Study of the inheritability of the yeast trait “interaction with natural antioxidant activity of red wine” in four generations of *Saccharomyces cerevisiae* and its enhancing by spore clone selection and hybridization

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Abstract The aim of this research was to study the inheritability of the yeast trait “interaction with natural antioxidant activity of red wine” and to enhance this trait by spore clone selection and hybridization of wine yeasts. This research was carried out using as a model three strains of *Saccharomyces cerevisiae* (wild types), 24 derived spore clones, three hybrids obtained crossing the derived spore clones and 34 spore clones derived from the three hybrids. Using these yeast strains, micro-winemaking trials were carried out utilizing grape must of the black varieties, *Cabernet* and *Magliocco*: the wines showed significant differences, due to the wine starter used, both for Folin–Ciocalteu’s index and for DPPH analysis. Data validate the main role that wine yeast selection plays to enhance red wine content in antioxidant compounds. Data also demonstrate that using spore clone selection and hybridization, it is possible to significantly enhance the natural antioxidant activity of wines, so improving their quality and stability.

Keywords Antioxidant activity · Red wine · *Saccharomyces cerevisiae* · Spore clone selection · Yeast hybridization · Yeast trait inheritability

Introduction

Red wine quality is primarily dependent on its phenolic content that confers colour, flavour and healthy properties. In fact, it is well recognized the contribution provided by phenolic compounds to wine sensorial characteristics [1] and also in preventing cancers [2, 3] and cardiovascular diseases [4] due to their antioxidant effect [5–7]. The phenolic content of red wine depends on a variety of factors, such as the vineyard climatic conditions and agronomic techniques [8, 9], the harvest time [10], the postharvest sunlight exposure [11] and interactions between polyphenols and other compounds under the wine yeast activity [12–16].

Wine antioxidant activity varies considerably depending on grape cultivar, environmental factors in the vineyard and wine processing techniques; also the must matrix and the oenological treatments affect the natural antioxidant activity of wines [17–19]. Several studies have recently evaluated the effect of wine yeasts on the antioxidant activity of wines [20–24].

The antioxidant activity assessment is usually based on the evaluation of the ability of an agent to induce an oxidative damage to a substrate; in the presence of antioxidant compounds, this ability is inhibited or reduced. The main elements of any test for antioxidant activity evaluation are as follows: (a) an appropriate substrate to monitor the inhibition of the oxidation, (b) an initiator of the oxidation (free radical) and (c) an appropriate measure of the oxidation endpoint [25]. Among the different methodologies proposed to assess the antioxidant activity, DPPH (2,2-diphenyl-1-picrylhydrazyl) method [26] is one of the most used to evaluate wine antioxidant activity. An essential part of the total red wines' scavenging radical activity is attributed to polymeric phenolic compounds; regarding the remaining part, anthocyanins and flavan-3-ol are the most active, followed by phenolic acids and flavonols [27]. It seems advantageous to select yeast starter cultures for winemaking considering their positive correlation with total antioxidant activity.

Despite the existence of several studies describing interaction of yeasts with phenolic compounds and antioxidant activity of wine, the knowledge of the inheritability of these characteristics remains limited. Taking into consideration all the facts mentioned above, the aim of this research was to study the inheritability of the yeast trait "interaction with natural antioxidant activity of red wine" and to enhance this trait by spore clone selection and hybridization of wine yeasts, using as a model four generations of *Saccharomyces cerevisiae*: three wild types and 61 descendants.

Materials and methods

Materials

Sixty-four strains of *S. cerevisiae* of four different generations were used. In particular: (a) three Calabrian parental strains: RC026, RC029 and RC039; (b) 24 monosporal cultures, obtained from the parental strains; (c) three hybrids obtained from monosporal cultures; and (d) 34 monosporal cultures derived from the hybrids. The strains were studied in Petri plates for the following biochemical characteristics: H_2S production on BiGGY agar at 25 °C for 48 h [28] and acetic acid production on calcium carbonate agar at 25 °C for 7 days [29]. H_2S production was expressed using an arbitrary scale from 1 to 5, according to the colour intensity of the yeast biomass, directly correlated to the H_2S production. This is one of the parameters to take into account not only because the yeasts producing a high quantity of this compound could contribute to undesirable sulphurated compounds in wine, but also—and especially—since H_2S exhibits potent antioxidant capacity [30], it may interact with the evaluation of the antioxidant activity. Acetic acid production was expressed using an arbitrary scale from 1 to 5, according to the halo around the yeast biomass, directly correlated to the acetic acid production. This is another important parameter to consider because a great amount of acetic acid in a wine is negatively correlated to its quality.

Micro-winemaking trials

Black grapes of the *Cabernet* and *Magliocco* varieties were separately destemmed, crushed, cold soaked at 0 °C for 3 days and punched down twice per day. The musts obtained after pressing (16 and 27°Brix, respectively) were adjusted to pH 3.50 (original values 3.66 and 4.18, respectively) and each lot divided in 192 test tubes (5 ml/each) and in 192 bottles (95 ml/each); after the addition of paraffin oil (10 ml/bottle), the grape musts were heated at 110 °C for 5 min and, and after cooling to room temperature, potassium metabisulfite was added to each bottle to achieve a final SO_2 concentration of 100 ppm. The 64 yeast strains were inoculated in triplicate in the 192 aliquots of 5 ml of each must and incubated at 25 °C. After 2 days, each preculture was used to inoculate the bottles. The micro-winemaking trials were carried out at 25 °C for 40 days measuring the production of CO_2—by the loss of weight—after 3, 7 and 40 days in order to monitor the fermentation. At the end of the winemaking, the wines were analysed for Folin–Ciocalteu's index, according to Singleton and Rossi [31], and for total antioxidant activity, measuring the percentage of inactivation of DPPH according to Molyneux et al. [32].

Statistics

All the analyses were performed in triplicate; data were subjected to statistical analysis using StatGraphics Centurion XVI for Windows XP (StatPoint Technologies, Inc., Warrenton, VA, USA). For each value, Fisher's LSD (least significant difference) intervals were scaled in, declaring their significant differences ($p < 0.05$).

Results and discussion

Table 1 shows for each parameter the values of the three wild types and, for the progeny, mean, range and percentage of descendants included in homogeneous groups that do not include the parental strain.

In detail, for the H_2S production, a great number of descendants are significantly different from the corresponding parental strain.

Also for the acetic acid production, a large number of descendants are significantly different from the corresponding parental strain.

For the Folin–Ciocalteu's index and for the DPPH inactivation, data are reported for each grape variety. Concerning the *Cabernet* variety, 75 and 50 % of the descendants are significantly different from the parental strain RC026 for the Folin–Ciocalteu's index and for the DPPH inactivation, respectively. Fifty per cent of the descendants are significantly different from the parental strain RC029 for both the Folin–Ciocalteu's index and the DPPH inactivation. Twenty-five per cent of the descendants are significantly different from the parental strain RC039 for both the Folin–Ciocalteu's index and the DPPH inactivation. Concerning the *Magliocco* variety, 25 and 50 % of the descendants are significantly different from the parental strain RC026 for the Folin–Ciocalteu's index and for the DPPH inactivation, respectively. Of the descendants of parental strain RC029, 12.5 and 87.5 % are significantly different for the Folin–Ciocalteu's index and for the DPPH inactivation, respectively. One hundred per cent and 37.5 % of the descendants are significantly different from the parental strain RC039 for the Folin–Ciocalteu's index and for the DPPH inactivation, respectively.

Table 2 shows for each parameter the values of the three hybrids and, for the progeny, mean, range and percentage of descendants included in homogeneous groups that do not include the hybrid strain.

In detail, for the H_2S production, the majority of the descendants are significantly different from the corresponding hybrid strain.

Also for the acetic acid production, the great majority of the descendants are significantly different from the corresponding hybrid strain.

For the Folin–Ciocalteu's index and for the DPPH inactivation, data are reported for each grape variety. Concerning the *Cabernet* variety, 87.5 and 68.75 % of the descendants are significantly different from the hybrid RC026-3CxRC039-3C for the Folin–Ciocalteu's index and for the DPPH inactivation, respectively. Of the descendants of hybrid RC029-1DxRC039-3C, 11.11 and 77.78 % are significantly different from the for the Folin–Ciocalteu's index and for the DPPH inactivation, respectively. Of the descendants of hybrid RC029-2CxRC039-3C, 44.44 and 11.11 % are significantly different for the Folin–Ciocalteu's index and for the DPPH inactivation, respectively.

Concerning the *Magliocco* variety, 6.25 and 100 % of the descendants are significantly different from the hybrid RC026-3CxRC039-3C for the Folin–Ciocalteu's index and for the DPPH inactivation, respectively. Of the descendants of hybrid RC029-1DxRC039-3C, 11.11 and 55.55 % are significantly different for the Folin–Ciocalteu's index and for the DPPH inactivation, respectively. Of the descendants of hybrid RC029-2CxRC039-3C, 33.33 and 11.11 % are significantly different for the Folin–Ciocalteu's index and for the DPPH inactivation, respectively.

These data demonstrate that using spore clone selection and hybridization it is possible to significantly enhance these yeast traits, so improving wine quality and stability.

In detail, concerning the Cabernet variety, Folin–Ciocalteu's index can be improved by at least:

• 7 %, from 1.50 (strain RC026) to 1.61 (strain RC026-3CxRC039-3C–1B);

• 5 %, from 1.48 (strain RC029) to 1.55 (strain RC029-2C x RC039-3C–3B);

• 17 %, from 1.37 (strain RC039) to 1.61(strain RC026-3CxRC039-3C–1B).

Moreover, DPPH (% of inactivation) can be improved by at least:

• 17 %, from 50.54 (strain RC026) to 59.22 % (strain RC026-3CxRC039-3C–1B);

• 14 %, from 51.64 (strain RC029) to 58.85 % (strain RC029-2C x RC039-3C–2B;

• 16 %, from 51.06 (strain RC039) to 59.22 % (strain RC026-3CxRC039-3C–1B).

Concerning the Magliocco variety, Folin–Ciocalteu's index can be improved by at least:

• 23 %, from 9.68 (strain RC026) to 11.89 (strain RC026-3CxRC039-3C–2B);

• 18 %, from 10.25 (strain RC029) to 12.16 (strain RC029-2D);

• 24 %, from 9.75 (strain RC039) to 12.16 (strain RC029-2CxRC039-3C–3A).

Moreover, DPPH (% of inactivation) can be improved by at least:

• 15 %, from 46.81 (strain RC026) to 54.15 % (strain RC026-3CxRC039-3C–1A);

• 20 %, from 44.84 (strain RC029) to 53.79 % (strain RC029-1DxRC039-3C–3B);

• 18 %, from 45.86 (strain RC039) to 54.15 % (strain RC026-3CxRC039-3C–1A).

Conclusion

In general, among all the wines produced, there are significant differences—due to the wine starter used—both for Folin–Ciocalteu's index and for DPPH value. Data validate the main role that wine yeast selection plays to enhance red wine content in antioxidant compounds.

Thus, a specific spore clone selection and hybridization allows wine starter to be obtained with enhanced ability to produce red wines with high natural antioxidant activity. This yeast trait is inheritable and stable in *S. cerevisiae* strains.

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Conflict of interest None.

Compliance with Ethics Requirements This article does not contain any studies with human or animal subjects.

References

1. Arnold RA, Noble AC, Singleton VL (1980) Bitterness and astringency of phenolic fraction in wine. J Agric Food Chem 28:675–678

2. Gali HU, Perchellet EM, Gao XM, Karchesy JJ, Perchellet JP (1994) Comparison of the inhibitory effects of monomeric, dimeric, and trimeric procyanidins on the biochemical markers of skin tumor promotion in mouse epidermis in vivo. Planta Med 60:235–239

3. Zhao J, Wang J, Chen Y, Agarwal R (1999) Anti-tumor-promoting activity of a polyphenolic fraction isolated from grape seeds in the mouse skin two-stage initiation-promotion protocol and identification of procyanidin B5-3′-gallate as the most effective antioxidant constituent. Carcinogenesis 20:1737–1745

4. Dell'Agli M, Buscialà A, Bosisio E (2004) Vascular effects of wine polyphenols. Cardiovasc Res 63:593–602

5. Rice-Evans CA, Miller NJ, Paganga G (1997) Antioxidant properties of phenolic compounds. Rev Trends Plant Sci 2:152–159

6. Netzel M, Strass G, Bitsch I, Konitz R, Christmann M, Bitcsh R (2003) Effect of grape processing on selected antioxidant phenolics in red wines. J Food Eng 56:223–228

7. Echeverry C, Ferreira M, Reyes-Parada M, Abin-Carriquiry JA, Blasina F, González-Neves G, Dajas F (2005) Changes in antioxidant capacity of Tannat red wines during early maturation. J Food Eng 69:147–154

8. Jackson DI, Lombard PB (1993) Environmental and management practices affecting grape composition and wine quality a review. Am J Enol Vitic 44:409–430

9. Reynolds AG, Price SF, Wardle DA, Watson BT (1994) Fruit environment and crop level effects on Pinot noir. I. Vine performance and fruit composition in British Columbia. Am J Enol Vitic 45:452–459

10. Bindon K, Varela C, Kennedy J, Holt H, Herderich M (2013) Relationships between harvest time and wine composition in Vitis vinifera L. cv. Cabernet Sauvignon 1. Grape and wine chemistry. Food Chem 138:1696–1705

11. Peinado J, López de Lerma N, Peralbo-Molina A, Priego-Capote F, de Castro C, McDonagh B (2013) Sunlight exposure increases the phenolic content in postharvested white grapes. An evaluation of their antioxidant activity in *Saccharomyces cerevisiae*. J Funct Foods 5:1566–1575

12. Lopez-Toledano A, Villano-Valencia D, Mayen M, Merida J, Medina M (2004) Interaction of yeasts with the products resulting from the condensation reaction between (+)-catechin and acetaldehyde. J Agric Food Chem 52:2376–2381

13. Morata A, Gómez-Cordovés MC, Calderón F, Suárez JA (2006) Effects of pH, temperature and SO_2 on the formation of pyranoanthocyanins during red wine fermentation with two species of *Saccharomyces*. Int J Food Microbiol 106:123–129

14. Morata A, Gómez-Cordovés MC, Suberviola J, Bartolomé B, Colomo B, Suárez JA (2003) Adsorption of anthocyanins by yeast cell walls during the fermentation of red wines. J Agric Food Chem 51:4084–4088

15. Medina K, Boido E, Dellacassa E, Carrau F (2005) Yeast interactions with anthocyanins during red wine fermentation. Am J Enol Vitic 56:104–109

16. Sidari R, Postorino S, Caparello A, Caridi A (2007) Evolution during the wine aging colour and tannin induced by wine starters. Ann Microbiol 57:197–201

17. Villaño D, Fernández-Pachón MS, Troncoso AM, García-Parrilla MC (2006) Influence of enological practices on the antioxidant activity of wines. Food Chem 95:394–404

18. Kostadinović S, Wilkens A, Stefova M, Ivanova V, Vojnoski B, Mirhosseini H, Winterhalter P (2012) Stilbene levels and antioxidant activity of Vranec and Merlot wines from Macedonia: effect of variety and enological practices. Food Chem 135:3003–3009

19. Comuzzo P, Battistutta F, Vendrame M, Silvina Páez M, Luisi G, Zironi R (2015) Antioxidant properties of different products and additives in white wine. Food Chem 168:107–114

20. Caridi A, Cufari A, Lovino R, Palumbo R, Tedesco I (2004) Influence of yeast on polyphenol composition of wine. Food Technol Biotechnol 42:37–40

21. Brandolini V, Fiore C, Maietti A, Tedeschi P, Romano P (2007) Influence of *Saccharomyces cerevisiae* strains on wine total antioxidant capacity evaluated by photochemiluminescence. World J Microbiol Biotechnol 23:581–586

22. Gallardo-Chacón JJ, Vichi S, Urpí P, López-Tamames E, Buxaderas S (2010) Antioxidant activity of lees cell surface during sparkling wine sur lie aging. Int J Food Microbiol 143:48–53

23. Loira I, Vejarano R, Morata A, Ricardo-da-Silva JM, Laureano O, González MC, Suárez-Lepe JA (2013) Effect of *Saccharomyces* strains on the quality of red wines aged on lees. Food Chem 139:1044–1051

24. Ivanova-Petropulos V, Ricci A, Nedelkovski D, Dimovska V, Parpinello GP, Versari A (2015) Targeted analysis of bioactive phenolic compounds and antioxidant activity of Macedonian red wines. Food Chem 171:412–420

25. Antolovich M, Prenzler PD, Patsalides E, McDonald S, Robards K (2002) Methods for testing antioxidant activity. Analyst 127:183–198

26. Lachman J, Sŭlc M, Schilla M (2007) Comparison of the total antioxidant status of Bohemian wines during the wine-making process. Food Chem 103:802–807

27. Fernández-Pachón MS, Villaño D, García-Parrilla MC, Troncoso AM (2004) Antioxidant activity of wines and relation with their polyphenolic composition. Chim Acta 513:113–118

28. Nickerson WJ (1953) Reduction of inorganic substances by yeast. I. Extracellular reduction of sulphide by species of *Candida*. J Infect Dis 93:43–48

29. Belarbi MA, Lemaresquier MH (1994) La caratterizzazione dei lieviti. Vignevini 21:57–59

30. Hamar J, Solymár M, Tanai E, Cseplo P, Springo Zs, Berta G, Debreceni B, Koller A (2012) Bioassay-comparison of the antioxidant efficacy of hydrogen sulfide and superoxide dismutase in isolated arteries and veins. Acta Physiol Hung 99:411–419

31. Singleton SL, Rossi JA (1965) Colorimetry of total phenolics with phosphomolybdic-phosphotungstic acid reagents. Am J Enol Vitic 16:144–158

32. Molyneux P (2004) The use of the stable free radical diphenylpicrylhydrazyl (DPPH) for estimating antioxidant activity. Songklanakarin J Sci Technol 26:211–219

Table 1 Phenotype of the three wild types of *Saccharomyces cerevisiae* and their progenies studied by Petri plate tests and micro-winemaking trials in *Cabernet* (C) and *Magliocco* (M) varieties

Parameters	RC026				RC029				RC039			
	Parent	Progeny			Parent	Progeny			Parent	Progeny		
		Mean	Range	%[a]		Mean	Range	%[a]		Mean	Range	%[a]
H_2S production[b]	3	3.50	3–5	37.50	3	2.10	1–3	75.00	5	4.25	4–5	75.00
Acetic acid production[c]	2	2.20	1–5	50.00	1	1.90	1–2	87.50	3	3	1–4	62.50
Folin–Ciocalteu's index in C	1.50	1.32	1.23–1.44	75.00	1.48	1.40	1.31–1.51	50.00	1.37	1.38	1.29–1.42	25.00
DPPH (% of inactivation) in C	50.54	53.13	48.11–56.89	50.00	51.64	51.70	49.00–55.38	50.00	51.06	52.92	51.53–55.19	25.00
Folin–Ciocalteu's index in M	9.68	9.98	9.44–10.56	25.00	10.25	10.82	10.07–12.16	12.50	9.75	11.04	10.76–11.39	100.00
DPPH (% of inactivation) in M	46.81	46.52	43.12–50.37	50.00	44.84	48.52	46.36–51.26	87.50	45.86	44.71	42.24–48.60	37.50

[a] Percentage of descendants included in homogeneous groups ($p < 0.05$ according to Least Significant Difference analysis) that do not include their parental strain

[b] Expressed using an arbitrary scale from 1 to 5, according to the colour intensity of the yeast biomass, directly correlated to the H_2S production

[c] Expressed using an arbitrary scale from 1 to 5, according to the halo around the yeast biomass, directly correlated to the acetic acid production

Table 2 Phenotype of the three hybrids of *Saccharomyces cerevisiae* and their progenies studied by Petri plate tests and micro-winemaking trials in *Cabernet* (C) and *Magliocco* (M) varieties

Parameters	RC026-3CxRC039-3C				RC029-1DxRC039-3C				RC029-2CxRC039-3C			
	Parent	Progeny			Parent	Progeny			Parent	Progeny		
		Mean	Range	%[a]		Mean	Range	%[a]		Mean	Range	%[a]
H_2S production[b]	4	4.06	3–5	56.25	3	3.33	2–4	55.55	4	3.67	3–5	55.55
Acetic acid production[c]	4	2.25	1–4	87.50	3	3.11	2–5	66.67	2	3.44	2–5	66.67
Folin–Ciocalteu's index in C	1.32	1.42	1.12–1.61	87.50	1.37	1.38	1.32–1.47	11.11	1.39	1.38	1.24–1.55	44.44
DPPH (% of inactivation) in C	56.45	53.96	49.81–59.22	68.75	56.75	53.73	45.98–58.53	77.78	57.78	56.71	53.34–58.85	11.11
Folin–Ciocalteu's index in M	10.81	10.70	10.01–11.89	6.25	10.76	10.48	9.83–10.88	11.11	11.12	11.24	10.32–12.16	33.33
DPPH (% of inactivation) in M	40.43	48.68	43.69–54.15	100.00	43.96	47.50	43.49–53.79	55.55	41.84	42.91	41.15–44.87	11.11

[a] Percentage of descendants included in homogeneous groups ($p < 0.05$ according to Least Significant Difference analysis) that do not include their parental strain

[b] Expressed using an arbitrary scale from 1 to 5, according to the colour intensity of the yeast biomass, directly correlated to the H_2S production

[c] Expressed using an arbitrary scale from 1 to 5, according to the halo around the yeast biomass, directly correlated to the acetic acid production