Vitis **46** (1), 39–43 (2007)

Metabolic activity and interactions between two strains, *Saccharomyces cerevisiae* r.f. *bayanus* (SBC2) and *Saccharomyces cerevisiae* r.f. *uvarum* (S6u), in pure and mixed culture fermentations

S. Favale, P. Pietromarchi and G. Ciolfi

C.R.A. - Istituto Sperimentale per l'Enologia S.O.P. Velletri, Velletri (RM), Italy

Summary

We have studied the metabolic activity of and interactions between two strains of *Saccharomyces cerevisiae* r.f. *bayanus* (SBC2) and *Saccharomyces cerevisiae* r.f. *uvarum* (S6u), fermenting in a synthetic must medium in pure culture, co-inoculation and sequential inoculation. The second strain was added with or without sterile filtration. We monitored the rate of fermentation; at the end, total and viable cells, percentage of each strain, alcohol, volatile acidity, total sulphur dioxide, glycerol, acetaldehyde and volatile compounds were determined. The rate of alcohol production was different during fermentation: at the onset, S6u was faster than SBC2, while lateron it was inverse. When fermentation was stopped simultaneously, S6u showed the highest total cell number when grown in pure culture and the highest percentage of viable cells in mixed culture fermentation. Moreover, S6u produced low amounts of alcohol, but more glycerol and volatile compounds (*i.e.* 2-phenylethanol, acetates, ethyl esters, and fatty acids) than SBC2. The co-inoculated and the sequentially inoculated sample, in which S6u was the first strain, gave values similar to the pure S6u culture. Hence, we conclude that S6u prevails over SBC2 when both strains ferment in a medium. It seems that sequential inoculation of SBC2 as the second strain is of advantage only with regard to the relatively fast ethanol production.

K e y w o r d s : co-inoculation, sequential inoculation, *Saccharomyces cerevisiae* r.f. *uvarum*, *Saccharomyces cerevisiae* r.f. *bayanus*, yeast interactions.

Introduction

Alcoholic fermentation of must may occur spontaneously by indigenous yeasts or by starter cultures of selected strains. The latter allows to minimize possible risks of stuck or sluggish fermentation and to obtain wine with specific characteristics (Romano *et al.* 2003, Vilanova *et al.* 2005). Some authors studied the indigenous flora in relation to species identification, their sequential growth pattern and metabolic activity with particular attention to secondary metabolites which sometimes are not desirable (Plata *et al.* 2003, Romano *et al.* 2003, Nikolaou *et al.* 2006). In the early stages of fermentation the dominant species are non-*Saccharomyces* yeasts, *i.e. Metschnikowia, Candida* and *Kloeckera spp.* When alcoholic concentration increases *Saccharomyces* are prevalent (Combina *et al.* 2005). Over the last few years many yeast strains have been isolated and their oenological behaviour is subject of biotechnological research, as well as of breeding and genetic manipulation (Cunha *et al.* 2006, Marullo *et al.* 2006). There are many yeast strains available with different oenological characteristics; moreover inoculation of mixed or pure yeast strains to control the fermentation process has become popular. Although microbiological interactions among yeast species during fermentation have been described (Zohre *et al.* 2002, Fleet 2003, Jemec and Raspor 2005, Garde-Cerdán and Ancin Azpilicueta 2006, Xu *et al.* 2006), only few authors have studied the interactions between two or more different yeast strains of oenological interest (Cheraiti *et al.* 2005, Howell *et al.* 2005). Thus, we studied the metabolic activity and some interactions between two strains, *Saccharomyces cerevisiae* r.f. *bayanus* (SBC2) and *Saccharomyces cerevisiae* r.f. *uvarum* (S6u), by analyzing their oenological behaviour, *i.e.* the production of secondary metabolites, during fermentation of a synthetic must medium by pure or mixed culture, with co-inoculation or sequential inoculation.

Material and Methods

We used a synthetic medium for fermentations with the following composition per liter: Na_2MoO_4 $2H_2O$ 200 µg; $ZnSO_4$ $7H_2O$ 400 µg; $CuSO_4$ 5 H_2O 40 µg; H_3BO_3 500 µg; KI 100 µg; $FeCl_3$ $6H_2O$ 400 µg; $MnSO_4$ H_2O 400 µg; $NiCl_2$ 6 H_2O 400 µg; $K_2Cr_2O_7$ 20 µg; $CaCl_2$ 0.1 g; NaCl 0.1 g; KH_2PO_4 1 g; $MgSO_4$ $7H_2O$ 0.5 g; $(NH_4)_2SO_4$ 0.944 g; $(NH_4)_2HPO_4$ 0.943 g; tartaric acid 3 g; sucrose 220 g; yeast extract 0.5 g; KOH to adjust pH to 3.2, pyridoxine hydrochloride 40 µg; thiamine hydrochloride 40 µg; myo-inositol 2 mg; biotin 20 µg; D-pantothenic acid calcium salt 400 µg; nicotinamide 40 µg; p-aminobenzoic acid 20 µg.

Thirty litres of the synthetic medium were sterilized with membrane filter (0.2 µm), and put in 14 sterile 2 l bottles that were used for fermentation. The rest of the medium was employed to start the culture growth in Erlenmeyer flasks at room temperature (about 20 °C). We used two strains of the yeast collection of C.R.A. Istituto Speri-

Correspondence to: Dr. G. Ciolfi, C.R.A. - Istituto Sperimentale per l'Enologia S.O.P. Velletri, Via Cantina Sperimentale 1, 00049 Velletri (RM)-Italy. Fax:+39-06-963-4020. E-mail: isesopciolfi@virgilio.it

mentale per l'Enologia di Velletri (RM): *Saccharomyces cerevisiae* r.f. *uvarum* (S6u) and *Saccharomyces cerevisiae* r.f. *bayanus* (SBC2) (nomenclature according to YARROW 1984).

The predominant oenological characteristics of the two strains are as follows: S6u, high capacity to ferment at low temperature (> 5 °C), alcoholic power about 15 % v/v, high production of glycerol and floral aromas (2-phenylethanol), low production of acetaldehyde, volatile acidity and sulphurous compounds, good production of succinic acid (CIOLFI 1994); SBC2, high alcohol resistance, low production of H_2S and SO_2, high resistance to added sulphur dioxide (sulphites), optimal fermentation temperature 25 °C, normal production of acetic esters and fatty acids, a successful strain to ferment both, white and red musts (CIOLFI *et al.* 2002).

We performed 7 fermentation trials in double which differed by the way of inoculation, as indicated in Tab. 1. A volume of pure starter culture, grown on the same synthetic medium, was inoculated to have a final concentration of 2 x 10^6 cells ml^{-1} for the first inoculation and co-inoculation, and 4 x 10^6 cells ml^{-1} for the second inoculation. Fermentation temperature was 20 °C; the loss of CO_2 was daily monitored by weighing the sample. We stopped fermentations at the same time except for the two samples filtered before the second inoculation. On that date, we evaluated the number of total and viable cells by direct light microscopy and for samples with both yeasts their relative percentage was determined by the raffinose fermentation test, after isolation of the pure culture on a solid agar medium (DELFINI 1995).

The following analyses were performed: alcohol, volatile acidity and total sulphur dioxide according to official methods (GAZZETTA UFFICIALE DELL'UNIONE EUROPEA 1990); glycerol and acetaldehyde by enzymatic methods (analytical kit, Chema Italia). Volatile compounds were extracted and determined by GC analysis as proposed by GIANOTTI *et al.* (1991) using a Thermoquest-GC8000; detector: FID; helium flow: 2.5 ml min^{-1}; column: HP-FFAP 50 m x 0.320 mm x 0.52 µm; injection temperature: 220 °C; oven temperature: 45 °C held for 5 min, then 2 °C min^{-1} to 220 °C; detector temperature: 245 °C.

Data were processed by analysis of variance (ANOVA) and least significant differences test (LSD) with the software STATISTICA 5.1 (STATSOFT ITALIA 1997).

Results and Discussion

Fermentation kinetics are expressed as volumetric percentage of alcohol produced per unit time, determined by weight losses due to CO_2 production (Figure). In the first hours the rate of fermentation was higher in samples containing the yeast S6u, both pure and mixed culture (50%S6uSBC2), than in SBC2 pure culture ones, producing about 4.5 vol. % of alcohol, 212 h and 236 h after inoculation, respectively. Then the second inoculation was started. The rate of fermentation was higher when SBC2 was inoculated first.

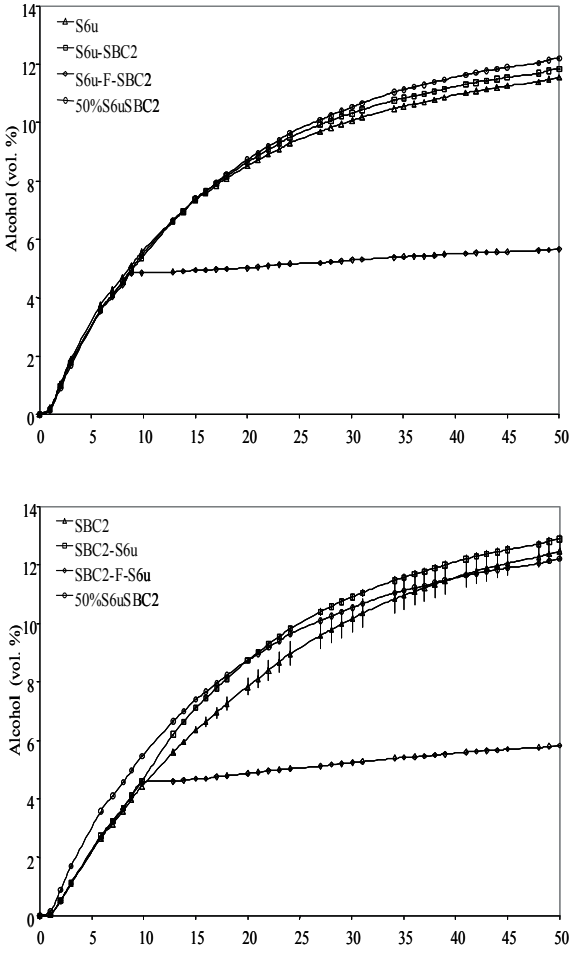

Figure: Alcohol production during days of fermentation (mean values and standard deviations).

Table 1

List of samples

Marks	Way of inoculation
S6u	Pure culture S6u
SBC2	Pure culture SBC2
S6u-F-SBC2	1st inoculation S6u; sterile filtration 0.45 µm, 2nd inoculation SBC2
SBC2-F-S6u	1st inoculation SBC2; sterile filtration 0.45 µm, 2nd inoculation S6u
50%S6uSBC2	Co-inoculation with 50 % of each strain
S6u-SBC2	1st inoculation S6u; 2nd inoculation SBC2
SBC2-S6u	1st inoculation SBC2; 2nd inoculation S6u

All the 2nd inoculation were made when alcohol was about 4.5 % v/v.

In fact, in the early stage of fermentation, while each strain was still alone in culture, the difference in alcohol production between S6u and SBC2 was 0.5 vol. % after 48 h; it was 1 vol. % before the second inoculation, S6u producing more alcohol. The opposite occurred after 684 h, when the alcohol was the same in the two pure cultures; at the end of fermentation it was still higher in SBC2 samples (about 1 vol.%).

Since we stopped fermentation at the same time, except for the filtered samples before the second inoculation, and because of the major rate of fermentation of SBC2 in the final stages, pure culture S6u had the lowest amounts of ethanol, while 50%S6uSBC2 was medium.

After sterile filtration the samples of the second inoculation had the lowest rate of alcohol production, which was expected, because we neither made a very strong inoculation nor added any nutrient; 5 months after the start, when we stopped the fermentations, the production of alcohol was 7.48±0.13 vol. % (S6u-F-SBC2) and 8.29±0.37 vol. % (SBC2-F-S6u).

At the end of our trial, we determined the number of total and viable cells (Tab. 2). In the first inoculation we had 2 x 10^6 cells ml^{-1} in each sample; thus S6u showed a greater growth than SBC2 (47 ±1.40 x 10^6 cells ml^{-1} and 29 ± 0.88 x 10^6 cells ml^{-1}, respectively). In the samples where both strains fermented together from the start and after the second inoculation (addition of 4 x 10^6 cell ml^{-1}), the total cell number was less than pure S6u culture. In samples, where fermentation took place with the two yeast strains in sequence by inoculating the second strain after sterile filtration, total cell number was 8 ± 0.94 x 10^6 cells ml^{-1} (S6u-F-SBC2) and 5 ±0.27 x 10^6 cells ml^{-1} (SBC2-F-S6u). Thus, under conditions, where the medium was previously modified by the other strain, even if the alcohol was the same, initial SBC2 growth was higher than that of S6u. Similarly this tendency has been observed in samples where the strains were added by a second inoculation without filtering (S6u-SBC2 and SBC2-S6u). Besides, both strains inoculated after filtration, but without any addition of nutrients, only survived in the modified medium.

Table 2

Total and viable cells after fermentation (10^6 cells ml^{-1})

Samples	Total cells	Viable cells
S6u	47.03 ± 1.40	30.50 ± 1.12
SBC2	29.38 ± 0.88	11.84 ± 1.71
S6u-F-SBC2	8.67 ± 0.94	0.47 ± 0.15
SBC2-F-S6u	5.26 ± 0.27	0.21 ± 0.06
50%S6uSBC2	31.30 ± 5.38	16.61 ± 2.64
S6u-SBC2	39.27 ± 0.59	15.52 ± 1.86
SBC2-S6u	34.79 ± 4.71	6.67 ± 0.96

The percentage of the two strains in the samples fermented together is shown in Tab. 3. The number of S6u viable cells prevailed over that of SBC2: mean values are always higher than 60 %. It seems that the percentage of S6u is lower when the strain is added later rather than when it is first inoculated. Besides, the percentage of S6u was higher in 50%S6uSBC2.

Table 3

Percentage of viable cells of the two strains after mixed fermentations

Samples	S6u mean	SBC2 mean	Standard deviation
50%S6uSBC2	92.5	7.5	10.6
S6u-SBC2	72.5	27.5	38.9
SBC2-S6u	65.0	35.0	35.4

The mean values, standard deviations and statistical estimations of analytical determinations we made after stopping fermentation are summarized in Tab. 4. S6u was the best producer of glycerol and volatile compounds, with the peculiar production of 2-phenylethanol, both in pure and in mixed culture when it was used as first inoculation. It is well known from literature that S6u is a good producer of secondary metabolites. Amounts of glycerol are always higher than with pure SBC2 culture fermentation; a lower content was observed in the SBC2-S6u sample. This low value with regard to S6u but high value with regard to pure SBC2 culture may be explained by the evidence that the major production of glycerol occurs in the early phase of fermentation. Besides, the glycerol content of SBC2-S6u was lower than that of SBC2-F-S6u, too. Thus, it seems that S6u produces more glycerol when added after removing the first strain than together with it.

2-phenylethanol is produced at high concentration by S6u and together with glycerol, under our experimental conditions, may be used as marker of it. It is interesting to note that in all samples containing the S6u strain the production of both, glycerol and 2-phenylethanol, was higher than with the pure SBC2 culture, but it was lower when S6u was inoculated as the second strain. Thus, we can assume that by inoculating S6u after SBC2 started fermentation these compounds increased only slightly, while we noted higher values compared to pure SBC2 culture in the sample S6u-F-SBC2; this is in agreement with the results of the other samples where S6u was inoculated first.

The same considerations are suitable for the other secondary metabolites, *i.e.* acetates, ethyl esters, fatty acids. The high production of 2-phenylethanol, isoamyl alcohol, as well as fatty acid, is correlated with their esters, acetates and ethyl ones, which are higher when S6u has started fermentation. Usually the acetate:acetic acid and ethyl esters: fatty acid ratios are considered as indices of secondary metabolism. By comparing these indices of pure and mixed culture fermentations, it seems that there is few variation, as there was a metabolic competition between strains. In fact, in the sample 50%S6uSBC2, the indices are similar to pure culture and first inoculation of S6u; these results are in agreement with the percentage of the cell of strains we found.

We conclude that when the two yeasts grow on the same medium, S6u is a dominant strain because of its enhanced metabolism in the early phase of fermentation; when these two strains are used together for fermentation, the oenological characteristics of the wine, *i.e.* secondary

Table 4

Final analysis after fermentation – Mean values, standard deviations (SD) – ANOVA and LSD test (α = 0.05)

		S6u		SBC2		S6u-F-SBC2		SBC2-F-S6u		50%S6uSBC2		S6u-SBC2		SBC2-S6u	
		mean	SD	mean	SD	mean	SD	mean	SD	mean	SD	mean	SD	mean	SD
Alcohol (% v/v)	***	11.55^{a}	0.04	12.48$^{e, f}$	0.32	7.48^{c}	0.13	8.29^{b}	0.37	12.22$^{d, e}$	0.04	11.85$^{a, d}$	0	12.90^{f}	0.12
Total SO_2 (mg l^{-1})	ns	12.80	4.53	9.60	0	19.20	0	12.16	3.62	10.40	3.39	11.20	2.26	12.80	0
Volatile acidity (g l^{-1})	ns	0.31	0.01	0.40	0.02	0.51	0.01	0.35	0.15	0.35	0	0.34	0.01	0.36	0.02
Glycerol (g l^{-1})	***	6.5^{a}	0	3.9^{f}	0.2	5.3^{c}	0.1	4.8^{d}	0.3	6.0^{b}	0.1	6.6^{a}	0.1	4.3^{e}	0.1
Acetaldehyde (mg l^{-1})	***	28^{a}	0	35$^{a, b}$	5.7	52^{c}	11.3	50$^{b, c}$	9.9	25^{a}	0.7	34^{a}	5.7	27^{a}	0.7
Volatile compounds (μg l^{-1})															
Isobutyl acetate	ns	119	25.9	125	2.5	42	12.7	43	7.9	62	82.8	117	6.3	88	8.4
Isoamyl acetate	***	538$^{a, b}$	28.8	342^{c}	1.6	105^{d}	12.4	77^{d}	11.8	580^{a}	89.6	489^{b}	0.6	352^{c}	0.2
2-Phenyethyl acetate	***	1853^{a}	5.7	204^{b}	5.9	273^{b}	50.2	162^{b}	15.2	1800^{a}	334.2	1588^{a}	149.4	292^{b}	6.9
∑ acetates		2510	60.4	671	10.0	420	75.3	282	11.3	2441	327.4	2194	156.2	733	15.1
(Acetates/Acetic acid)x10^{-3}		8.1	0.17	1.7	0.07	0.8	0.17	0.9	0.42	7.0	0.94	6.6	0.60	2.1	0.08
Ethyl hexanoate	***	440^{a}	18.1	268$^{b, c}$	34.7	187$^{c, d}$	42.5	122^{d}	1.8	487^{a}	25.1	441^{a}	66.1	275^{b}	0.3
Ethyl octanoate	***	660^{a}	7.4	277^{b}	31.9	152^{d}	5.2	153^{d}	0.3	694^{a}	28.0	644^{a}	90.5	300^{b}	2.9
Ethyl decanoate	***	370^{a}	13.2	56^{b}	14.3	105^{b}	2.7	34^{b}	48.5	384^{a}	127.2	387^{a}	112.6	83^{b}	0.2
∑ Ethyl esters		1471	38.7	602	80.9	443	45.1	309	46.4	1564	130.1	1472	269.2	659	2.8
C6 Acid	***	2820^{a}	113	1489$^{c, d}$	135	2071^{c}	401	903^{d}	8	3700^{b}	257	3732^{b}	645	1709^{c}	7
C8 Acid	***	7338^{a}	197	4028^{b}	448	2813$^{b, c}$	323	2249^{c}	144	8624^{a}	1178	8589^{a}	1397	4438^{b}	46
C10 Acid	***	5609^{a}	288	1021^{b}	141	1583^{b}	25	938^{b}	84	5792^{a}	864	5751^{a}	1101	1412^{b}	13
∑ fatty acids		15767	598	6538	724	6467	750	4090	236	18115	2300	18071	3143	7559	65
Ethylic esters/fatty acids		0.093	0.001	0.092	0.002	0.069	0.001	0.075	0.007	0.087	0.004	0.081	0.001	0.087	0.001
C4 Acid	***	56$^{a, b}$	22.1	0^{e}	0.0	40$^{b, c}$	13.8	10$^{d, e}$	14.6	83^{a}	6.4	38$^{b, c, d}$	0.3	19$^{c, d, e}$	3.7
C12 Acid	***	289^{a}	31.4	16^{b}	23.0	32^{b}	5.0	99^{b}	4.8	341^{a}	74.6	341^{a}	65.8	28^{b}	39.9
3-OH-ethyl butyrate	***	15^{a}	0.9	0^{c}	0	0^{c}	0	14$^{a, b}$	2.7	0^{c}	0.0	6$^{b, c}$	9.0	0^{c}	0
4-OH-ethyl butyrate	***	7002^{a}	827	594$^{d, e}$	82	183^{e}	7	553$^{d, e}$	38	5746^{b}	217	4916^{c}	18	1163^{d}	21
Ethyl lactate	***	611^{a}	137	285^{d}	67	423$^{b, c, d}$	6	493$^{a, b, c}$	41	544$^{a, b}$	14	376$^{c, d}$	32	316^{d}	26
γ-butyrrolactone	***	69^{a}	21	0^{c}	0	0^{c}	0	0^{c}	0	54$^{a, b}$	0	22$^{b, c}$	31	0^{c}	0
Diethyl succinate	***	347^{a}	14.8	34^{d}	48.4	213^{c}	2.3	235$^{b, c}$	15.2	314^{a}	49.5	305$^{a, b}$	31.5	78^{d}	0.4
2-Phenyl ethanol	***	223278^{a}	9667	47111^{c}	2510	117043^{b}	15792	45136^{c}	433	225296^{a}	46398	213265^{a}	39955	58479^{c}	531
Isoamyl alcohol	***	308044^{a}	14569	121026^{d}	12714	101551^{e}	6003	81884^{f}	3511	272464^{b}	1893	241660^{c}	1621	130391^{d}	1916

ns = not significant differences; *** = statistical significant differences; α = 0.05; values with the same letter belong to the same group.

metabolites, are produced by the dominant yeast. S6u is able to enhance the complexity of wine taste and aroma. In fact, the S6u strain fermenting a synthetic medium produces about 6.5 g l^{-1} of glycerol, 2.5 mg l^{-1} of acetates, 1.5 mg l^{-1} of ethyl esters, and 15.0 to 20.0 mg l^{-1} of fatty acids; SBC2 produces lower amounts of these compounds, about 4.0 g l^{-1}, 0.6 mg l^{-1}, 0.6 mg l^{-1}, and 6.0 to 7.5 mg l^{-1}, respectively. In addition, S6u produces about 225 mg l^{-1} of 2-phenylethanol, a compound with the characteristic rose odour, with a perception threshold of 200 mg l^{-1} (Franco *et al. 2004*). The values of these compounds are similar in the samples where S6u was present at the start of fermentation, while there seems to be a small difference in the rate of alcohol production. Thus the gain in time, few days, to metabolize all the sugar in the medium, by choosing SBC2, would mean a loss of production of secondary metabolites. Furthermore it seems that the use of SBC2 in association with S6u gives results similar to those which can be obtained with S6u pure culture.

This is the first study analysing metabolic interactions between the S6u and SBC2 strains in a synthetic must. It would be interesting to verify their behaviour when they ferment natural grape must.

References

Cheraiti, N.; Guezenec, S.; Salmon, J. M.; 2005: Redox interactions between *Saccharomyces cerevisiae* and *Saccharomyces uvarum* in mixed culture under enological conditions. Appl. Environ. Microbiol. **71**, 255-260.

Ciolfi, G.; 1994: Selezione di uno stipite di lievito *Saccharomyces* della razza fisiologica *uvarum* e suo impiego enologico allo stato secco (S6u). L'Enotecnico **11**, 71-75.

Ciolfi, G.; Garofolo, A.; Lombardi, D. S.; Favale, S.; 2002: Comparazione del metabolismo di sette lieviti commerciali più uno di nuovo isolamento. Industrie delle Bevande **178**, 123-132.

Combina, M.; Elía, A.; Mercado, L.; Catania, C.; Ganga, A.; Martinez, C. P.; 2005: Dynamics of indigenous yeast populations during spontaneous fermentation of wines from Mendoza, Argentina. Int. J. Food Microbiol. **99**, 237-243.

Cunha, A. F.; Missawa, S: K.; Gomes, L. H.; Reis, S. F.; Pereira G. A. G.; 2006: Control by sugar of *Saccharomyces cerevisiae* flocculation for industrial ethanol production. FEMS Yeast Res. **6**, 280-287.

Delfini, C.; 1995: Scienza e tecnica di microbiologia enologica. Ed. "Il Lievito"- Asti- Italia, 533-539.

Fleet, G.H.; 2003: Yeast interactions and wine flavour. Int. J. Food Microbiol. **86**, 11-22.

Franco M.; Peinado R.A.; Medina M.; Moreno J.; 2004: Off-vine grape drying effect on volatile compounds and aromatic series in must from Pedro Ximénez grape variety. J. Agric. Food Chem. **52**, 3905-3910.

Garde-Cerdán, T.; Ancín- Azpilicueta, C.; 2006: Contribution of wild yeasts to the formation of volatile compounds in inoculated wine fermentations. Eur. Food Res. Technol. **222**, 15-25.

Gazzetta ufficiale dell'Unione europea; 1990. Regolamento (CE) N 2676/90. G.U.U.E. IT 3/Ottobre/1990, L 272.

Gianotti, S.; Di Stefano, R.;1991: Metodo per la determinazione dei composti volatili di fermentazione. L'Enotecnico **10**, 61-64

Howell, K. S.; Cozzolino, D.; Bartowsky, E. J.; Fleet, G. H.; Henschke, P. A.; 2005: Metabolic profiling as a tool for revealing *Saccharomyces* interactions during wine fermentation. FEMS Yeast Res. **6**, 91-101.

Jemec, K.; Raspor, P.; 2005: Initial *Saccharomyces cerevisiae* concentration in single or composite cultures dictates bioprocess kinetics. Food Microbiol. **22**, 293-300.

Marullo, P.; Bely, M.; Masneuf-Pomarède, I.; Pons, M.; Aigle, M.; Dubourdieu, D.; 2006: Breeding strategies for combining fermentative qualities and reducing off-flavor production in a wine yeast model. FEMS Yeast Res. **6**, 268-279.

Nikolaou, E.; Soufleros, E.H.; Oulompasi , E.;Tzanetakis N.;2006: Selection of indigenous *Saccharomyces cerevisiae* strains according to their oenological characteristics and vinification results. Food Microbiol. **23**, 205-211.

Plata, C.; Millan, C.; Mauricio, J. C.; Ortega, J. M.; 2003: Formation of ethyl acetate by various species of wine yeasts. Food Microbiol. **20**, 217-224.

Romano, P.; Fiore, C.; Paraggio, M.; Caruso, M.; Capece, A.; 2003: Function of yeast species and strains in wine flavour. Int. J. Food Microbiol. **86**, 169-180.

StatSoft Italia; 1997: *STATISTICA per Windows*. [Manuale programma per computer]. StatSoft Italia S.r.l., Vigonza (Padova)-Italia. WEB: http://www.statsoft.com.

Vilanova, M.; Musneuf-Pomarède, I.; 2005: Effect of three *Saccharomyces cerevisiae* strains on the volatile composition of Albariño wines. Ital. J. Food Sci. **2**, 221-227.

Xu, Y.; Zhao, C. A.; Wang, L. P.; 2006: Controlled formation of volatile components in cider making using a combination of *Saccharomyces cerevisiae* and *Hanseniaspora valbeyensis* yeast species. J. Ind. Microbiol. Biotechnol. **33**, 192-196.

Yarrow, D; 1984. *Saccharomyces* Meyen ex Reess. In: N. J. W. Kreger-van Rij (Ed.): The Yeasts - a Taxonomic Study, 379-395. Groningen, The Netherlands.

Zohre, D. E.; Erten, H.; 2002: The influence of *Kloeckera apiculata* and *Candida pulcherrima* yeasts on wine fermentation. Proc. Biochem. **38**, 319-324.

Received May 8, 2006