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Effect of yeast strain and some nutritional factors on tannin composition and potential astringency of model wines

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ABSTRACT

Nine *Saccharomyces cerevisiae* cultures, isolated from different sources, were tested for their ability to reduce tannins reactive towards salivary proteins, and potentially responsible for wine astringency. Strains were preliminary genetically characterized and evaluated for physiological features of technological interest. Laboratory-scale fermentations were performed in three synthetic media: CT) containing enological grape tannin; CTP) CT supplemented with organic nitrogen sources; CTPV) CTP supplemented with vitamins. Adsorption of total tannins, tannins reactive towards salivary proteins, yellow pigments, phenolics having antioxidant activity, and total phenols, characterizing the enological tannin, was determined by spectrophotometric methods after fermentation. The presence of vitamins and peptones in musts greatly influenced the adsorption of tannins reactive towards salivary proteins (4.24 g/L gallic acid equivalent), thus promoting the reduction of the potential astringency of model wines. With reference to the different phenolic classes, yeast strains showed different adsorption abilities. From a technological point of view, the yeast choice proved to be crucial in determining changes in gustative and mouthfeel profile of red wines and may assist winemakers to modulate colour and astringency of wine.

1. Introduction

Wine flavour is the result of many factors concerning both grape (variety, soil and climate, vineyard management, ecology) and processes (fermentations and winemaking practices) (Noble, 1994). During the alcoholic fermentation, yeasts perform the biotransformation of grape derivates into wine compounds, by converting sugars into ethanol and other metabolites as well as into a wide range of volatile and non-volatile end products that significantly contribute to the sensory properties of the wine (Fleet, 2003). The management of yeast metabolic activities during alcoholic fermentation can help winemakers to creatively engineer wine character (Fleet, 2008) according to their own wine project.

During red wine production, the maceration process is generally concomitant with the alcoholic fermentation and the extraction of compounds responsible for wine antioxidant activity, colour, taste and mouthfeel from the solid parts of the grape occurs in this stage. In particular, the extraction of monomeric, oligomeric and polymeric flavan-3-ols (condensed tannins) from skins and seeds plays a relevant role to define the sensory characteristics of red wines, by contributing to wine bitterness and astringency (Vidal et al., 2003; Cheynier et al., 2006). Astringency is a tactile sensation mainly due to the interactions of tannins with salivary proteins: the formation of complexes, followed by precipitation, leads to a reduction of the lubricating properties of saliva (Breslin et al., 1993). Consequently, sensations of dryness, hardness, and constriction are felt in the mouth (Lee and Lawless, 1991).

Many studies have been focused on the yeast role to influence the concentration and the composition of wine phenolic compounds (Caridi et al., 2004), above all by adsorption on cell wall. In particular, yeasts may retain anthocyanins (Morata et al., 2003), reduce total phenolics (Boisson et al., 2002) and modify the antioxidant capacity of wine (Brandolini et al., 2007). Few studies were carried out on the

Abbreviations: GAE, Gallic Acid Equivalent; EGT, Enological Grape Tannin; PCA, Principal Component Analysis; SDS-PAGE, Sodium Dodecyl Sulfate Gel Electrophoresis; SPI, Saliva Precipitation Index; T_{RSP}, Tannins Reactive Towards Salivary Proteins; YCC-DA, Yeast Culture Collection of Department of Agriculture.

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adsorption of tannins on yeasts, especially on living cells during fermentation. Yeast lees were found to deplete condensed tannins from wine during a simulation of wine aging (Mazauric and Salmon, 2005). Carew et al. (2013) evaluated tannins concentration and composition in Pinot noir at bottling as well as after 8 months of ageing, proving the yeast impact on the wine tannin profile.

Although the yeasts influence on chromatic properties (Cuinier, 1988), phenolic profile and antioxidant power of wine (Caridi et al., 2004) has been pointed out, the effect on tannins reactivity towards salivary proteins has not yet been established. In the present work, nine *Saccharomyces* (*S.*) *cerevisiae* strains were tested for their ability to reduce tannins reactive towards salivary proteins (T_{RSP}), which are responsible for wine potential astringency. Laboratory-scale alcoholic fermentations were carried out in synthetic musts containing 3 g/L of enological grape tannin (EGT), and supplemented with organic nitrogen sources and/or vitamins.

Wine potential astringency was evaluated by a method based on the Sodium Dodecyl Sulfate Polyacrylamide Gel Electrophoresis (SDS-PAGE) of salivary proteins after the interaction of saliva with wine (Saliva Precipitation Index, SPI) (Rinaldi et al., 2012). In addition, adsorption of tannins, yellow pigments, phenolics having antioxidant activity, and total phenols of model wines were determined by spectrophotometric methods.

The aim of this work is to evaluate the ability of yeasts in reducing phenolics after fermentation, in particular tannins responsible for astringency, and to state if media nutritional factors can influence this phenomenon.

2. Material and methods

2.1. Yeasts

2.1.1. Yeast strains and culture conditions

Nine *Saccharomyces cerevisiae* strains belonging to the Yeast Culture Collection of the Department of Agriculture (YCC-DA) of the University of Naples Federico II were used (Table 1). Hybrid strains were generated during the research project "*Wine strain improvement strategies to enhance red wine safety based on parietal adsorption activity*" (PRIN 2007 – prot. 2007FB4EZF). For long-term maintenance, strains were stored at −20 °C in YPD broth (Oxoid, Basingstoke, UK) plus 20% (w/v) glycerol (Sigma, Milan, Italy). Before experimental use, cultures were propagated twice in YPD broth.

2.1.2. Strains molecular identification and typing

DNA was isolated as previously described by Pennacchia et al. (2008). Preliminary molecular identification of yeast strains was achieved by ITS (ITS1-5.8S-ITS2)-rDNA RFLP analysis with the restriction endonucleases *Hae* III, *Hinf* I, *Cfo* I and *Dde* I (Esteve-Zarzoso et al., 1999). Identifications were confirmed by ITS-rDNA sequencing. For molecular biotyping two markers were considered: interdelta analysis (Legras and Karst, 2003), *DAN*4 minisatellites analysis (Marinangeli et al., 2004).

2.1.3. Strains technological characterization

Fermentation rate (FR) and fermentation power (FP) were assessed by micro-fermentation (100 mL) trials in red must at 25 °Brix following procedures described in the resolution OIV-OENO 370-2012 (OIV, 2012). FR was expressed as grams of CO_2 produced in 100 mL of must during the first 96 h of fermentation, while FP was expressed as alcoholic degree (% vol/vol = g CO_2 produced/100 mL × 1.25) reached at the end of fermentation. Acetic acid and glycerol in wines were quantified by means of a Gilson 307 Series HPLC system fitted with a MetaCarb 68H (Varian) column at 65 °C. Columns were eluted at 0.6 mL/min by a solution of H_2SO_4 (0.0125 mol/L) in ultrapure water. A refractometer (RID 133, Gilson) was used as detector. External standards (Sigma) were used for the quantification of the substances in the samples. Colour intensity (CI) of wines was evaluated by determining absorbance at wavelengths of 420, 520 and 620 *nm* of diluted samples (1:5, in 9 g/L tartaric acid, pH 3.20 ± 0.1). CI was expressed as sum of absorbance values of the three wavelengths ($CI = A_{420} + A_{520} + A_{620}$).

Hydrogen sulphide (H_2S) production was evaluated on Biggy agar (Oxoid) after incubation at 28 °C for 48 h. For browning description, the following codes were used: 1, Snow; 2, White; 3, Hazelnut; 4, Brown; 5, Rust; 6, Coffee (1, lowest and 6, highest, level of H_2S production). Type of growth was estimated in YPD broth (pH 3.50) after 4 days at 28 °C. Sulphur dioxide resistance was estimated in YPD broth (pH 3.50) containing potassium metabisulphite in concentrations ranging from 100 up to 400 mg/L (50 mg/L increments). All evaluations were carried out in triplicate.

2.2. Fermentations

Laboratory-scale fermentations were performed in three different synthetic media: C) synthetic must (glucose 100 g/L, fructose 100 g/L,

Table 1
Strains used during this study.

Strain	Origin	Production area	YCC-DA[a] id number	Identification[b]	ITS-RFLP type[c]	Molecular biotype[d]	Used in fermentation trial
M26	Passito wine (Moscato of Sarcena)	Saracena (Calabria, Italy)	NA026	*S. cerevisiae*	A	A-a	Y1
M33v	Passito wine (Moscato of Sarcena)	Saracena (Calabria, Italy)	NA028	*S. cerevisiae*	A	B-b	Y2
F45	Wine (Gragnano DOC)	Gragnano (Campania, Italy)	NA040	*S. cerevisiae*	A	C-b	Y3
SP5 (D2)	Soppressata (Fermented meat product)	Frascineto (Calabria, Italy)	NA067	*S. cerevisiae*	A	D-c	Y4
AL41	Wine (Catalanesca, Caprettone, Falanghina)	Bosco Tre Case (Campania, Italy)	NA072	*S. cerevisiae*	A	E-d	Y5
99 (DB7)	Wine (Pollino DOC)	Frascineto (Calabria, Italy)	NA116	*S. cerevisiae*	A	F-e	Y6
1	(DB5BA)	Wine (Pollino DOC) Frascineto (Calabria, Italy)	NA131	*S. cerevisiae*	B	G-a	Y7
F3-H1	Hybrid (Moscato of Sarcena × Gaglioppo)[a]		NA239	*S. cerevisiae*	A	H-f	Y8
F3-H12	Hybrid (Gragnano DOC × Montepulciano)[a]		NA250	*S. cerevisiae*	A	I-g	Y9

[b]YCC-DA: yeast Culture Collection of Department of Agriculture.
[a] Origin of parental strains.
[b] Identification was obtained by ITS-RFLP analysis and ITS sequencing.
[c] Classification on the basis of ITS-RFLP-*Hae* III patterns (Figure S1, panel B).
[d] Molecular biotyping performed by interdelta (Capital letter) and ministatellite DAN-4 (Lower case) analyses (Figure S1, panels C and D).