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Evolution during wine aging of colour and tannin differences induced by wine starters

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Abstract - The ability of three *Saccharomyces cerevisiae* strains, with different colour adsorption aptitude, to induce and maintain colour differences in wines obtained from the Calabrian Gaglioppo and Magliocco black grape varieties was studied during one year of aging. The evolution of wine tannin content was also considered. Total polyphenols, colour parameters and total tannin values exhibited, both for Gaglioppo and Magliocco wines, significant ($P < 0.05$) or highly significant ($P < 0.01$) differences among strains. It is interesting to note that yeasts appear to exhibit a different adsorption aptitude for anthocyanins and tannins. The strain that gave wine with high values for the colour parameters was not the same as the one that produced wine with high values of tannins. The obtained results suggest that the choice of yeast strain in winemaking affects, in a significant way, the phenolic composition of wines with direct consequences on their colour and tannin content. Moreover, the interaction between grape cultivar and yeast is close and important, because grape variety, due to its phenolic composition, modulates yeast strain adsorption activity.

Key words: grape variety; tannins; wine aging; wine colour; wine yeast.

INTRODUCTION

Phenolic compounds play an important role in red wine sensory characteristics, particularly concerning colour and taste (Somers, 1971; Arnold *et al.*, 1980). Wine colour is determined mainly by the content and composition of anthocyanin in black grapes and is an important quality parameter. Anthocyanins also determine wine mouthfeel and astringency, as does tannin content and composition. Both of these phenolic compounds are modified during aging; tannin polymerisation and anthocyanin and tannin condensation take place, thus determining production of phenolic compounds of higher molecular weight. This phenomenon stabilizes wine colour (Ribéreau-Gayon *et al.*, 1998; Pérez-Lamela *et al.*, 2007), improves sensory characteristics and increases antioxidant effects (Rice-Evans *et al.*, 1997; Netzel *et al.*, 2003).

Anthocyanin and tannin profiles are peculiar attributes of each grape variety and determine, together with other factors, the production of wine with different sensory characteristics and evolution during aging.

Wine yeasts play an important role in wine colour, since they are able to affect it in different ways (Castino, 1982; Conterno *et al.*, 1997). They can influence wine colour tint and intensity during winemaking by different mechanisms: anthocyanin adsorption on cell walls (Vasserot *et al.*, 1997); enzymatic hydrolysis of anthocyanin-3-O-glucosides (Delcroix *et al.*, 1994; Ortega *et al.*, 2003); SO_2 production (Fornachon, 1968; Eschenbruch, 1974); accumulation of secondary metabolites such as pyruvic acid and acetaldehyde (Dallas *et al.*, 1996; Fulcrand *et al.*, 1998); protection against browning (Merida *et al.*, 2005); combination between anthocyanins and mannoproteins (Ferrari *et al.*, 1997; Escot *et al.*, 2001; Caridi, 2006).

The yeast adsorption activity during alcoholic fermentation has important consequences for wine quality because of the amount of pigment removed (Mazauric and Salmon, 2005). Therefore, a simple and quick Petri plate method was developed that permits yeast biomass colour to be evaluated (Caridi *et al.*, 2002). Moreover, the enological trait wine colour adsorption (WCA) was recently defined as an inheritable and polygenic character in yeasts (Caridi *et al.*, 2007).

Significant differences in colour, polyphenolic index and anthocyanins have been reported for wines fermented with different yeast strains (Cuinier 1988; Dumont *et al.*, 1993; Morata *et al.*, 2003a; Caridi *et al.*, 2004; Medina *et al.*, 2005; Morata *et al.*, 2005). However, other authors have hypothesized that anthocyanin derivatives are not directly correlated to yeast adsorption activity (Eglinton *et al.*, 2004) and that wine colour modifications depend on the time of wine aging (Hernández *et al.*, 2006).

So far, only two papers have described the yeast's ability to adsorb tannins on cell walls and their preference towards polar condensed tannins (Mazauric and Salmon, 2005; Salmon, 2006).

The aim of the present work was to validate the role played by selected wine yeasts in the evolution of colour and tannin differences during wine aging.

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MATERIALS AND METHODS

Microorganisms. The study was carried out using three strains of *Saccharomyces cerevisiae* - Sc226, Sc2489, and Sc2717 - belonging to our yeast collection. They were selected for their main enological traits, in particular, for biomass colour on a medium made with grape skins: strains Sc226 and Sc2717 are characterised by low anthocyanin adsorption, while strain Sc2489 is characterised by medium anthocyanin adsorption (Rizzo *et al.*, 2006).

Grape varieties and winemaking trials. The Calabrian Gaglioppo and Magliocco black grape (*Vitis vinifera L.*) varieties, respectively characterised by low and high content in phenolic compounds were used. In autumn 2005, Gaglioppo winemaking was performed at the Malaspina winery (Melito Porto Salvo, RC, Italy) and Magliocco winemaking at the Caparello winery (Lametia Terme, CZ, Italy). Using the conventional technology for red winemaking, grapes were harvested at optimal maturity, destemmed and crushed. After the addition of potassium metabisulphite, to achieve an SO_2 concentration of 23 ppm (Gaglioppo) and 15 ppm (Magliocco), each must was divided in three lots and inoculated with 5% of 48-h precultures of the three strains. Fermentation caps were punched down three times per day. After seven (Gaglioppo) and five (Magliocco) days of maceration, samples were pressed, separated from grape pomace and barrelled to complete alcoholic fermentation. In all cases, the malolactic fermentation was spontaneous and fully finished, which was facilitated by the low levels of SO_2 used. The wines were subsequently bottled and stored in the dark.

Wine analyses. Ethanol production was determined at the end of winemaking using standard methods (Ough and Amerine, 1988).

Colour parameters were determined after four, eight and 12 months of wine aging. Wines were centrifuged at 4500 rpm for 5 min and diluted 1:2 (v/v) and 1:5 (v/v) for Gaglioppo and Magliocco cultivars, respectively, with a pH 3.5 buffer (citric acid monohydrate 0.1 M, Na_2HPO_4 0.2 M). The absorbance was read using an Anadeo1 spectrophotometer (Bibby Sterilin Ltd). The colour intensity was given by the sum of the absorbance at 420, 520, and 620 nm; the colour tint was expressed by the ratio of the absorbance at 420 nm and 520 nm.

The total polyphenol content was determined after four months of wine aging using the Folin-Ciocalteu Index according to Singleton and Rossi (1965).

The determination of wine tannins, expressed as g/L of total tannins, was performed at the end of fermentation and after 12 months of wine aging using a Lambda2 spectrophotometer (Perkin-Elmer). Two solutions containing 50 µL of wine, 3 mL of distilled water and 3 mL of chloridric acid 12 N were prepared. One solution was treated at 100 °C for 30 min while the other was left at room temperature. Five hundred µL of 95% ethanol were added to each solution and the absorbance was read at 470, 520, and 570 nm (Ribéreau-Gayon *et al.*, 1998).

All the analyses were performed in triplicate; data were subjected to statistical analysis using StatGraphics Centurion XV for Windows XP from StatPoint.

RESULTS AND DISCUSSION

Wines obtained from the Gaglioppo cultivar showed lower ethanol content than those from the Magliocco cultivar. The former wines showed an ethanol content of 12.60% (v/v) for strain Sc226 and 13.20% (v/v) for strains Sc2489 and Sc2717; the latter wines exhibited an ethanol content of 14.60, 14.80 and 13.90% (v/v) for strains Sc226, Sc2489 and Sc2717, respectively.

Table 1 shows mean values, standard deviations and significant differences of the colour parameters during aging of Gaglioppo wines obtained with the three different yeast strains.

Strains Sc2717 and Sc2489 produced wines with higher values of colour parameters than those produced with strain Sc226; moreover, during aging these differences were maintained. The 420 nm parameter increased during wine aging for all the strains; however, strain Sc226 exhibited the lowest values and, consequently, seems to be able to protect wine against an increase in yellow tones. The 520 nm and 620 nm parameters also increased during aging; strains Sc2489 and Sc2717 gave higher values, showing the ability to protect the red and blue tones of wines.

Table 2 shows mean values, standard deviations and significant differences of the colour parameters during aging of Magliocco wines obtained with the three different yeast strains.

After four months of wine aging, strain Sc2489 produced wine with the highest values of colour parameters, whereas after eight and 12 months of aging, strain Sc226 exhibited the highest values. Therefore, this second strain showed the best ability to protect Magliocco colour during wine aging. The 420 nm parameter decreased during aging for all the wines, reporting the lowest value for strain Sc2717. The 520 nm parameter decreased for all the wines, showing the highest value for strain Sc226. The 620 nm parameter decreased in the same way for all the strains.

Overall, Magliocco wines exhibited higher colour intensity values than Gaglioppo wines, due to the cultivar characteristics. Moreover, while strain Sc226 maintained the highest value of colour intensity for Magliocco wine after 12 months of aging, a similar activity was observed with strains Sc2489 and Sc2717 for Gaglioppo after 12 months of aging. For both Gaglioppo and Magliocco wines, significant ($P < 0.05$) or highly significant ($P < 0.01$) differences among strains were found. Gaglioppo wines (Table 1) maintained significant or highly significant differences among strains for the different colour parameters until the end of the trial. Similarly, Magliocco wines (Table 2) maintained significant or highly significant differences among strains only until eight months of aging; after 12 months of aging significant differences were limited to 520 nm, intensity and tint parameters. It is interesting to note that the data highlight a different behaviour of the three yeast strains, based on the grape variety; the strain that determined a more strongly coloured wine was not the same for the two grape varieties considered. This observation suggests that the grape variety, due to its phenolic composition, modulates yeast strain adsorption activity.

Table 3 reports, for Gaglioppo and Magliocco varieties, mean values, standard deviations and significant differences of the Folin-Ciocalteu Index and total tannins measured after four and twelve months of wine aging, respectively.

TABLE 1 - Mean values, standard deviations and significant differences among yeast strains regarding Gaglioppo colour parameters during wine aging

Time (months)	Strain	Colour parameters				
		420 nm	520 nm	620 nm	Intensity	Tint
4	Sc226	1.68 ± 0.03[a]	1.34 ± 0.02[a]	0.38 ± 0.03	3.40 ± 0.06[a]	1.26 ± 0.03[b]
	Sc2489	2.03 ± 0.03[b]	1.73 ± 0.02[b]	0.44 ± 0.03	4.20 ± 0.08[b]	1.18 ± 0.01[a]
	Sc2717	2.03 ± 0.01[b]	1.73 ± 0.02[b]	0.44 ± 0.03	4.20 ± 0.04[b]	1.18 ± 0.02[a]
8	Sc226	1.93 ± 0.00[a]	1.67 ± 0.01[A]	0.36 ± 0.00[a]	3.97 ± 0.00[A]	1.15 ± 0.01[b]
	Sc2489	2.24 ± 0.02[b]	2.08 ± 0.01[C]	0.39 ± 0.01[b]	4.71 ± 0.02[C]	1.08 ± 0.00[a]
	Sc2717	1.92 ± 0.02[a]	1.79 ± 0.00[B]	0.37 ± 0.01[ab]	4.08 ± 0.01[B]	1.08 ± 0.01[a]
12	Sc226	2.04 ± 0.00[A]	1.54 ± 0.00[A]	0.38 ± 0.00[a]	3.96 ± 0.01[A]	1.32 ± 0.00[C]
	Sc2489	2.39 ± 0.01[B]	1.85 ± 0.02[B]	0.41 ± 0.02[b]	4.65 ± 0.05[B]	1.29 ± 0.00[B]
	Sc2717	2.47 ± 0.01[C]	1.95 ± 0.01[C]	0.44 ± 0.01[c]	4.85 ± 0.03[C]	1.27 ± 0.00[A]

Significant ($P < 0.05$) or highly significant ($P < 0.01$) differences according to LSD are indicated, in the same column, by small and capital letters, respectively.

TABLE 2 - Mean values, standard deviations and significant differences among yeast strains regarding Magliocco colour parameters during wine aging

Time (months)	Strain	Colour parameters				
		420 nm	520 nm	620 nm	Intensity	Tint
4	Sc226	2.47 ± 0.03[B]	3.73 ± 0.08[b]	0.74 ± 0.07	6.94 ± 0.16[b]	0.66 ± 0.01[a]
	Sc2489	2.64 ± 0.04[C]	3.75 ± 0.04[b]	0.82 ± 0.06	7.21 ± 0.12[b]	0.70 ± 0.01[b]
	Sc2717	2.29 ± 0.05[A]	3.36 ± 0.03[a]	0.68 ± 0.09	6.33 ± 0.16[a]	0.68 ± 0.01[ab]
8	Sc226	2.29 ± 0.02[b]	3.62 ± 0.02[C]	0.73 ± 0.02[c]	6.63 ± 0.05[b]	0.63 ± 0.00[a]
	Sc2489	2.18 ± 0.04[a]	3.26 ± 0.03[A]	0.61 ± 0.02[a]	6.05 ± 0.07[a]	0.67 ± 0.01[b]
	Sc2717	2.16 ± 0.02[a]	3.35 ± 0.02[B]	0.67 ± 0.04[b]	6.18 ± 0.07[a]	0.64 ± 0.00[a]
12	Sc226	2.33 ± 0.00	3.10 ± 0.02[b]	0.67 ± 0.01	6.10 ± 0.01[b]	0.75 ± 0.01[a]
	Sc2489	2.31 ± 0.06	2.89 ± 0.05[a]	0.65 ± 0.03	5.84 ± 0.14[a]	0.80 ± 0.01[c]
	Sc2717	2.24 ± 0.04	2.91 ± 0.02[a]	0.64 ± 0.02	5.78 ± 0.07[a]	0.77 ± 0.01[b]

Significant ($P < 0.05$) or highly significant ($P < 0.01$) differences according to LSD are indicated, in the same column, by small and capital letters, respectively.

The highest values of the Folin-Ciocalteu Index were determined by strains Sc2717 and Sc2489 in Gaglioppo and Magliocco wine, respectively. All strains produced different polyphenol content and their behaviour was clearly related to the grape variety. Significant differences were observed for all the strains.

At the end of fermentation the tannin content of the Magliocco wines was 1.17, 0.97 and 1.07 for strains Sc226, Sc2489 and Sc2717, respectively. For Gaglioppo wines, the tannin content was 1.85, 1.36 and 1.33, respectively. It is evident that after 12 months of aging (Table 3) there was a strong increase of the tannin content values, due to polymerisation and condensation reactions. For Gaglioppo wines, strain Sc2717 showed the highest value of total tannins followed by strains Sc226 and Sc2489, while for Magliocco wines, strain Sc226 showed the highest value of total tannins followed by strains Sc2489 and Sc2717. Significant differences were observed between strains for Magliocco wines, but not for Gaglioppo.

It is interesting to note that yeasts appear to exhibit a different adsorption aptitude between anthocyanins and tannins. The strain that gave wine with high values for the colour parameters was not the same as the one that produced wine with high values of tannins.

Although these analytical characteristics may be affected by spontaneous malolactic fermentation, in our opinion, this is improbable because, taking into account the important effects of the interaction, ranging from inhibitory to stimulatory, between the yeast strains used to conduct alcoholic fermentation and lactic acid bacteria (Alexandre *et al.*, 2004), it is possible to affirm that the tested yeast strains strongly influenced the proceeding of the malolactic fermentation in different ways.

It is well known that colour and phenolic content of wines are influenced by yeasts, not only by their WCA trait, but also by a variety of factors, as previously stated, that can be present in each strain to a different level, and independent of their WCA trait. Consequently, it is possible to

TABLE 3 - Mean values, standard deviations and significant differences of the Folin-Ciocalteu Index and total tannins measured after four and 12 months of aging, respectively, in Gaglioppo and Magliocco wines

Yeast	Folin-Ciocalteu Index (mg/L)		Total tannins (g/L)	
	Gaglioppo	Magliocco	Gaglioppo	Magliocco
Sc226	60.33 ± 0.55[a]	36.17 ± 0.42[ab]	3.68 ± 0.15[a]	2.00 ± 0.08[b]
Sc2489	66.13 ± 0.21[b]	36.27 ± 0.57[b]	3.51 ± 0.54[a]	1.88 ± 0.09[ab]
Sc2717	67.07 ± 1.42[b]	35.30 ± 0.36[a]	3.73 ± 0.40[a]	1.78 ± 0.08[a]

Significant ($P < 0.05$) differences according to LSD are indicated, in the same column, by small letters.

find two strains with a very similar WCA aptitude but producing different colour and phenolic content, and vice versa (Caridi *et al.*, 2007).

Wines of each variety obtained with the three yeast strains had diverse chromatic profiles, total polyphenol and tannin contents, showing a different evolution of these parameters. It is important to notice that these differences were exclusively due to the yeast strain used, since this was the only variable in the present experimental design.

These results allow us to assert that the interaction between grape cultivar and yeast is closer and more important than was believed until now. Therefore, our findings confirm that the choice of yeast strain in winemaking significantly affects the phenolic composition of wines with direct consequences on their colour and tannin content.

It is, therefore, possible to propose the selection of yeast strains able to fully exploit grape cultivar characteristics.

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