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Influence of Yeast on Polyphenol Composition of Wine**

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Summary

Two strains of *Saccharomyces cerevisiae* were employed for winemaking of must from red grapes. Twenty-two parameters were determined in the red wines produced. Very significant ($p<0.01$) differences were observed for colour intensity, total polyphenols, and non-anthocyanic flavonoids. Moreover, significant ($p<0.05$) differences were observed for colour and monomeric anthocyanins.

Key words: fermentation, phenolics, red wine, wine yeast

Introduction

In the production of red wine, the type and quantity of phenolics play a major role in the quality of wine. Anthocyanins, flavonols, catechins and other flavonoids contribute to the sensory characteristics of wine, particularly colour and astringency; in addition, they possess a wide range of antioxidant and pharmacological effects.

Phenolics vary notably according to several parameters (*1,2*) such as the grape variety (*3*), the maceration temperature (*4*), the length of grape pomace contact (*5*) and other vinification conditions (*6–8*). During ageing, phenolics evolve and monomeric anthocyanins polymerize by reaction with other flavonoid compounds and aldehydes (*9*).

It is well known that wine yeasts are among the causes that decrease the phenolic content of wines (*10*). This mechanism can be exclusively physical, involving the establishment of weak and reversible interactions mainly between anthocyanins and yeast walls by absorption (*11*). Various yeast metabolites, such as pyruvic acid (*12*) and acetaldehyde (*13,14*), were shown to react with different classes of phenolics, suggesting that they offer an important way of stabilizing pigments during the maturation and ageing of wine. Furthermore, an enzymatic hydrolysis involving a yeast periplasmic anthocyanin-β-D-glucosidase, followed by a decolourizing activity connected to the loss of A_{520nm}, was described (*15*). Heterologous expression of an anthocyanin-β-D-glucosidase in a wine yeast strain was performed, but without obtaining wines with different physicochemical characteristics (*16*). The effect of four strains of *Saccharomyces cerevisiae* on phenolic glycosides was evaluated during fermentation and during ageing of yeast for 40 months,

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but no differences among strains were observed (*17*). The influence of yeast used for winemaking on phenolics is still poorly understood; so we decided to verify if different wine yeasts can somewhat modify chromatic properties, phenolic profile and antioxidant power of wine.

In this paper, we have analyzed the wines produced using two different wine yeasts and Gaglioppo must from red grapes. This variety was employed because it has limited content of anthocyanins; so Gaglioppo wines are very susceptible to browning and, when elaborated for young wines, they lose their vivid colour rapidly.

Material and Methods

Microorganisms

The work was performed using two different strains of *Saccharomyces cerevisiae*, Sc2659 and Sc1483; these wine yeasts belong to the Department STAFA collection (Reggio Calabria, Italia).

Winemaking test

About 1600 kg of Gaglioppo grapes (*Vitis vinifera* L.), cultivated in Cirò Marina (South of Italy), were harvested at optimum maturity and transported to the winery. Grapes were processed using a mechanical crusher/destemmer. Crushed grapes were subdivided into four 400-kg lots and dispensed into stainless steel vessels of 600 L to provide two replicates of the two wine yeasts used. Then, the must was treated with SO_2 (50 mg/kg) and inoculated with 5 % of 48-h yeast precultures. The contact with grape pomace was prolonged for the complete fermentation period of 18 days; fermentation caps were punched down six times the first day, five times the second day, four times the third day, three times the fourth day, twice the fifth, sixth and seventh days, and once the following days. Fermentations were monitored by determining the increase of temperature and the decrease of °Brix. At the end of fermentation the grape pomace was pressed and, after spontaneous sedimentation, the wines were racked, bottled and immediately analyzed.

Wine analysis

On the whole, 22 parameters were determined in the wines obtained with the two yeasts. Acetic acid, succinic acid and glycerol were analyzed using specific Boehringer kits on diluted samples. The chromatic properties of the wines were determined according to the EEC methods (*18*) and expressed as follows: the colour was given by the value of A_{520nm}, the colour intensity was given by the sum of the A_{420nm}, A_{520nm}, and A_{620nm}, the tint was expressed by the ratio of the A_{420nm} and A_{520nm}. The total polyphenol content was determined using the Folin-Ciocalteu reagent according to Singleton and Rossi (*19*). The non-flavonoid compounds were determined according to Kramling and Singleton (*20*). The flavonoid compound content was calculated by difference between the total polyphenols and the non-flavonoid compounds. Values were expressed as concentration (μM) of gallic acid equivalent. In order to determine *in vitro* antioxidant properties of red wines, a colourimetric method based on the reduction of a ferric tripyridyl-s-triazine complex to its ferrous form, namely FRAP (ferric reducing antioxidant power) assay (*21*), was employed. A volume of 10 μL of wine (diluted in ratio 1:10 with ethanol 10 %) was added to the volume of 1 mL of FRAP assay solution, prepared by mixing 25 mL of 300 mM acetate buffer (pH=3.6), 2.5 mL of 2,4,6-tripyridyl-s-triazine (TPTZ) solution (10 mM TPTZ in 40 mM HCl) and 2.5 mL of 20 mM $FeCl_3 \cdot 6H_2O$. Antioxidant power was calculated as difference in the A_{593nm} after 0 and 6 min. The values were correlated to a standard curve made with a pure solution of quercetin. All the other parameters were determined using standard methods (*22,23*). Analyses were performed in triplicate for each sample and data were subjected to mathematical and statistical analysis.

Results and Discussion

The grape must had the following characteristics: 23.6 °Brix, 7.31 g/L titratable acidity, pH=3.63. During the 18 days of winemaking the temperature oscillated from a minimum of 20 °C to a maximum of 24 °C, with similar profiles for all the four vessels. The decrease of the °Brix was somewhat faster for the two vessels inoculated with strain Sc2659, according to its higher fermentation rate. The physicochemical parameters of the red wines produced with the two wine yeasts are reported in Table 1; strain Sc2659 would be preferable compared to strain Sc1483 because it produced significantly higher levels of ethanol ($p<0.01$) and lower levels of acetic acid ($p<0.05$). Significantly higher ($p<0.01$) differences in total dry extract clearly depend on the different content in residual reducing sugars ($p<0.01$); effectively, a comparison between the sugar-free extracts, *i.e.* the difference between the total dry extract and the total sugars, did not show significant differences.

The chromatic properties, the phenolic profile, and the antioxidant power of the red wines produced with the two wine yeasts are reported in Table 2. Strain Sc2659, compared to strain Sc1483, produced a wine with significantly higher values of colour, colour intensity, total polyphenols and monomeric anthocyanins. Also, the content of flavonoids, total anthocyanins, flavans and proanthocyanidins was higher in the wine produced by strain Sc2659, but the differences from the strain Sc1483 were not significant. The levels of non-anthocyanic flavonoids were significantly lower. Therefore, strain Sc2659 protects, during winemaking, the phenolics and, above all, the anthocyanins of the must better than strain Sc1483. Obviously, colour and colour intensity are also notably influenced by this behaviour, because anthocyanins are the major components responsible for red wine colour (*24*). In the wine produced with strain Sc2659 the monomeric anthocyanins and flavans are higher: this probably indicates low absorption of these classes of phenolics on the yeast walls, which probably delayed the polymerization process. This effect could be very useful in winemaking of grape must with low levels of anthocyanins and high percentages of a few stable di-substituted anthocyanins, such as the Gaglioppo variety (*25*). Low level of non-anthocyanic flavonoids and, at the same time, high content of

Table 1. Physicochemical parameters in red wines obtained with two different wine yeasts

Parameters	Sc2659			Sc1483		
	Mean		SD	Mean		SD
pH	3.74		0.01	3.69		0.03
φ(ethanol)/%	14.20	A	0.14	12.89	B	0.09
γ(reducing sugars)/(g/L)	8.5	B	0.21	23.0	A	0.85
γ(total dry extract)/(g/L)	45.0	B	0.07	59.7	A	1.13
γ(sugar-free extract)/(g/L)	37.5		0.14	37.7		0.28
γ(titratable acidity)/(g/L)	6.51		0.01	6.60		0.04
γ(total SO_2)/(mg/L)	15		1.41	11		1.41
γ(acetic acid)/(g/L)	0.37	b	0.04	0.59	a	0.01
γ(succinic acid)/(g/L)	0.44		0.12	0.64		0.15
γ(glycerol)/(g/L)	7.05		0.33	7.50		0.08

A, B: $p<0.01$; a, b: $p<0.05$ indicate the significance of the comparison in the same row

Table 2. Chromatic properties, phenolic profile, and antioxidant power in red wines obtained with two different wine yeasts

Parameters	Sc2659			Sc1483		
	Mean		SD	Mean		SD
Colour	2.49	a	0.05	1.95	b	0.08
Tint	1.03		0.06	1.14		0.09
Colour intensity	5.76	A	0.08	4.67	B	0.10
γ(total polyphenols)/(mg/L)	3900	A	96.87	3156	B	28.28
c(flavonoids equiv. to gallic acid)/μM	22.40		0.71	16.30		2.26
c(non-flavonoids equiv. to gallic acid)/μM	0.57		0.01	0.60		0.01
γ(non-anthocyanic flavonoids)/(mg/L)	1419	B	7.78	2059	A	87.68
γ(total anthocyanins)/(mg/L)	80.2		2.40	74.0		1.13
γ(monomeric anthocyanins)/(mg/L)	23.4	a	1.48	16.3	b	0.28
γ(flavans)/(mg/L)	2625		219.20	2171		34.65
γ(proanthocyanidins)/(mg/L)	4367		236.17	3816		357.09
c(FRAP value equiv. to quercetin)/μM	6.00		0.42	4.13		0.67

A, B: $p<0.01$; a, b: $p<0.05$ indicate the significance of the comparison in the same row

proanthocyanidins could produce a more astringent wine. Interestingly, the strain 1483, which fermented at a slower rate, gave lower phenolic protection; Mazza *et al*. (*24*) found a similar behaviour for strain Wädenswil 27 used to ferment Pinot noir must, however, they concluded that yeast used for fermentation had minimal effect.

The antioxidant properties of wines are usually investigated according to Rice-Evans and Miller (*26*); nevertheless, there are many methods to determine antioxidant capacity (*27*). Methods differ in terms of the assay principles and experimental conditions; consequently, in different methods specific antioxidants have varying contributions to total antioxidant potential (*28*). Previous works reported that FRAP assay is a valid method to determine the antioxidant properties of red wine (*29*,*30*). Therefore, we used this method to measure the antioxidant activity of red wines obtained with the two different strains. As indicated in Table 2, wine obtained with strain Sc2659 showed the highest FRAP value; the antioxidant effects appear to depend on the concentration of flavonoids but not on simple phenolic compounds, according to a previous work (*31*).

These preliminary results showed interesting correlations between yeast strain used for winemaking and phenolic composition of wine, elucidating that strain behaviour can somewhat modify chromatic properties, phenolic profile and antioxidant power of wine.

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Utjecaj sojeva kvasca na sastav polifenola u vinu

Sažetak

Za proizvodnju vina iz mošta dobivenog od crvenoga grožđa upotrijebljena su dva soja *Saccharomyces cerevisiae*. U proizvedenim crvenim vinima određena su 22 fizikalno-kemijska i polifenolna parametra. Od polifenolnih parametara vrlo značajne razlike ($p<0,01$) opažene su u intenzitetu boje, ukupnim polifenolima i neantocijanskim flavonoidima. Nadalje, značajne razlike ($p<0,05$) uočene su za boju i monomerne antocijanine.