content (up to 32 %) and to obtain a much more aromatic wine (*2*). They are then crushed with a beam press and the must is fermented in casks of capacities not exceeding 10 hectolitres. The final product is a gold coloured wine, with honey and apricot smell and with an aromatic, harmonic, lightly sweet taste (*3*). Limited studies have been carried out to improve the process of production and the characteristics of this wine, with particular regards to the use of alternative yeasts. Many studies have established that the yeast species is a prominent factor in determining the wine composition (*4–6*).

Saccharomyces cerevisiae is the species mainly responsible for the alcoholic fermentation, but non-*Saccharomyces* yeasts can usually be present at different levels, both in spontaneous and inoculated wine fermentations, contributing to wine taste and aroma with their peculiar characteristics (*4,7–11*). The prolonged exposure of grapes to air results in a high colonization of the peel by yeasts such as species of *Candida, Hanseniaspora* and *Metschnikowia*; these microorganisms, especially during the first step of the fermentative process, may produce large

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amounts of volatile acidity with possible negative effects on wine quality (*5*). On the other hand, some cryotolerant *Saccharomyces*, belonging to physiological races *uvarum* and *bayanus*, have previously been studied and they were characterized for their ability to carry out alcoholic fermentation at low temperature with low production of acetic acid, high levels of glycerol and succinic acid, when compared with non-cryotolerant *Saccharomyces* (*12–14*). Due to their oenological characteristics, Castellari *et al.* (*12*) suggested the use of such strains for production of high quality wines.

The aim of this work was to evaluate the possibility to carry out alcoholic fermentation with a strain of *Saccharomyces uvarum*, characterized by low acetic acid and low acetaldehyde production (*14,15*), in order to keep these undesirable secondary metabolites in the wine within acceptable amounts, even though they are present in high amounts in the must. The final characteristics of the wine were assessed, in comparison with a wine produced with non-cryotolerant *Saccharomyces* strain, by chemical and sensory analyses.

Materials and Methods

Microrganisms and fermentation conditions

Two yeast strains belonging to *S. cerevisiae* and *S. uvarum* species were used. The strain 12233 of *S. uvarum*, belonging to the DIPROVAL collection (University of Bologna), was selected on the basis of previous studies (*13,16*); the commercial *S. cerevisiae* strain was produced by Bio Springer (Maisons-Alfort, France), product code: Levures oenologiques 823, distributed by Chimica Franke S.a.s. (Torino, Italy) with the commercial name Zymoferm – *Saccharomyces cerevisiae*.

The Malvasia grapes were harvested in two consecutive years (referred as vintage #1 and #2) on the island of Salina (Aeolian Islands, Italy). The undamaged grape berries were partially dried on bamboo canes and no *Botrytis cinerea* was present. The grape berries were crushed into 40 L of must and poured into two stainless steel tanks with a capacity of 50 L each. The must was clarified by filtration with a sack filter using a pump (Spagni S.n.c, Reggio Emilia, Italy) and sterilized with cardboard filters up to 0.45 μm diameter. The clarified must for each vintage was poured into four 10-litre stainless-steel fermentors, two of which were inoculated with a 48--hour *S. cerevisiae* preculture (5 % of volume), the remaining two with a 48-hour *S. uvarum* preculture (5 % of volume). The room temperature where fermentation took place was maintained at (18±1) °C. At the end of fermentation, the wine was poured into 0.5-litre glass bottles (typical for Malvasia wine), corked and stored at cellar temperature ((18±1) °C) until chemical and sensory analyses. The wine produced with *S. cerevisiae* was marked M1, the one produced with *S. uvarum* M2.

The physicochemical analyses were carried out for wine samples of the two vintages, while gas chromatographic analyses and sensory tests were carried out for the wines of vintage #2.

Chemical analyses

Ethanol content, total acidity, volatile acidity, reducing sugars and pH were determined for musts and wines according to the official methods of the Office International de la Vigne et du Vin (*17*).

To analyse the aromatic components, 200 mL of wine, mixed with internal standards (*n*-amyl acetate; 1-heptanol; β-citronellol) were put in a liquid/liquid extractor together with a solvent mixture pentane/dichloromethane (2:1). Aromatic compounds were extracted by evaporation at 45 °C and then condensed at –15 °C.

The extraction was carried out for about 20 h, then the aromatic extract was concentrated in a Rotavapor at room temperature, and any solvent traces were removed with a weak nitrogen flow.

The volume of the extract was adjusted to 5 mL with pentane/dichloromethane (2:1) and 1 μL of each was injected in the gas chromatograph and in the GC-MS to identify the components.

A Shimadzu GC-17A gas chromatograph with a flame ionisation detector was used including a CP-WAX 52 CD capillary column measuring 50 m × 0.25 mm × 0.25 μm. Injector temperature: 220 °C, detector temperature: 250 °C, temperature program: 70 °C for 3 min, 4 °C/min, 200 °C for 10 min; carrier gas: He; carrier speed: 24 cm/s; make up: He 75 kPa; split ratio 1:50.

Peak identifications were made by comparing retention times and electron impact (EI) mass spectra with published data or with authentic compounds, using a Shimadzu GCMS-QP5050A system. Injector temperature: 60–220 °C; temperature program: 45 °C for 3 min, 4 °C/min up to 220 °C; 200 °C for 20 min; carrier gas: He; carrier speed: 24 cm/s.

All analytical determinations were performed in duplicate for each tank; statistical processing of chemical analysis was carried out using Statgraphics Plus software, version 5, from Manugistic Incorporated (Rockville, Maryland, USA).

Sensory analyses

Wines were assessed by 36 trained judges (*18*) recruited among students of Food Science and Technology Department at the University of Catania with previous experience in wine sensory analysis. At first, a discriminant analysis (triangle test) was performed to determine possible significant differences among the samples (*S. cerevisiae* and *S. uvarum* fermented wines) (*19*).

In order to define the sensory profile (*20*) thirty judges were trained in five sessions using the existing terminology and reference standard over four commercial Malvasia wines. A list of descriptors was selected on the basis of occurrence (%) of the terms used. The final set comprised twenty six descriptors, grouped as aroma (citrus fruit, apple, apricot, dry apricot, raisin, broom flower, orange blossom, vanilla, clove, cinnamon, almond, hazelnut, caramel, honey, sourdough, alcohol, vinegar), taste (acid and sweet), mouthfeel (spicy and alcoholic), and flavour (apple, apricot, dry apricot, raisin and aged) terms. The different descriptors were quantified using an intensity scale ranging from 1 (minimum) to 9 (maximum) (*21*). Each judge evaluated the wines in

triplicate and in random order. A volume of 20 mL of wine was evaluated in ISO approved wine glasses labelled with a 3-digit code and covered to prevent volatile loss. All evaluations were conducted at 20 °C from 10.00 to 12.00 pm in individual booths (22) illuminated with white light. The data were analyzed with StatView statistical software for Windows (ver. 5.0.1; SAS® Institute Incorporated, Cary, NC, USA). ANOVA was carried out to verify significantly different attributes among the samples studied. Spider diagrams were used to graphically represent the data.

Results and Discussion

Chemical analyses

Table 1 presents the results of chemical analyses of musts and wines produced in vintages #1 and #2: almost all analytical data resulted significantly different in the two wines for each batch (p<0.01). Total acidity, ethanol content and pH were all typical of fermentations using Malvasia grape musts, pointing out the efficiency of fermentation in all batches. Total acidity in wines was always higher than in musts, and fermentation with cryotolerant *Saccharomyces* led to higher amounts of acids with respect to the non-cryotolerant one. This is in agreement with the results of previous studies (13,14), which underlined that different strains of *S. uvarum* produce high amounts of succinic and malic acid, balanced by a lower alcohol yield.

High values of volatile acidity were found in wines fermented by the commercial strain of *S. cerevisiae*, especially in vintage #1. These results are in agreement with those found by Nicolosi Asmundo *et al.* (3) in Malvasia wine produced with spontaneous fermentation, and underline that high volatile acidity is a peculiar characteristic of traditionally produced Malvasia wine. Volatile acidity increased as a result of fermentation using both yeasts, but the use of strain 12233 of *S. uvarum* allowed to obtain a product with lower volatile acidity (37 and 33 % lower in vintage #1 and #2, respectively) if compared with the wine fermented with the commercial strain of *S. cerevisiae*. This result confirmed that cryotolerant strains of *Saccharomyces* are low acetic acid producers (12).

Table 2 shows the average of aromatic compounds, divided into esters, terpenes, acids, alcohols and other components, and the relative standard deviation of wines produced in vintage #2 with the two different yeast species.

In the ester class, monoethyl succinate and diethyl succinate were significantly higher in M2 wine, as a probable consequence of the higher production of succinic acid by the cryotolerant strain, thus strengthening the results of previous studies (13,14). Similarly, we could explain higher recovery of ethyl lactate in M2 wine, hypothesizing a higher production of lactic acid, although this feature of cryotolerant *Saccharomyces* has not been studied yet. Higher amounts of ethyl acetate were recovered in M2 wine, although the concentrations recovered were much lower than the odour threshold displayed by other authors (23). On the basis of a previous study (3), some compounds such as isoamyl acetate, diethyl succinate, ethyl lactate, ethyl butyrate and 3(OH)--ethyl butyrate are not present in the freshly-squeezed Malvasia must, therefore their presence and possible differences in concentration are entirely due to fermentation, underlining differences between the yeasts. *n*-ethyl acetate, responsible for fruity aroma, and 2-phenyl ethyl acetate, responsible for floral aroma, were increased in M2 wine. On the other hand, ethyl hexanoate and ethyl octanoate, referable to fruit and/or mature fruit, and ethyl pyruvate, increased in M1 wine.

Among alcohols, in the wine fermented with *S. uvarum* increased amounts were recovered for isoamyl and 2-phenyl ethyl alcohol, responsible for vegetable and floral aroma, respectively. 2-phenyl ethanol has been recognized as a major aroma component in muscadine wines, and its presence is to be ascribed mainly to biosynthesis during fermentation. Even though its presence has been determined in muscadine grape skin, the contribution of this fraction to the overall aromatic content is unlikely to be significant, especially in white wines produced without skin contact (24). Higher recovery of 2-phenyl ethanol in M2 wine, therefore, underlines a higher biosynthetic power of this important compound by *S. uvarum* 12233. This theory is emphasized by Bertolini *et al.* (25), who found a higher production of 2--phenylethyl alcohol by cryotolerant *Saccharomyces*, among which *S. uvarum* 12233, in comparison with two strains of *S. cerevisiae*.

Sensory analyses

Wines fermented with the *S. cerevisiae* and *S. uvarum* analysed by triangle test resulted significantly different, with p<0.01. Sensory profile of the samples confirmed a marked difference between the two wines (Figs. 1 and 2).

Table 1. Physicochemical analyses

	Vintage #1			Vintage #2		
	Must	**M1**	**M2**	**Must**	**M1**	**M2**
pH	3.96±0	4.04±0[b]	3.97±0[a]	3.83±0	3.73±0.01[a]	3.69±0.03[a]
Ethanol content/%	–	18.65±0.21[b]	14.12±0[a]	–	15.30±0.08[b]	14.65±0.04[a]
Total acidity/(g/L)	5.32±0.01	5.62±0.03[a]	7.05±0.01[b]	5.80±0.01	6.30±0.08[a]	6.90±0.04[b]
Volatile acidity/(g/L)	0.69±0.02	1.35±0.06[b]	0.85±0.03[a]	0.63±0.02	0.96±0.01[b]	0.64±0.01[a]
Reducing sugar/(g/L)	367.85±0.55	61.50±1.27[a]	100.55±0.95[b]	343.30±0.45	76.30±0.16[a]	79.20±0.33[a]

Mean values of 4 determinations±standard deviation
Means followed by different letters are significantly different for p<0.01

Table 2. Aromatic compounds (mg/L) in the Malvasia delle Lipari wines fermented in vintage #2 by *Saccharomyces cerevisiae* (M1) and *Saccharomyces uvarum* (M2)±standard deviations

Esters	M1	M2
Ethyl butyrate	0.096±0.037[a]	0.064±0.003[a]
Isoamyl acetate	0.589±0.027[a]	0.592±0.037[a]
Ethyl hexanoate	0.329±0.0319[b]	0.199±0.068[a]
Ethyl pyruvate	0.712±0.070[b]	0.479±0.032[a]
Ethyl lactate	7.547±0.502[a]	15.179±1.494[b]
Ethyl octanoate	0.446±0.057[b]	0.287±0.083[a]
3-Hydroxyethyl butyrate	0.228±0.0103[a]	0.279±0.037[a]
γ-Butyric lactone	14.584±2.673[a]	10.627±1.564[a]
n-Ethyl acetate	0.616±0.059[a]	1.117±0.068[b]
Diethyl succinate	1.149±0.158[a]	1.739±0.142[b]
2-Phenyl ethyl acetate	0.084±0.034[a]	0.123±0.024[b]
n-3-Butyl methyl acetamide	3.450±0.606[a]	2.781±0.513[a]
Diethyl malate	1.197±0.281[a]	1.567±0.263[a]
Monoethyl succinate	11.256±1.839[a]	15.748±1.2669[b]
Terpenes		
Linalool	0.217±0.074[a]	0.169±0.002[a]
α-Terpineol	0.120±0.020[a]	0.085±0.020[a]
Acids		
Propionic	0.266±0.052[a]	0.182±0.025[a]
Butyric	1.994±0.279[a]	1.411±0.114[a]
Isovalerianic	1.962±0.455[a]	1.398±0.226[a]
Hexanoic	2.014±0.540[a]	1.268±0.027[a]
Octanoic	1.774±0.473[a]	1.839±0.156[a]
Decanoic	1.000±0.0791[a]	0.980±0.045[a]
Alcohols		
Propanol	5.960±2.191[a]	2.562±0.923[a]
Isoamyl alcohols	355.332±9.116[a]	408.580±10.811[b]
Isobutanol	52.679±11.196[a]	34.695±7.289[a]
Butanol	0.903±0.168[a]	0.901±0.024[a]
Pentanol	0.147±0.081[a]	0.131±0.008[a]
Hexanol	2.288±0.158[a]	2.065±0.161[a]
Ethoxypropanol	0.447±0.0449[a]	0.365±0.030[a]
Methyl pentanol	0.111±0.007[a]	0.139±0.004[a]
cis-Hexenol	0.272±0.031[a]	0.192±0.069[a]
2,3-Butanediol (D,L)	333.670±10.052[a]	346.086±14.574[a]
Butanediol (*meso*)	49.735±10.829[a]	57.554±14.384[a]
Propanediol	0.332±0.071[a]	0.546±0.137[a]
3-Ethoxy-1-propanol	0.795±0.082[a]	0.788±0.072[a]
α-3-Methyl thiopropanol	1.073±0.182[a]	1.062±0.169[a]
4-Hydroxy-2-butanol	1.773±0.284[a]	1.087±0.091[a]
3-Mercapto-1-hexenol	17.695±2.182[a]	15.288±1.358[a]
Phenyl ethanol	79.634±12.109[a]	101.490±11.119[b]
Benzylic alcohol	0.1102±0.001[a]	0.079±0.007[a]
Other components		
Acetoin	4.152±0.700[a]	3.310±0.4593[a]
Decanal	0.353±0.054[a]	0.336±0.0532[a]
3,7-Octadien-2,6-diol	0.601±0.003[a]	0.792±0.0128[a]

Means followed by different letters are significantly different for $p < 0.01$

Comparing the aroma sensory profile of wines fermented with *S. uvarum* or *S. cerevisiae*, significant differences ($p < 0.001$, Table 3) occurred in the following attributes: apple, raisin, clove, broom flower, orange blossom, cinnamon, hazelnut, sourdough, alcohol and vinegar, while the significant difference for vanilla and honey was slightly lower ($p < 0.05$). Some of the oral descriptors were found to be different ($p < 0.001$, Table 3), such as dry apricot, acid, sweet, spicy, alcoholic and aged. In particular, the wine fermented with *S. uvarum* showed much higher scores for the following smell attributes: apple, raisin, broom flower and clove, while the smell attributes orange blossom, sourdough and vinegar, the last certainly negative, were noticeably higher for the wine fermented with *S. cerevisiae*. Among oral attributes, acid, spicy and aged, the last of which is also negative for the quality of the product, were much higher in the wine produced with the commercial strain of *S. cerevisiae*, while the flavour of dry apricot was significantly higher in the M2
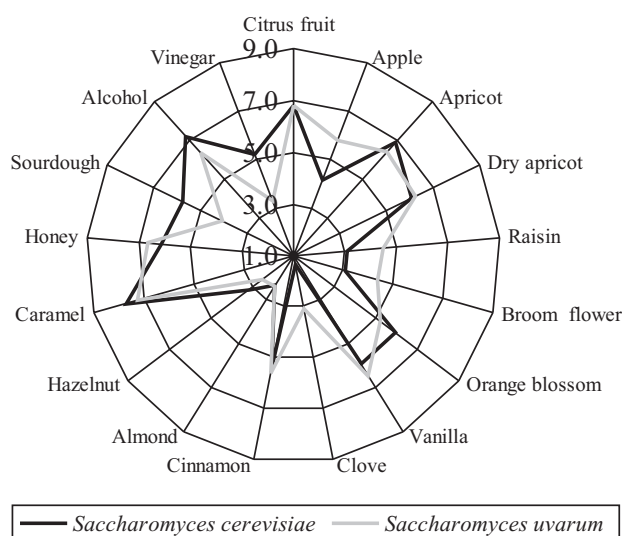

Fig. 1. Descriptive analysis: aroma profile for Malvasia wines. Sensory impact of yeast strains
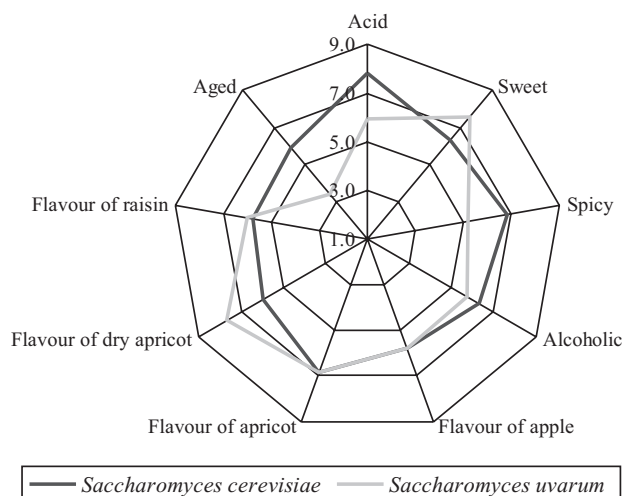

Fig. 2. Descriptive analysis: oral perceptions profile for Malvasia wines. Sensory impact of yeast strains

Table 3. Statistical analysis of sensory profile: F-values

		Wine	Judge	Repetition	Wine × Judge
Aroma	Citrus fruit	3.49	26.18***	3.25	1.25
	Apple	161.54***	31.39***	3.41*	18.75***
	Apricot	1.01	25.59***	2.73	2.76*
	Dry apricot	1.99	24.37***	7.86**	1.56
	Raisin	94.92***	21.97***	1.29	9.66***
	Broom flower	120.43***	37.33***	3.47*	17.68***
	Orange blossom	21.26***	32.61***	0.35	3.51**
	Vanilla	10.39**	28.86***	0.10	4.99***
	Clove	140.37***	27.89***	4.89*	9.73***
	Cinnamon	16.99***	29.44***	1.84	0.94
	Almond		28.61***	4.13*	3.21**
	Hazelnut	31.48***	57.39***	0.26	5.25***
	Caramel	3.52	21.35***	0.74	5.03***
	Honey	8.67**	10.93***	0.20	3.30**
	Sourdough	45.01***	20.58***	0.46	3.91***
	Alcohol	43.29***	16.31***	0.03	7.19***
	Vinegar	163.93***	18.68***	0.97	12.35***
Taste	Acid	99.87***	7.82***	2.15	8.57***
	Sweet	65.97***	10.91***	4.13*	5.04***
Mouthfeel	Spicy	95.36***	14.75***	1.79	10.70***
	Alcoholic	22.23***	4.82***	2.90	3.40**
Flavour	Apple	0.51	40.33***	0.98	1.45
	Apricot	1.71	56.47***	1.82	1.26
	Dry apricot	178.57***	14.54***	2.00	18.84***
	Raisin	2.58	15.33***	2.35	4.96***
	Aged	299.26***	15.44***	0.53	4.18***

*significant at p<0.05, **significant at p<0.01, ***significant at p<0.001

wine. Neither of the wines differed substantially regarding the yield at harvest, microbiological quality of the grapes, grape maturity or oenological treatments. Thus, the significant variation in aroma composition between wines suggests a strong impact of yeast strain used to carry out alcoholic fermentation.

Conclusions

On the basis of the present study, interesting considerations can be drawn by the comparison of the two different yeast strains employed to carry out alcoholic fermentation and the general characteristics of the Malvasia wines.

The main chemical composition was dependent on the yeast strain employed. In particular, the acetic acid content varied with the yeast used to carry out alcoholic fermentation. The recovered amounts of acetic acid confirmed the ability of *S. uvarum* strains to maintain the values of volatile acidity at low levels. The differences in chemical and aromatic composition were so significant that they could be perceived by sensory analysis. The wine fermented with *S. uvarum* yeast was the most positively valued and received a higher score in desirable

sensory attributes, while the traditionally fermented wine recorded higher values in the negative characters »vinegar« and »aged«.

On the basis of experimental data obtained, it was possible to assign a role of primary importance to the yeast strain used to carry out fermentation as a biological control of volatile acidity and aroma. Results broaden the range of employment of such strains by showing the successful application in the production of higher quality special wines, such as Malvasia wine.

References

1. Nuova disciplina delle denominazioni di origine dei vini (New regulation of the denominations of origin of wines), *Official Gazzette of the Republic of Italy no. 164* (1998).

2. Riconoscimento della denominazione di origine controllata del vino »Malvasia delle Lipari« (Assignment of the denomination of controlled origin of »Malvasia delle Lipari« wine), *Official Gazzette of the Republic of Italy no. 28* (1974).

3. C. Nicolosi Asmundo, M.C. Cataldi Lupo, S. Campisi, C. Russo, La Malvasia delle Lipari: Influenza delle tecnologie di produzione sui componenti volatili dell'aroma (Malvasia delle Lipari: Influence of production technologies on the aromatic volatile components), *Vigne Vini, 4* (1990) 33–37.

4. G.H. Fleet, G.M. Heard: Yeasts: Growth During Fermentation. In: *Wine Microbiology and Biotechnology*, G.H. Fleet (Ed.), Harwood Academic Publishers, Chur, Switzerland (1993) pp. 27–54.

5. P. Romano, C. Fiore, M. Paraggio, M. Caruso, A. Cepece, Function of yeast species and strains in wine flavour, *Int. J. Food Microbiol. 86* (2003) 169–180.

6. M. Vilanova, I. Masneuf-Pomarède, D. Dubourdieu, Influence of *Saccharomyces cerevisiae* strains on general composition and sensorial properties of white wines made from *Vitis vinifera* cv. Albariño, *Food Technol. Biotechnol. 43* (2005) 79–83.

7. M. Ciani, G. Picciotti, The growth kinetics and fermentation behaviour of some non *Saccharomyces* yeast associated with wine-making, *Biotechnol. Lett. 17* (1995) 1247–1250.

8. M. Ciani, F. Maccarelli, Oenological properties of non-*Saccharomyces* yeasts associated with wine-making, *World J. Microbiol. Biotechnol. 14* (1998) 199–203.

9. B. Esteve-Zarzoso, P. Manzanares, D. Ramón, A. Querol, The role of non-*Saccharomyces* yeasts in industrial wine-making, *Int. Microbiol. 1* (1998) 143–148.

10. I. Pardo, M.I. Garcia, M. Zuniga, F. Uruburu, Dynamics of microbial populations during fermentation of wines from the Utiel-Requena region of Spain, *Appl. Environ. Microbiol. 50* (1989) 539–541.

11. P. Romano, G. Suzzi, P. Domizio, F. Fatichenti, Glycerol and other fermentation products of apiculate wine yeasts, *J. Appl. Microbiol. 82* (1997) 615–618.

12. L. Castellari, G. Pacchioli, C. Zambonelli, V. Tini, L. Grazia, Isolation and initial characterization of cryotolerant *Saccharomyces* strains, *Ital. J. Food Sci. 3* (1992) 179–186.

13. L. Castellari, M. Ferruzzi, A. Magrini, P. Giudici, P. Passarelli, C. Zambonelli, Unbalanced wine fermentation by cryotolerant *vs.* non-cryotolerant *Saccharomyces* strains, *Vitis, 33* (1994) 49–52.

14. P. Giudici, C. Zambonelli, P. Passarelli, L. Castellari, Improvement of wine composition with cryotolerant *Saccharomyces* strains, *Am. J. Enol. Vitic. 46* (1995) 143–147.

15. C. Zambonelli, L. Castellari, M. Ferruzzi, A. Magrini, P. Passarelli, P. Giudici, L. Grazia, V. Tini, I ceppi criotolleranti di *Saccharomyces* per il miglioramento della composizione chimica dei vini (Cryotolerant *Saccharomyces* strains for the improvement of the chemical composition of wines), *Vitivinicoltura, 37* (1994) 10–17.

16. P. Giudici, C. Altieri, G. Gambini, Influenza del ceppo di lievito sui prodotti minoritari della fermentazione alcolica (Influence of the yeast strain on the minor products of alcoholic fermentation), *Industrie delle Bevande, 22* (1993) 303– 306.

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

18. Sensory analysis–General guidance for the selection, training and monitoring of assessors–Part 1: Selected assessors, ISO 8586–1 (1993).

19. Analisi sensoriale–Metodo triangolare (Sensory analysis–Triangular method), UNI U590A2520 (2000).

20. Sensory analysis–Methodology–General guidance for establishing a sensory profile, ISO 13299 (2003).

21. Analisi sensoriale–Linee guida per l'utilizzo di scale quantitative di risposta (Sensory analysis–Guidelines for the utilization of quantitative scales), UNI ISO/WD 4121 (2001).

22. Analisi sensoriale–Criteri generali per la progettazione di locali destinati all'analisi sensoriale (Sensory analysis–General criteria for the design of rooms intended for sensory analysis), UNI ISO 8589 (1990).

23. L. Zea, L. Moyano, J. Moreno, B. Cortes, M. Medina, Discrimination of the aroma fraction of Sherry wines obtained by oxidative and biological ageing, *Food Chem. 75* (2001) 79–84.

24. O. Lamikanra, C.C. Grimm, I.D. Inyang, Formation and occurrence of flavor components in Noble muscadine wine, *Food Chem. 56* (1996) 373–376.

25. L. Bertolini, C. Zambonelli, P. Giudici, L. Castellari, Higher alcohol production by cryotolerant *Saccharomyces* strains, *Am. J. Enol. Vitic. 47* (1996) 343–345.