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Test of four generations of *Saccharomyces cerevisiae* concerning their effect on antioxidant phenolic compounds in wine

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Abstract The aim of this research was to study the behaviour of 70 different *Saccharomyces cerevisiae* strains on the antioxidant compounds level in wines by RP-HPLC/DAD. Micro-winemaking was carried out in *Cabernet Sauvignon* grape must testing eight Italian wild strains, 12 derived monosporal cultures, 15 hybrids obtained by monosporal spore-to-spore conjugation, 34 monosporal cultures derived from the hybrids, and Zymaflore F15 as control strain. At the end of the winemaking, the wines show significant differences concerning their antioxidant levels in relation to the strain used. Catechin and epicatechin were the principal antioxidant compounds for all the samples. In particular, the catechin content varied from 0 to 79.53 mg/L, while epicatechin varied from 0 to 70.51 mg/L. The vanillic acid level varied from 3.10 to 12.71 mg/L. Gallic and caffeic acids varied, respectively, from 2.54 to 6.77 mg/L and from 0 to 10.63 mg/L. The rutin and quercetin content varied from 0 to 11.77 mg/L and from 0 to 2.09 mg/L, while *trans*-resveratrol level varied from 0 to 0.85 mg/L. Data validate the main role that wine yeast selection plays to enhance red wine content in antioxidant phenolic compounds.

Keywords Antioxidant phenolic compounds · Hybrids · Monosporal cultures · *Saccharomyces cerevisiae* · Wine yeast selection

Introduction

Red wine quality depends mainly on its polyphenol content that contributes to color, flavour, healthy properties, and natural antioxidant activity. Anthocyanins, catechins, proanthocyanidins, flavonols, stilbenes, and other phenolics are known for their biological properties and ability to induce beneficial effects on human health and

to prevent diseases [1, 2]. In particular, *trans*-resveratrol (3,5,4'-trihydroxy-*trans*-stilbene) can prevent or reduce a wide range of diseases, including cancer, cardiovascular disease, and ischemic damage [3–7]. The *trans*-resveratrol content in grape and wine depends on many different factors, including grape variety, harvest year, harvest date, climatic conditions, UV light, and winemaking technology; many authors have studied the content in *trans*-resveratrol of different grape varieties and wines [8–11]. It was reported that maceration of grape skins increased the *trans*-resveratrol concentration ten times in comparison with the winemaking without maceration [12]. Different authors investigated the influence of the fermentation process on aroma composition [13], phenolics, antioxidants, and volatiles in red wines [14, 15], as well as the effects of different yeast strains on volatile content of red wines [16]. During alcoholic fermentation, yeasts affect the content of polyphenols by producing different substances [17–19] and by the interaction between the negative charge of their cell wall and polyphenols [20–23]. Yeast strain activity influences the antioxidant level in wine [24, 25]; in details, compounds with a greater degree of methoxylation are more retained than those more hydroxylates beside different polarity and porosity of cell walls [26]. A simple method is used to screen the polyphenols yeast adsorption activity on cell walls [27], and the impact on red wine production has been described [28]. Recently, it has been demonstrated that, analogously to the yeast biofilm-like properties [29], wine yeast can decrease or increase its polyphenol parietal adsorption activity according to the must nutrient availability [30]. It is well known that the effect of the adsorption of antioxidant phenolic compounds by yeast is strictly dependent on the yeast strain. This effect is mainly due to the ability of the yeasts to generate and release high amounts of compounds, mainly polysaccharides, able to interact with phenolic compounds. Studying the polysaccharide and phenolic composition in *Syrah* red wines, a different behaviour between two strains of *Saccharomyces cerevisiae* was described: one of them released higher amounts of polysaccharides during both alcoholic fermentation and aging period [31]. In *Cabernet Sauvignon* red wines, this yeast during alcoholic fermentation allowed a faster release of polysaccharides than the other [32]. Recently, it was demonstrated that yeasts belonging to the *Schizosaccharomyces* genus release—by the end of the alcoholic fermentation—a quantity of polysaccharides of cell wall origin approximately 3–7 times higher than that released by a commercial *S. cerevisiae* yeast strain under the same fermentative conditions [33]. Whole yeast cells were found to exhibit a high capacity to irreversibly adsorb tannins from grape and wines [34]. Yeasts and proanthocyanidins were found to interact; this was probably due to the passage of the proanthocyanidins through the cells and to the

interaction of the proanthocyanidins with the plasma membrane [35]. Therefore, taking into account the factors above mentioned, the selection of a good yeast strain is a prerequisite for the production of high-quality wine [36–39]. Several authors, using metabolic engineering techniques, transferred in *S. cerevisiae* genes from other microorganisms [40, 41]; the resulting activity is the neo-production of polyphenols, in particular *trans*-resveratrol. However, it is interesting to note that, at least in Europe, the release of genetically modified microorganisms into the environment—in our case at the end of winemaking—is possible only if it is in compliance with part B of Directive 2001/18/EC [42]. It is generally asserted that wine yeasts are homothallic [43, 44], and for this reason, it is useful to carry out a strain genetic improvement by conjugation of spores [45]. The yeast interaction with the antioxidant activity of red wines was studied by DPPH demonstrating the usefulness of monosporal culture selection and hybridization in enhancing the natural antioxidant activity of wines [46]. Among other methods, HPLC-DAD technique has been positively used to detect phenolics in grapevine red berry skin and in wine [47, 48]. The aim of this research was to study the behaviour of 70 different *S. cerevisiae* strains regarding their effect on *Cabernet Sauvignon* wine content in *trans*-resveratrol; according to the analytical methodology adopted, the following antioxidant compounds have also been analysed: (+)-catechin, epicatechin, vanillic acid, gallic acid, caffeic acid, rutin, and quercetin.

Materials and methods

Chemicals

Gallic acid ($\geq 99\%$ HPLC grade), vanillic acid (97% HPLC grade), and caffeic acid (98% HPLC grade) were purchased from Sigma–Aldrich Chem. Co. (Milwaukee, WI, USA). Quercetin ($\geq 99\%$ HPLC grade), rutin ($\geq 99\%$ HPLC grade), (–)-epicatechin ($\geq 99\%$ HPLC grade), (+)-catechin ($\geq 99\%$ HPLC grade), and *trans*-resveratrol ($\geq 99\%$ HPLC grade) were supplied by Extrasynthese (Genay-France). Acetonitrile, formic acid, and water were solvent HPLC grade, obtained from Carlo Erba Reagents (Milano, Italia). A standard mixture was prepared by adding accurately weighed amount of each antioxidant compound (about 100 mg) to a 100 mL volumetric flask and brought to the mark using methanol: distilled water (90:10, v/v) acidified to pH 3 with concentrated formic acid (98–100%). A calibration straight for each standard was obtained by analysing the

standard solution diluted at different concentrations. All solutions were filtered through a 0.45 µm Millipore filter (GMF Whatman) and inject to HPLC system for retention times determination.

Yeast strains

For the present research were used *S. cerevisiae* Zymaflore F15 (Laffort Oenologie, France) as control strain, eight Italian wild strains of *S. cerevisiae* (NA14, NA15, NA93, RC26, RC29, RC39, RE49, and RE78), and the progeny obtained by micromanipulation: 12 monosporal cultures—RC026B-1A, RC026B-1B, RC026B-1C, RC026B-1D, RC029A-1A, RC029A-1B, RC029A-1C, RC029A-1D, RC039B-1A, RC039B-1B, RC039B-1C, and RC039B-1D—15 hybrids obtained by monosporal culture's spore to spore conjugation—RC026C-1C × RC039C-1C (4), RC029A-1D × RE078C-1C (4), RC029A-1D × RC039C-1C (4), RC029B-1C × RE078C-1C (4), RC029B-1C × RC039C-1C (7), RC026C-1C × RC039C-1C (9), RC029B-1C × NA093B-1C (6), NA014C-1D × RC039C-1C (3), NA014C-1D × RC039C-1C (2), NA015A-1B × RC039C-1C (5), NA015A-1B × NA093B-1C (2), RE049B-1A × NA093B-1C (1), RE049B-1A × NA093B-1C (5), RE049B-1A × RC039C-1C (8), and RE049B-1A × RC039C-1C (9)—34 monosporal cultures derived from the hybrids—[RC029B-1C × RC039C-1C (7)]-1A, [RC029B-1C × RC039C-1C (7)]-1B, [RC029B-1C × RC039C-1C (7)]-1C, [RC029B-1C × RC039C-1C (7)]-2A, [RC029B-1C × RC039C-1C (7)]-2B, [RC029B-1C × RC039C-1C (7)]-2C, [RC029B-1C × RC039C-1C (7)]-3A, [RC029B-1C × RC039C-1C (7)]-3B, [RC029B-1C × RC039C-1C (7)]-3C, [RC026C-1C × RC039C-1C (4)]-1A, [RC026C-1C × RC039C-1C (4)]-1B, [RC026C-1C × RC039C-1C (4)]-1C, [RC026C-1C × RC039C-1C (4)]-1D, [RC026C-1C × RC039C-1C (4)]-2A, [RC026C-1C × RC039C-1C (4)]-2B, [RC026C-1C × RC039C-1C (4)]-2C, [RC026C-1C × RC039C-1C (4)]-2D, [RC029A-1D × RC039C-1C (4)]-1A, [RC029A-1D × RC039C-1C (4)]-1B, [RC029A-1D × RC039C-1C (4)]-1C, [RC029A-1D × RC039C-1C (4)]-2A, [RC029A-1D × RC039C-1C (4)]-2B, [RC029A-1D × RC039C-1C (4)]-2C, [RC029A-1D × RC039C-1C (4)]-3A, [RC029A-1D × RC039C-1C (4)]-3B, [RC029A-1D × RC039C-1C (4)]-3C, [RC026C-1C × RC039C-1C (9)]-1A, [RC026C-1C × RC039C-1C (9)]-1B, [RC026C-1C × RC039C-1C (9)]-1C, [RC026C-1C × RC039C-1C (9)]-1D, [RC026C-1C × RC039C-1C (9)]-2A, [RC026C-1C × RC039C-1C (9)]-2B, [RC026C-1C × RC039C-1C (9)]-2C, [RC026C-1C × RC039C-1C (9)]-2D. Yeast Peptone Dextrose broth (YPD—10 g/L yeast extract, 20 g/L peptone, and 20 g/L glucose) and Yeast Peptone Dextrose agar (YPD—10 g/L yeast extract, 20 g/L peptone, 20 g/L glucose, and 20 g/L agar) were used to grow and micromanipulate the

strains. Sodium acetate agar (sodium acetate anhydrous 1 g/L and agar 20 g/L) was used to induce strain sporulation at 25 °C for 10 days [49]. The wild strains and their progeny (Fig. 1) were micromanipulated (MSM System 400, Singer Instrument Co Ltd, UK) to obtain monosporal cultures and hybrids: each sporified strain was treated with Zymolyase (20U/mL) for 10 min; then, a small quantity of the suspension was spread on one side of Petri plates containing YPD agar and the spores from individual ascus were picked up and placed at defined positions on the plates (according to the MSM System instruction). The plates were then incubated at 25 °C for 2 days. The needle of the MSM System was used to obtain hybrids by spore to spore conjugation.

Micro-winemaking trials

A total of 213 micro-winemaking trials—70 strains inoculated in triplicate and the spontaneous fermentation in triplicate—were carried out using the eight wild strains, the 12 derived monosporal cultures, the 15 hybrids, the 34 monosporal cultures derived from the hybrids, and the Zymaflore F15 control strain. Black grapes of the *Cabernet Sauvignon* variety were destemmed, crushed, and cold soaked at 4 °C for 5 days to allow the release of polyphenols. The must obtained after pressing (27 °Brix) was adjusted to pH 3.50 (original value 3.98) using hydrochloric acid 5 N, dispensed (12 mL/each) in 15-mL plastic conical sterile centrifuge tubes, inoculated in triplicate using 0.6 mL of 2-day pre-cultures in pasteurized must of the 70 yeast strains, and incubated at 25 °C for 2 months. At the end of fermentation, the wines were centrifuged at 5000 rpm for 10 min to remove the lees and subsequently analysed for the antioxidant compounds.

Liquid chromatographic analysis of antioxidant compounds

HPLC-DAD technique was used to detect simultaneously different classes of phenolic compounds. Analyses of antioxidant compounds in different wines were performed on a Knauer (Asi Advanced Scientific Instruments, Berlin) system equipped with two pumps Smartline Pump 1000, a Rheodyne injection valve (20 µL), and a photodiode array detector UV/VIS equipped with a semi micro-cell. Processing data were carried out using the *Clarity* Software (Chromatography Station for Windows). Compounds were separated on a Knauer RP C18 (250 × 4.6 mm, 5 µm particle size). The chromatographic method used was a gradient elution, using acidified water (pH 3, solvent A) with 0.1% (v/v) formic acid and acidified acetonitrile (pH 3, solvent B) with 0.1% (v/v) formic acid [50]. The gradient was used as follows: 0.01–20.00 min 5% B isocratic; 20.01–50.00 min, 5–40% B;

50.01–55.00 min, 40–95% B; and 55.01–60.00 min 95% B isocratic. The column temperature was 30 °C, and the flow rate was 1.0 mL/min. The wines were filtered through a 0.45 µm Millipore filter (GMF Whatman) before injection. The injection volume was 20 µL. Peaks were detected at 254 nm (vanillic acid, rutin, and quercetin), 280 nm (gallic acid, (+)-catechin, and epicatechin), and 305 nm (*trans*-resveratrol and caffeic acid).

Statistical analysis

All the analyses were performed in duplicate; data were subjected to statistical analysis using StatGraphics Centurion XVI for Windows XP (StatPoint Technologies, Inc., USA) according to Fisher's LSD (Least Significant Difference) ($P < 0.05$).

Results and discussion

The effect of wild strains of *S. cerevisiae* and their progeny—monosporal cultures, their hybrids, and hybrids' monosporal cultures—on the antioxidant power of red wines was investigated. Catechin and epicatechin were the principal antioxidant compounds among all the samples. In particular, the catechin content varied from 0 to 79.53 mg/L (mean 31.67), while epicatechin varied from 0 to 70.51 mg/L (mean 19.24). Regarding benzoic and hydroxybenzoic acids in general, the vanillic acid level varied from 3.10 to 12.71 mg/L (mean 8.72), while gallic and caffeic acids varied, respectively, from 2.54 to 6.77 mg/L (mean 4.82) and from 0 to 10.63 mg/L (mean 1.28). The rutin and quercetin content varied from 0 to 11.77 mg/L (mean 3.46) and from 0 to 2.09 mg/L (mean 1.62), while *trans*-resveratrol level varied from 0 to 0.85 mg/L (mean 0.27). Tables 1, 2 and 3 report, for each parameter, the values of the wild strains and, for the progeny, mean, range, and percentage of descendants, included in homogeneous groups that do not include the parental strain. As expected, the different yeast strains modified antioxidant level in wine. In fact, the different metabolic pathways of the yeast can affect the phenolic content (e.g., tyrosol, pyruvic acid, and vinylphenol) and modify their adsorption properties [22, 26].

Concerning the catechin content of the wines, the majority of the parental strains fully remove this compound; they are significantly different from the parental strains RE49, RC29, and NA15 and from many other strains. The derived strains RC29B-1C × RE78C-1C (4), RC29B-1C × RC39C-1C (7), NA14C-1D × RC39C-1C (2), [RC26C-1C × RC39C-1C (4)]-1D, and RE49B-1A × NA93B-1C (5) produce the highest values, significantly higher than their parental strains. The strains are distributed in 56 homogeneous groups.

Concerning the epicatechin content of the wines, the parental strains RE49, RE78, and NA93 fully remove this compound and they are significantly different from the parental strains NA14, NA15, RC26, RC29, and RC39 and from many other strains. Compared to the parental strains, the derived strains RC26C-1C \times RC39C-1C (9), NA15A-1B \times NA93B-1C (2), [RC29A-1D \times RC39C-1C (4)]-1A, and NA14C-1D \times RC39C-1C (3) produce the lowest values (0.00 mg/L), significantly lower than their parental strains. In addition, compared to the parental strains, the derived strains RE49B-1A \times NA93B-1C (1), RC29B-1C \times NA93B-1C (6), RC29B-1C \times RE78C-1C (4), [RC29B-1C \times RC39C-1C (7)]-3B, and RC26B-1C produce the highest values, significantly higher than their parental strains. The strains are distributed in 51 homogeneous groups. Concerning the vanillic acid content of the wines, the eight parental strains are significantly different among them and compared to the majority of the derived strains. Compared to the parental strains, the descendants NA15A-1B \times NA93B-1C (2), RC26B-1D, RE49B-1A \times NA93B-1C (1), and [RC29B-1C \times RC39C-1C (7)]-2C produce the highest values, significantly higher than their parental strains. The strains are distributed in 50 homogeneous groups.

Concerning the gallic acid content of the 71 wines, the eight parental strains are significantly different among them and compared to the majority of the derived strains. Compared to the parental strains, the derived strains NA15A-1B \times RC39C-1C (5), RC29B-1C \times NA93B-1C (6), [RC26C-1C \times RC39C-1C (4)]-1D, RC29B-1C \times RC39C-1C (7), and RE49B-1A \times RC39C-1C (8) produce the highest values, significantly higher than their parental strains. The strains are distributed in 46 homogeneous groups. Concerning the rutin content of the 71 wines, the parental strains are significantly different among them—with the exception of the strains RC26 and RC29—and from many other derived strains. Compared to the parental strains, the derived strains NA15A-1B \times NA93B-1C (2), RC26C-1C \times RC39C-1C (4), [RC29A-1D \times RC39C-1C (4)]-2C, and RC29B-1C \times RE78C-1C (4) produce the highest values, significantly higher than their parental strains. The strains are distributed in 35 homogeneous groups.

Concerning the quercetin content of the 71 wines, the parental strains have a wide distribution and show significant differences among the majority of them and many other derived strains. Compared to the parental strains, the descendants [RC29B-1C \times RC39C-1C (7)]-1B, RE49B-1A \times NA93B-1C (5), [RC29B-1C \times RC39C-1C (7)]-1A, [RC26C-1C \times RC39C-1C (9)]-1C, NA15A-1B \times NA93B-1C (2), and NA14C-1D \times RC39C-1C (2) produce the lowest values, significantly lower than their parental strains. Moreover, compared to the parental strains, the derived strains RC29B-1C \times RE78C-1C (4), RE49B-1A \times NA93B-1C (1), RC26B-1D,

and [RC29B-1C \times RC39C-1C (7)]-2C produce the highest values, significantly higher than their parental strains. The strains are distributed in 39 homogeneous groups. Concerning the caffeic acid content of the 71 wines, the majority of the strains—parental and derived strains—fully remove this compound. Compared to the parental strains, the descendants RE49B-1A \times RC39C-1C (8), [RC26C-1C \times RC39C-1C (4)]-2C, NA14C-1D \times RC39C-1C (3), RC29A-1D \times RE78C-1C (4), and [RC29B-1C \times RC39C-1C (7)]-3B produce the highest values, significantly higher than their parental strains. The strains are distributed in 18 homogeneous groups. It is interesting to note that caffeic acid levels were higher with respect to the values found in *Cabernet Sauvignon* red wines from Romania [51]. Concerning the trans-resveratrol content of the 71 wines, the parental strains have a wide distribution and show significant differences among the majority of them and many other derived strains. Compared to the parental strains, the descendants [RC29A-1D \times RC39C-1C (4)]-3A, RE49B-1A \times NA93B-1C (5), [RC26C-1C \times RC39C-1C (4)]-2A, and NA15A-1B \times RC39C-1C (5) produce the highest values, significantly higher than their parental strains. In addition, compared to the parental strains, the derived strains RC29B-1C \times RE78C-1C (4), RE49B-1A \times RC39C-1C (8), NA14C-1D \times RC39C-1C (2), NA15A-1B \times NA93B-1C (2), [RC26C-1C \times RC39C-1C (4)]-1C, RC29B-1C \times RC39C-1C (7), and RC29A-1A produce the highest values, significantly higher than their parental strains. The strains are distributed in 23 homogeneous groups. The present results agree with those reported by some authors who found polyphenol in a similar concentration or in higher content than control wines [52, 53]. Differences in our data are due exclusively to the wine starter used; this validates the main role that wine yeast selection plays to enhance antioxidant phenolic content in red wines. The strain behaviour towards the polyphenolic compounds is due to a defined cell wall composition in terms of mannoproteins and quantity of phosphate [54].

Conclusion

Since the aim of this research was to study the behaviour of different *S. cerevisiae* strains regarding their effect on *Cabernet Sauvignon* wine content in trans-resveratrol and, incidentally, also other antioxidant compounds, the best strain to use is RC29A-1A. This strain has produced a wine with the highest content in trans-resveratrol (0.85 mg/L), a low content in (+)-catechin (12.20 mg/L), a medium content in gallic acid (4.48 mg/L) and vanillic acid (7.67 mg/L), and a high content in caffeic acid (3.77 mg/L), rutin (10.45 mg/L), quercetin (1.63 mg/L), and epicatechin (60.11 mg/L). Results have shown that the strategy adopted—to get

hybrids by spore-to-spore conjugation—allows obtaining strains able to produce wine with significantly different amount of antioxidant compounds, compared to the parental strains. Consequently, the clonal selection will allow enhancing the antioxidant capacity of the wine. Moreover, it is possible to affirm that the significant differences observed are due exclusively to the wine starter used, so validating the main role that wine yeast selection can play to enhance red wine content in antioxidant phenolic compounds.

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Compliance with ethical standards

Conflict of interest None.

Compliance with ethics requirements This article does not contain any studies with human or animal subjects.

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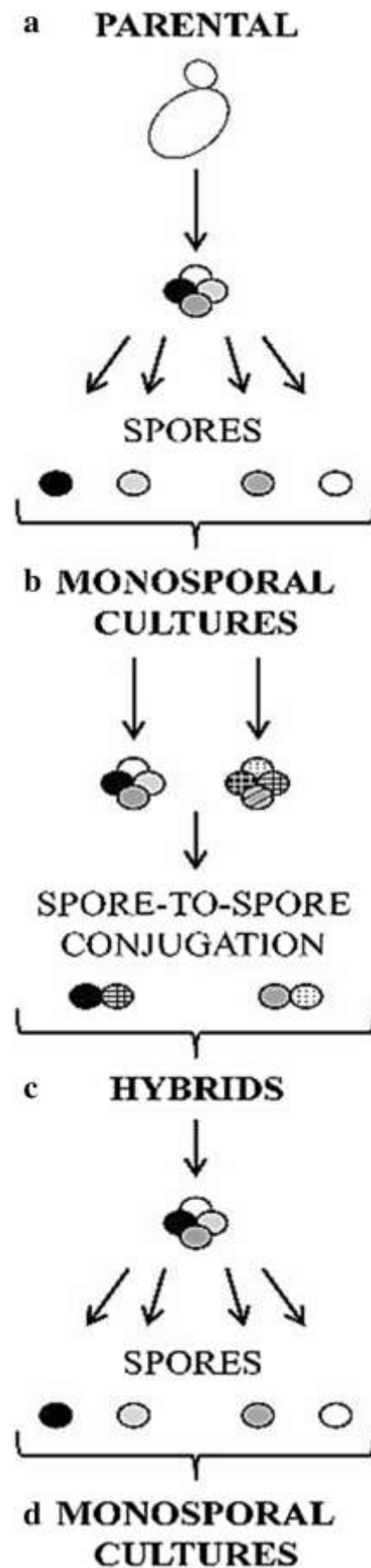


Fig. 1 Rationale of the experimental scheme to obtain yeast strains: **a** parental strain with high/low character to test, **b** monosporal culture obtained by micromanipulation of the parental strain, **c** hybrids obtained by spore-to-spore conjugation, and **d** monosporal cultures from hybrids.

Table 1 Antioxidant content (mg/L) of the wines obtained using the strains NA14, NA15, and NA93 and their progeny

Parameters (mg/L)	NA14				NA15				NA93			
	Parent	Progeny			Parent	Progeny			Parent	Progeny		
		Mean	Range	% ^a		Mean	Range	% ^a		Mean	Range	% ^a
Catechin	0.00	64.43	64.30–64.56	100.00	48.10	31.26	28.89–33.62	100.00	0.00	53.45	33.62–75.94	100.00
Epicatechin	22.69	10.58	0.00–21.15	100.00	20.56	0.00	0.00	100.00	0.00	8.10	0.00–18.22	50.00
Vanillic acid	10.04	8.73	8.71–8.75	100.00	9.16	9.73	8.76–10.69	100.00	7.42	10.78	9.21–12.28	100.00
Gallic acid	5.35	2.97	2.57–3.36	100.00	4.20	4.17	3.91–4.43	100.00	3.88	4.59	2.67–6.10	75.00
Rutin	6.70	1.26	0.00–2.51	100.00	5.58	3.67	1.65–5.69	100.00	8.07	3.38	0.00–5.69	100.00
Quercetin	1.84	1.81	1.79–1.83	50.00	1.88	1.73	1.64–1.81	100.00	2.00	1.23	0.00–1.81	100.00
Caffeic acid	0.00	4.02	0.00–8.04	50.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
<i>trans</i> -resveratrol	0.00	0.47	0.45–0.48	100.00	0.22	0.26	0.00–0.52	100.00	0.40	0.20	0.00–0.52	100.00

^aPercentage of descendants included in homogeneous groups ($p < 0.05$) according to least significant difference analysis that does not include their parental strain

Table 2 Antioxidant content (mg/L) of the wines obtained using the strains RC26, RC29, and RC39 and their progeny

Parameters (mg/L)	RC26				RC29				RC39			
	Parent	Progeny			Parent	Progeny			Parent	Progeny		
		Mean	Range	% ^a		Mean	Range	% ^a		Mean	Range	% ^a
Catechin	0.00	28.95	0.00–75.76	72.73	45.12	30.71	0.00–56.42	100.00	0.00	35.99	0.00–75.76	87.23
Epicatechin	33.36	21.96	0.00–70.51	100.00	37.81	19.67	0.00–60.89	100.00	41.44	16.63	0.00–60.89	100.00
vanillic acid	8.34	8.56	5.55–11.94	100.00	7.22	8.74	3.10–12.71	100.00	8.92	9.44	4.75–12.71	97.87
gallic acid	4.52	5.04	2.54–6.31	100.00	6.19	4.84	2.89–6.33	100.00	4.88	5.01	2.54–6.43	89.36
Rutin	6.85	2.11	0.00–9.09	100.00	6.84	3.31	0.00–11.77	100.00	5.96	1.73	0.00–10.25	100.00
Quercetin	1.64	1.65	1.15–1.91	59.09	1.86	1.59	0.00–2.09	88.89	1.56	1.62	0.00–2.09	89.36
Caffeic acid	0.00	1.38	0.00–6.07	31.82	0.00	1.52	0.00–10.63	22.22	0.00	1.59	0.00–10.63	29.79
<i>trans</i> -resveratrol	0.44	0.27	0.00–0.70	90.91	0.42	0.29	0.00–0.85	81.48	0.00	0.25	0.00–0.84	82.98

^aPercentage of descendants included in homogeneous groups ($p < 0.05$) according to least significant difference analysis that do not include their parental strain

Table 3 Antioxidant content (mg/L) of the wines obtained using the strains RE49, RE78, and their progeny

Parameters (mg/L)	RE49				RE78			
	Parent	Progeny			Parent	Progeny		
		Mean	Range	% ^a		Mean	Range	% ^a
Catechin	26.65	62.09	47.25–75.94	100.00	0.00	37.19	32.58–41.79	100.00
Epicatechin	0.00	6.87	0.00–14.17	50.00	0.00	13.28	0.00–26.55	50.00
vanillic acid	8.76	10.90	9.21–12.28	100.00	9.85	5.33	3.10–7.56	100.00
gallic acid	4.38	5.23	2.67–6.43	100.00	6.77	4.24	3.13–5.34	100.00
Rutin	9.25	1.32	0.00–3.63	100.00	7.77	9.52	7.27–11.77	100.00
Quercetin	1.75	1.27	0.00–1.81	75.00	1.16	1.76	1.72–1.79	100.00
Caffeic acid	0.00	1.28	0.00–5.11	25.00	0.00	4.31	0.00–8.61	50.00
<i>trans</i> -resveratrol	0.31	0.17	0.00–0.40	75.00	0.20	0.27	0.23–0.31	50.00

^aPercentage of descendants included in homogeneous groups ($p < 0.05$) according to least significant difference analysis that does not include their parental strain