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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.
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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).

tartaric acid 4 g/L, malic acid 2 g/L, NH_4SO_4 0.5 g/L, $(NH_4)_2HPO_4$ 0.665 g/L, KH_2PO_4 0.5 g/L, $CaCl_2$ $2H_2O$ 0.31 g/L, NaCl 0.1 g/L, YNB (Oxoid) 6.7 g/L, pH 3.20) sterilized by tyndallization (100 °C × 5 min × 3) to prevent sugar caramelization; CP) tyndallized C supplemented with filter-sterilized organic nitrogen solution (peptone 12.5 mg/L, tryptone 12.5 mg/L); CPV) tyndallized C supplemented with filter-sterilized organic nitrogen and vitamins solutions (biotin 0.0125 mg/L, pyridoxine 0.25 mg/L, meso-inositol 1.875 mg/L, calcium pantothenate 2.5 mg/L, thiamine 2.2 mg/L, peptone 12.5 mg/L, tryptone 12.5 mg/L). Before yeast strains inoculation, 3 g/L of EGT were added at each medium to obtain CT, CTP, and CTPV. Strains were grown in YPD medium (yeast extract 10 g/L, peptone 20 g/L, dextrose 20 g/L) at 28 °C for 48 h. Pre-inocula were prepared from YPD cultures (0.1 mL) in 10 mL of sterile CT medium for 48 h at 25 °C. Fermentations were started by inoculating 2 mL (about 1 OD_{600}/mL) of such cultures in 100 mL of CT, CTPV or CTP in 250 mL Erlenmeyer flask. Flasks were then closed with a Müller valve, previously filled with sulphuric acid and incubated at 25 °C. Fermentations were carried out in duplicate. During the first three days of fermentation, flasks were handle stirred for 30 s every 12 h. Fermentation kinetics were monitored by weight loss as a result of CO_2 escaping from the system. At the end of fermentation - flasks weight does not change within 24 h—50 mL the upper phase were collected and stored at 12 °C until analyses.

2.3. Evaluation of the effect of EGT on yeasts growth

Yeast strains were grown in 10 mL of YPD (pH 3.50) with or without EGT (3 g/L). Optical densities (OD_{600}) of pre-inocula in YPD (48 h at 25 °C) were estimated and cultures were diluted with fresh YPD to obtain suspensions with an OD_{600} of 1. Two hundred μL of each suspension were used to inoculate YPD with or without EGT. Cultures were then incubated at 25 °C for 5 days in static conditions (vortex for 30 s every 24 h). Viable counts were evaluated by standard plating procedure on WL nutrient agar (Oxoid), after one and 5 days of fermentation. Results were expressed as means ± standard deviation of three independent experiments.

2.4. Biomass production in synthetic must supplemented or not with EGT

Six different media were used to evaluate biomass production of some strains (Y1-Y4-Y5-Y8): a) synthetic musts with 3 g/L EGT (CT, CTP and CTPV); b) synthetic musts without EGT (C, CP and CPV). Fermentations were carried out as described in the Section 2.2. Produced biomass of each strain in each medium, at 14 days (end of fermentation), was evaluated collecting cells of 10 mL of fermentation mixture after vigorous shaking. Pellets were recovered by centrifugation (ALC PK130) a 6500 *rpm* (T541 rotor) for 10 min, twice washed with 10 mL sterile water and then freeze dried. Freeze dried pellets were weighed using an analytical balance (Gibertini E42S) and results were expressed as mg/10 mL. Results were expressed as means and standard deviation of three independent experiments.

2.5. Adsorbed phenolics quantification

Phenolics characterising EGT (Laffort, France) used for experiments were measured as described below. Phenolics having antioxidant activity (Folin—Ciocalteu Index, FCI) were evaluated according to Waterhouse (2002) and were expressed as g/L of gallic acid equivalent (GAE). Total tannins were evaluated as described by Ribereau-Gayon and Stonestreet (1966). Total phenols were measured by absorbance at 280 *nm* (A_{280}) according to the OIV Compendium of International Methods of Analysis of Wine and Musts (2007). Yellow pigmented compounds were measured by absorbance at 420 *nm* (A_{420}) according to Glories (1984). T_{RSP} were measured by the Saliva Precipitation Index (SPI), in detail described in paragraph 2.6.

Adsorbed phenolics (AP) were quantified as difference between the amount in the samples and in the corresponding controls according to the following formula:

$$AP = P_{control} - P_{sample}$$

$P_{control}$ being the amount of the phenolics measured in the medium without yeast cells, P_{sample} the amount of the phenolics in the supernatant recovered after yeast fermentation.

2.6. The Saliva Precipitation Index (SPI)

2.6.1. Salivary proteins

Human saliva used for binding reactions was obtained by mixing resting saliva samples from different individuals. Saliva was collected from six non-smoking volunteers (three males and three females) by expectorating saliva into a pre-weighted ice-cooled tube for 5 min. The resulting mix was centrifuged at 10,000 *g* for 10 min at 4 °C to remove any insoluble material, and the salivary proteins in the supernatant were used for the analyses.

2.6.2. Binding assays

Interaction mixtures (75 μL final volume) contained 50 μL of saliva and 25 μL of model wines obtained after fermentation, which was previously filtered at 0.45 μm (Millipore; Rome, Italy). Binding assays were performed in Eppendorf tubes maintained at 37 °C for 5 min. The mixture was then centrifuged at 4 °C for 10 min at 10,000 *g*. Electrophoresis was performed on the resulting saliva supernatant.

2.6.3. SDS—PAGE electrophoresis

The SDS—PAGE electrophoresis of saliva was performed on a Bio-Rad Protean II xi Cell electrophoresis apparatus (Bio-Rad, Milano, Italy) using a PowerPac 1000 Bio-Rad power supply set at 150 V/gel for the stacking gel and 180 V/gel for the resolving gel. Saliva samples mixed with an equal volume of 2× electrophoresis sample buffer (0.125 M Tris—HCl, 4% SDS; 20% v/v glycerol, 0.2 M DTT, 0.02% bromophenol blue, pH 6.8) and heated at 95 °C for 4 min were analysed by SDS—PAGE using 30% acrylamide/bisacrylamide (37.5:1) solution. The resolving gels were 14% acrylamide, stacking gels were 5% acrylamide. The gels were fixed with a mixture of ethanol, acetic acid, and deionised water (40:10:50) for 1 h. After washing in water for 5 min, the gels were stained with Coomassie Brilliant Blue R250 staining solution (Bio-Rad, Milano, Italy). The destain step was performed by incubation in the destain solution Coomassie Blue R250 (Bio-Rad, Milano, Italy).

2.6.4. Densitometry

Densitometric tracing of gels was performed with a Bio-Rad GS800 densitometer, and electrophoretic data were analysed by Quantity One analysis software, Version 4.5 (Bio-Rad).

2.6.5. Tannins reactive towards salivary proteins determination

The SPI, used to determine T_{RSP}, was based on SDS-PAGE patterns of salivary proteins before and after exposure to increasing tannin solutions, measuring the differences seen in the intensity of selected bands which in turn allows estimation of the percentage of proteins that have been precipitated by tannins (Rinaldi et al., 2012). The reactivity of salivary proteins towards tannins was expressed as g/L of GAE. All the data are expressed as means ± standard deviation of four replicates.

2.7. Statistical analysis

Analysis of variance (ANOVA) was carried out using the software XLSTAT 2012 (Addinsoft, Paris, France). The Tukey's test was applied to discriminate amongst the means of data at the 95% confidence level ($p \leq 0.05$). To highlight the effect of medium and yeast strain a multifactorial ANOVA was performed. Differences of $p < 0.05$ were considered significant. Principal Component Analysis (PCA) was used to examine any possible grouping of samples according to EGT phenolic composition. Elaborations were carried out by means of Statgraphics 5.0. (Manugistics, Inc.).

3. Results and discussion

3.1. Yeast identification and characterization

Nine strains (Table 1), presumptively identified as *S. cerevisiae* by morpho-physiological tests, were submitted to ITS–RFLP analysis, a reliable and simple method for yeast species identification and monitoring (Granchi et al., 1999). All strains showed the ITS pattern (850 bp; Supplementary Fig. S1A) of typical *Saccharomyces sensu stricto* strains (Esteve-Zarzoso et al., 1999). Moreover, 8 out 9 strains showed an ITS-RFLP-*Hae* III restriction pattern (Supplementary Fig. S1B) referable to *S. cerevisiae*/*S. paradoxus* species (Esteve-Zarzoso et al., 1999). However, all strains could be identified as *S. cerevisiae* by ITS region sequencing (Lopandic et al., 2007). Intedelta and *Dan-4* analyses (Supplementary Fig. S1C and S1D) evidenced a high genetic polymorphism within analysed strains. Several studies pointed out significant molecular polymorphisms within indigenous *S. cerevisiae* strains of different origin and a strong correlation among genotypic and phenotypic properties (Nadal et al., 1996). Different yeast strains differently contribute to the wine quality; therefore, biodiversity studies of wine yeasts within an ecological framework are essential to select strains with new molecular and enological attributes (Lopandic et al., 2007). As matter of fact, due to the molecular diversity, strains here analysed showed different enological traits including acetic acid, glycerol and H_2S productions, type of grow and effect on colour intensity of produced wines (Table 2).

In this study, the effect of EGT on yeasts growth was preliminarily evaluated in YPD media with and without 3 g/L of EGT at pH 3.5. Yeasts viable counts in YPD ranged from 6.95 ± 0.13 to 7.71 ± 0.21 log CFU/mL after 24 h of incubation and from 6.11 ± 0.41 to 7.37 ± 0.09 log CFU/mL after five days, independently by the EGT supplementation (data not shown), indicating that the concentration of EGT does not significantly affect yeast growth ($p < 0.05$).

3.2. Strains behaviour during fermentation in tannin enriched media

S. cerevisiae strains usually selected for winemaking are not able to secrete tannases and then to exploit tannins as carbon and/or energy source (Aguilar et al., 2001). Besides, wine yeast cultures may express different aptitudes to retain or adsorb phenolic compounds (Vasserot et al., 1997; Morata et al., 2003). Nine *S. cerevisiae* strains (Y1-Y9) were chosen to carry on alcoholic fermentations in EGT-enriched media (Table 1). Fermentation rates (FRs) at 1, 5 and 8 days were evaluated. After 24 h, FR values differed for strains and media: strain Y7 showed a longer lag phase (0.14 ± 0.07, 0.15 ± 0.02 and 0.22 ± 0.05 g of CO_2 for CT, CTP and CTPV, respectively) if compared to others (values ranging from 0.4 to 0.6 g of CO_2 in 24 h) (Data not shown). The presence of organic nitrogen sources in the medium (CTP) affected the length of the lag phase just for strains Y2, Y4, and Y9, while the addition of vitamins (CTPV) increased the FRs for all strains, except for Y1 and Y4 (Data not shown). After 5 days, FRs ranged from 1.12 ± 0.06 to 1.52 ± 0.04 g CO_2 per day with no significant differences among strains or media used (Data not shown). Similar results were even recorded after 8 days of fermentation: FRs ranged from 0.99 ± 0.01 to 1.18 ± 0.04 g of CO_2 per day (Data not shown). According to results, the fermentative behaviour of yeast strains is not affected by nutrients added to the medium.

The fermentation power (FP), an estimation of the ethanol produced during fermentation, is the main technological feature to be considered for the selection of wine strains (Rainieri and Pretorius, 2000). Usually, FP values range from 10 to 20% of ethanol for *S. cerevisiae* strains (Vincenzini et al., 2005). All strains, independently from the media, showed similar FP values (from 11.85 ± 0.13 to 12.52 ± 0.08) (Data not shown). All fermentations appeared successfully completed within 14 days, with an estimated ethanol production of about 12% (Data not shown).

3.3. Medium and yeast effect on the adsorption of EGT phenolics

The absorption ability of yeasts fermenting CT, CTP, and CTPV media was evaluated by measuring the following phenolic compounds: yellow pigments, that are compounds absorbing at the wavelength of 420 nm characterised by yellow colour (A_{420}), total phenols (A_{280}), phenolics having antioxidant activity (FCI), total tannins, and T_{RSP}.

Table 2
Some enological traits of *S. cerevisiae* strains.

ID	Strain	FP[a]	FR[b]	AAP[c]	GLYP[d]	CI[e]	SO_2R[f]	H_2SP[g]	TGR[h]
Y1	M26 (SCM8)	14.39 ± 0.35	2.48 ± 0.06	0.15 ± 0.00	6.22 ± 0.21	1.01 ± 0.07	400	1	Flocculent
Y2	M33v (SCM10)	14.54 ± 0.12	2.24 ± 0.05	0.41 ± 0.05	7.19 ± 0.18	1.04 ± 0.02	400	4	Dispersed
Y3	F45	13.84 ± 0.15	2.36 ± 0.07	0.15 ± 0.00	6.77 ± 0.31	1.15 ± 0.07	400	3	Dispersed
Y4	SP5 (D2)	13.62 ± 0.41	1.90 ± 0.03	0.32 ± 0.10	6.52 ± 0.08	0.31 ± 0.02	400	4	Dispersed
Y5	AL41	14.09 ± 0.17	2.06 ± 0.04	0.28 ± 0.08	7.59 ± 0.25	0.83 ± 0.05	400	3	Dispersed
Y6	99 (DB7)	14.34 ± 0.21	2.33 ± 0.08	0.32 ± 0.04	7.07 ± 0.34	0.61 ± 0.01	400	4	Dispersed
Y7	1 (DB5BA)	13.98 ± 0.18	2.20 ± 0.07	0.38 ± 0.10	7.89 ± 0.26	0.95 ± 0.01	400	1	Dispersed
Y8	F3–H1	14.43 ± 0.22	2.40 ± 0.01	0.25 ± 0.09	6.75 ± 0.12	1.23 ± 0.06	400	3	Dispersed
Y9	F3–H12	14.59 ± 0.31	2.48 ± 0.10	0.24 ± 0.07	6.42 ± 0.51	1.39 ± 0.04	400	5	Dispersed

[a] Fermentation Power (% vol.) in red must at 25 °Brix.
[b] Fermentation Rate (g CO_2/days; time period four days) in red must at 25 °Brix.
[c] Acetic acid production (g/L) in red must at 25 °Brix.
[d] Glycerol production (g/L) in red must at 25 °Brix.
[e] Colour intensity at the end of fermentation of red must at 25 °Brix.
[f] Resistance (ppm) to potassium meta-bisulphite in YPD (pH 3.50).
[g] H_2S production evaluated on BIGGY Agar: 1, lowest; 6, highest production level.
[h] Type of growth in liquid medium (YPD, pH 3.50).

The data sets corresponding to adsorbed phenolics were combined for medium (CT, CTP, and CTPV) and yeast (Y1-Y9) effect, and submitted to a two-way ANOVA analysis with factor 1 medium (M), and factor 2 yeast (Y), and interaction between them (M × Y). Mean table and significativity are showed in Table 3. The factor that mainly influenced the adsorption of phenolics during alcoholic fermentation is the medium ($p = 0.0000$), showing the highest effect on total phenols ($F = 921.17$). Without nutrients (CT), fermenting yeasts were able to adsorb more phenols than in presence of peptones (CTP) or peptones/vitamins (CTPV). However, since A_{280} measurement also reflects the presence of non phenolic molecules with a benzene ring (amino acid) in medium (Lorrain et al., 2013), it can be conjectured that some nutrients were not fully metabolized. This may depend on the uptake of such compounds. In peptone and tryptone, amino acids predominate as peptides and should thus enter into the cell by means of peptide permeases (Hauser et al., 2001), while the uptake of vitamins and cofactors occurs by cation symport or product antiport, and twelve families providing for these functions have already been identified (Saier, 2000).

Similarly, an increased adsorption of phenolics with antioxidant activity (FCI) by yeast cells was observed in CT. The interaction M × Y, in this case, was highly significant, meaning that yeasts in presence of small peptides as sources of assimilable nitrogen adsorbed less phenolics, thus preserving the relative antioxidant potential of model wines (Vinson and Hontz, 1995). Actually, the influence of *S. cerevisiae* strain on the antioxidant capacity was also stated by others (Brandolini et al., 2007).

Yellow pigmented phenolics (A_{420}), total tannins and T_{RSP} were mainly adsorbed by yeasts when grown in CTPV medium (Table 3). The effect of peptides and vitamins on yeast adsorption ability is likely due to a profitable interaction between phenolics and the cell wall and membrane constituents (Bennis et al., 2004). Moreover, during alcoholic fermentation some vitamins promote the synthesis of lipids (Casey and Ingledew, 1986), ergosterol (del Castillo Agudo, 1992) and other membrane components such as phospholipids (Chi et al., 1999). It has been postulated that membrane sterols could be involved in the yeast's ability to adsorb oxidized compounds characterized by a yellowish colour (Marquez et al., 2009). The efficacy of yeasts in adsorbing yellow pigmented phenolic compounds was also observed in white wines (Bonilla et al., 2001) and in oxidized wine model solutions (Salmon et al., 2003; Salmon, 2006; Marquez et al., 2009). In spite of slight differences emerged among yeast strains, the adsorption of these compounds was not significant for Y effect (Table 3).

A significant adsorption of total tannins, proanthocyanidins characterised by a high molecular weight, was observed after the fermentation in CT (0.48 g/L), CTP (0.74 g/L), and CTPV (0.96 g/L) musts (Table 3). The mean amount of tannins absorbed by yeasts was almost double when the medium was enriched with vitamins and peptones, compounds that can be easily metabolized by yeasts to obtain energy, for enzymatic reactions and to maintain cell wall integrity during alcohol production. Concerning tannins adsorption, the interaction with yeasts has been mainly attributed to cell walls (Salmon et al., 2003), and specifically to polysaccharides such as glucans, mannoproteins, and chitins. The ability of pronathocyanidins to pass thorough the wall pores and the possible interaction with plasma membrane has been recently hypothesized (Mekoue Nguela et al., 2014). However, the Y factor had a little influence on tannins concentration, meaning that yeasts adsorbed total tannins at the same amount.

Yeast strains were also tested for the ability to reduce T_{RSP}, estimated by means of the SPI. SDS-PAGE electrophoresis of CT, CTP, and CTPV media are shown in Supplementary Figs. S2, S3 and S4, respectively.

The effect of medium and yeast, as well as of the interaction between them, on the content of adsorbed T_{RSP} (g/L of GAE) was highly significant (Table 3). Both medium composition and yeast strain influenced the adsorption of astringent tannins. This ability appeared to be highly enhanced by addition of vitamins: 4.24, 3.14 and 2.20 g/L GAE for CTPV, CTP and CT, respectively (Table 3). In other words, yeast cell wall and membrane composition seem to play an important role in astringent tannin adsorption. Indeed, the essential role of vitamins on the lipid metabolism is well known (Rattray et al., 1975). Y5, isolated from white wine of Campania region, and Y8, an hybrid from strains isolated from the Moscato white wine and the Gaglioppo red wine, resulted to be the most capable of adsorbing the astringent tannins, so related model wines can be considered less astringent. It cannot be excluded that yeasts showing great affinity towards astringent tannins could release in the external medium cell constituents such as proteins or polysaccharides, thus affecting the binding of tannins with salivary proteins, and contributing to the lowering of astringency (Riou et al., 2002). However, this hypothesis needs to be further investigated.

Table 3
Comparison of Medium (M) and Yeast (Y) effect of the studied variables on adsorbed phenolics[a] by a two-way ANOVA analysis with repetitions ($n = 4$).

Mean table					
	A_{420}	A_{280}	FCI	tannins	T_{RSP}
Medium effect					
CT	0.94^{b}	31.47^{c}	0.61^{c}	0.48^{a}	2.20^{a}
CTP	0.81^{a}	19.07^{a}	0.38^{a}	0.74^{b}	3.14^{b}
CTPV	1.10^{c}	30.75^{b}	0.56^{b}	0.96^{c}	4.24^{c}
Yeast effect					
Y1	0.86^{a}	26.30^{a}	0.47^{a}	0.68^{a}	3.11^{ab}
Y2	0.94^{abc}	27.01^{ab}	0.52^{ab}	0.73^{ab}	3.04^{a}
Y3	0.93^{abc}	26.73^{ab}	0.49^{ab}	0.71^{ab}	3.05^{a}
Y4	0.98^{bc}	27.40^{ab}	0.54^{ab}	0.74^{b}	3.17^{ab}
Y5	0.91^{ab}	27.10^{ab}	0.51^{ab}	0.72^{ab}	3.43^{d}
Y6	0.98^{bc}	27.18^{ab}	0.54^{ab}	0.75^{b}	3.25^{bc}
Y7	0.91^{ab}	27.23^{ab}	0.54^{ab}	0.72^{ab}	3.15^{ab}
Y8	1.03^{c}	27.61^{b}	0.55^{b}	0.74^{b}	3.34^{cd}
Y9	0.99^{bc}	27.48^{ab}	0.50^{ab}	0.75^{b}	3.16^{ab}

Significativity	Medium (M)		Yeast (Y)		M × Y	
	p-value	F ratio	p-value	F ratio	p-value	F ratio
A_{420}	0.0000	41.03	0.1234	1.65	0.1621	1.40
A_{280}	0.0000	921.17	0.4790	0.95	0.5082	0.96
FCI	0.0000	76.50	0.3175	1.19	0.0028	2.58
tannins	0.0000	381.27	0.249	1.31	0.6405	0.84
T_{RSP}	0.0000	903.32	0.0002	4.82	0.0000	15.39

[a] yellow pigments (A_{420}), total phenols (A_{280}), phenolics having antioxidant activity (FCI), total tannins, and astringent tannins (T_{RSP}). Values followed by different letters along the row for each effect are significantly different ($p < 0.05$).

3.4. Relationship between adsorption ability and yeast strains

In order to individuate yeasts able to better perform in musts enriched of EGT, a PCA was applied on the studied variables (A_{420}, A_{280}, FCI, total tannins, and T_{RSP}), considering the mean table for Y effect. Two principal components (PC1 and PC2) accounted for the 87.71% of the total variance in the data. Fig. 1 revealed that the four yeasts Y1, Y5, Y8 and Y4 exhibited a different behaviour in adsorbing phenolics characterizing the EGT.

The evaluation of yeast dry biomass at the end of fermentation in the three different media (Table 4), allowed relating the increased phenolics adsorption just to the biomass production. Y1, isolated from the “passito” wine, expressed the lowest adsorbing

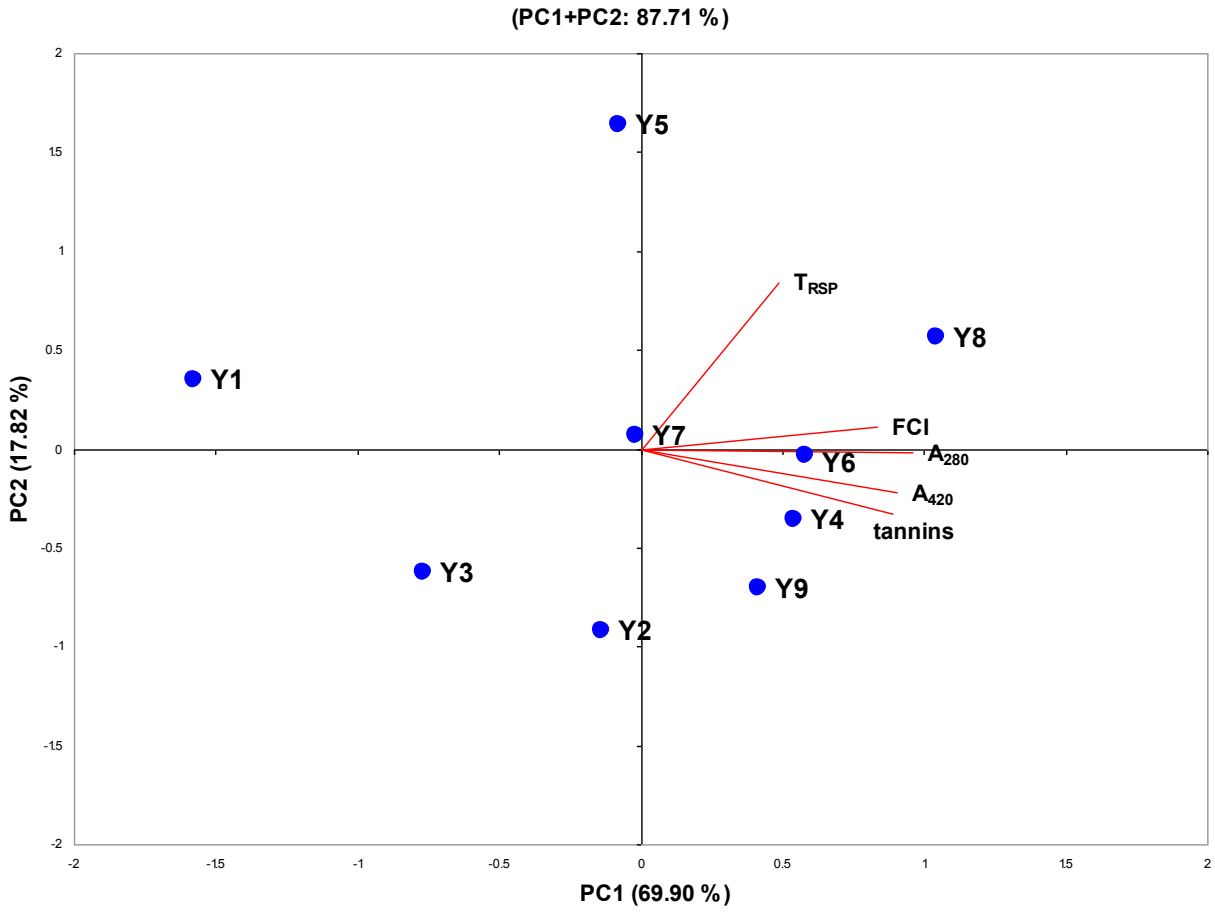

Fig. 1. The Principal Component Analysis (PCA) performed on yeasts (Y1-Y9) and adsorbed EGT phenolics such as yellow pigments (A_{420}), total phenols (A_{280}), phenolics having antioxidant activity (FCI), total tannins, and tannins reactive towards salivary proteins (T_{RSP}).

capacity, and appeared in an isolated position (Fig. 1). Unexpectedly, it was not the lowest biomass producer. On the opposite side in PCA, strain Y8 adsorbed all phenolics to a large extent. This may be attributed also to the high biomass production, which was not influenced by the presence or the absence of EGT in the medium (Table 4). Strain Y5 showed a marked specificity in adsorbing T_{RSP} and a high biomass production at the end of fermentation that may likely favour the adsorption phenomenon. Y4 seem to be specific for yellow pigmented phenolics and tannins. The lowest biomass production compared to others clearly indicate that aspect is crucial but, does not allow to fully explain the different behaviour recorded for the nine strains with reference to the phenolics adsorption. Since strain Y4 was isolated from Soppressata, a traditional fermented sausage, it could be conjectured that the rich-lipid environment could have induced modifications of the strain's membrane constituents, thus promoting a higher phenolics adsorption. According to results in Table 4, biomass production of yeasts Y5 and Y8 proved to be independent by the vitamins and peptones supplementation, while for the other strains slight differences were observed. At any rate, the presence of EGT never inhibited the biomass formation. The phenolics adsorption appeared to be related to a different biomass production, but membrane constituents seem to play a significant role as well.

4. Conclusions

In this study synthetic musts enriched of EGT were fermented by different yeast strains to evaluate the influence of both medium nutrients and yeast strains on the wine final composition. According to outcomes, no variation in fermentation rate within strains appeared to be related to nutritional factors. Consequently, differences in adsorbing phenolics are most likely to be a function of a different adsorption capacity among yeast strains. The presence of biotin, pyridoxine, meso-inositol, calcium pantothenate and thiamine in combination with peptone and tryptone in musts favours a remarkable reduction of yellow pigments, total tannins, and

Table 4
Dry biomass (mg) production at the end of fermentation in different media: CT) containing enological grape tannin; CTP) CT supplemented with organic nitrogen sources; CTPV) CTP supplemented with vitamins. C, CP, CPV controls without EGT.

Strains	C	CP	CPV	CT	CTP	CTPV
Y1	19.80 ± 0.71^{d}	21.44 ± 0.54^{bc}	23.39 ± 0.40^{ab}	22.91 ± 0.66^{bc}	23.42 ± 0.50^{ab}	25.06 ± 1.28^{a}
Y4	16.07 ± 0.64^{c}	15.57 ± 0.12^{c}	17.67 ± 0.15^{b}	17.93 ± 0.83^{b}	18.27 ± 0.49^{b}	19.73 ± 0.35^{a}
Y5	42.33 ± 3.36^{a}	38.97 ± 1.10^{a}	39.80 ± 2.72^{a}	42.13 ± 2.84^{a}	41.03 ± 3.75^{a}	42.23 ± 0.96^{a}
Y8	32.23 ± 6.05^{a}	30.73 ± 2.35^{a}	31.83 ± 0.93^{a}	30.96 ± 2.58^{a}	33.06 ± 0.12^{a}	31.03 ± 0.72^{a}

Values with different letters in the same row indicate statistical differences according to the Tukey's test at $p \leq 0.05$.

tannins responsible for the potential astringency in model wines. Some yeasts proved to be of particular enological interest because of the specificity in adsorbing some phenolic compounds. In detail, strains Y8 and Y5 can be used to reduce tannins especially in musts from grapes highly astringent; strain Y6 can be used in fermentation of grapes with a high content of yellow pigmented phenolics; strain Y4, not-derived-from wine, will represent an interesting strain to be applied in winemaking for its specificity in adsorbing both tannins and yellow pigmented phenolics.

In conclusion, yeasts selection for winemaking should always take into account their potential role in phenolics adsorption that can be helpful or detrimental depending on issues related to different grape varieties.

Conflicts of interest

All authors declare no financial/commercial conflicts of interest.

Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.fm.2015.09.013.

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