Università degli Studi Mediterranea di Reggio Calabria
Archivio Istituzionale dei prodotti della ricerca

Improved screening method for the selection of wine yeasts based on their pigment adsorption activity

This is the peer reviewd version of the followng article:

Original
Improved screening method for the selection of wine yeasts based on their pigment adsorption activity / Caridi, A.. - In: FOOD TECHNOLOGY AND BIOTECHNOLOGY. - ISSN 1330-9862. - 51:1(2013), pp. 137-144.

Availability:
This version is available at: https://hdl.handle.net/20.500.12318/4888 since: 2023-05-25T17:49:31Z

Published
DOI: http://doi.org/

The final published version is available online at:https://www.ftb.com.hr/archives/133-volume-51-issue-no-

Terms of use:
The terms and conditions for the reuse of this version of the manuscript are specified in the publishing policy. For all terms of use and more information see the publisher's website

Publisher copyright

This item was downloaded from IRIS Università Mediterranea di Reggio Calabria (https://iris.unirc.it/) When citing, please refer to the published version.

(Article begins on next page)

12 November 2026

Improved Screening Method for the Selection of Wine Yeasts Based on Their Pigment Adsorption Activity

*Andrea Caridi**
*Corresponding author; Phone/Fax: ++39 0965 680 578; E-mail: acaridi@unirc.it
Department AGRARIA, Mediterranea University of Reggio Calabria, Feo di Vito, I-89122 Reggio Calabria, Italy

Summary

The aim of this research is to improve an existing low-cost and simple but consistent culturing technique for measuring the adsorption of grape skin pigments on yeasts, comprising: (*i*) growing yeasts in Petri dishes on chromogenic grape-skin-based medium, (*ii*) photographing the yeast biomass, (*iii*) measuring its red, green, and blue colour components, and (*iv*) performing the statistical analysis of the data. Twenty strains of *Saccharomyces cerevisiae* were grown on different lots of the chromogenic medium, prepared using grape skins from dark cultivars Greco Nero, Magliocco and Nero d'Avola. Microscale wine fermentation trials were also performed. Wide and significant differences among wine yeasts were observed. The chromogenic grape-skin-based medium can be prepared using any grape cultivar, thus allowing the specific selection of the most suitable strain of *Saccharomyces cerevisiae* for each grape must, mainly for red winemaking. The research provides a useful tool to characterize wine yeasts in relation to pigment adsorption, allowing the improvement of wine colour.

Key words: adsorption, pigment, screening method, yeast

Introduction

Red wines contain different amounts of a variety of grape pigments, primarily anthocyanins. Pigment composition of wine depends on grape cultivar, vineyard management practices and environmental conditions, winemaking techniques, and time of wine ageing. Also wine yeasts interact, in different ways, with grape pigments by producing glycosidases (*1*,*2*) and pectinolytic enzymes (*3*) that increase colour extraction (*4–6*), by accumulating reactive metabolites such as pyruvic acid (*7*,*8*) and acetaldehyde (*8–11*), by releasing mannoproteins and different polysaccharides (*12*,*13*), by contributing to anthocyanin-derived pigment formation (*14*,*15*), and by adsorbing pigments on cell walls (*16–18*). Different methods to measure the adsorption of grape skin pigments by wine yeasts on cell walls have been proposed (*19–27*) and will be discussed later. The aim of this research is to improve an existing low-cost and simple but consistent culturing technique for measuring the adsorption of grape skin pigments on yeasts, comprising: (*i*) growing yeasts in Petri dishes on chromogenic grape-skin-based medium, (*ii*) photographing the yeast biomass, (*iii*) measuring its red, green, and blue colour components, and (*iv*) performing the statistical analysis of the data.

Materials and Methods

Culture media and yeast strains

Chemicals and culture medium components were purchased from Sigma-Aldrich (Milan, Italy). Twenty strains of wine yeasts with different grape pigment adsorption ability were used: 16 strains of *Saccharomyces sensu stricto*, selected for winemaking and belonging to the Department AGRARIA collection (Reggio Calabria, Italy), two laboratory strains: *S. cerevisiae* 1042 and *S. bayanus* 12233, selected for winemaking and belonging to the DISTAL collection of the University of Bologna (Bologna, Italy), and two commercial wine strains: Lalvin BM45 and Lalvin ICV-D254 (Lallemand, Montreal, Canada), which were reconstituted from dried yeast, as supplied by the manufacturer, and plated to provide single colonies.

The control medium, yeast extract-peptone-dextrose (YPD) broth, solidified with 20 g/L of agar when required, was sterilized by autoclaving at 121 °C for 15 min and contained the following (in g/L): yeast extract 10, peptone from casein 10, and dextrose 20. Dried grape skins, basic ingredient for the chromogenic grape-skin-based medium, were obtained as follows: the skins were manually removed from grapes, gently washed, dried with paper towels and then heated at 55 °C until constant mass was reached, and lastly finely ground in a blender (Oster®/Sunbeam® 890-48H, McMinnville, TN, USA). The final composition of the chromogenic grape-skin-based medium (pH=3.5) was (in g/L): dried grape skin 60, citric acid monohydrate 50, disodium hydrogen phosphate 25, dextrose 20, casein peptone 7.5, yeast extract 4.5, and agar 20. The medium – all ingredients without agar – was prepared and sterilized at double concentration as follows: a double amount of dried grape skins was suspended in distilled water, heated at 110 °C for 5 min to extract grape pigments, and filtered through a gauze. The filtered extract was measured using a graduate cylinder and the corresponding double amount of the other ingredients was added. The solution was divided into test tubes (5 mL per tube) and heated at 110 °C for 5 min. A double amount of agar was dissolved in distilled water, divided into test tubes (5 mL per tube) and sterilized by autoclaving at 121 °C for 15 min. Then, one test tube containing the medium and one containing the agar solution, both maintained at a temperature ≥50 °C in a water bath, were poured together in Petri dishes (60×15 mm). After careful mixing with a sterile L-shaped plastic spreader, the medium was allowed to solidify.

Optimisation of the chromogenic grape-skin-based medium

It is well known that anthocyanin properties, including colour expression, are highly influenced by pH: when the pH varies, anthocyanins provide colours ranging from salmon-pink through red and violet to nearly black. To buffer the medium at pH=3.5, thus reproducing the wine pH and allowing the best colour expression of grape pigments (especially anthocyanins), an appropriate ratio of citric acid monohydrate and disodium hydrogen phosphate was included in the chromogenic medium composition.
To optimize the dose of dried grape skin in the chromogenic grape-skin-based medium, and thus obtain an adequate colour intensity, the absorbance at 420, 520 and 620 nm of different heat-treated and filtered solutions, obtained using different concentrations of dried grape skin, was measured using a spectrophotometer Anadeo 1, Bibby Sterilin Ltd (Staffordshire, UK).
To study the interaction between the yeast strain and the grape pigment quality and quantity, three lots of dried grape skins were prepared, each using a different grape skin from the cultivars Greco Nero, Magliocco and Nero d'Avola, each with very different grape pigment content.

Methodology

The twenty wine yeasts were inoculated in Petri dishes containing the three lots of chromogenic grape-skin-based medium and the control medium (YPD agar). Using sterile loops, 10 mL of biomass of each strain, grown at 28 °C for 2 days on the YPD agar, were inoculated in each Petri dish, by spreading over the surface using a sterile L-shaped plastic spreader. The Petri dishes were put back into their packs, nitrogen gas was gently blown in for 1 min and then the packs were hermetically closed; this was both to simulate fermentation conditions and to inhibit pigment oxidation. After 10 days of incubation at 28 °C, the biomass was carefully mixed using a sterile loop and 10 mL of the collected sample. The careful mixing of the biomass was necessary to assess the colour of the whole biomass and not only of the superficial layer. The biomass collected in the loop was carefully spread, both to prepare a flat surface to be photographed, and also to ensure an equal quantity was collected each time.
The colour assessment was performed on the photographs of the yeasts, measuring their red, green, and blue components with Adobe Photoshop CS for Windows XP (Adobe Systems, Inc., San Jose, CA, USA). Yeasts were photographed with the digital camera HP Photosmart 945 (Hewlett Packard, Palo Alto, CA, USA) at 10-cm distance using the macro function; the camera was set at ISO-100 in automatic mode and was positioned orthogonally using a tripod. To spread the flashlight evenly, provide softer illumination and reduce shadows and reflections, a homemade soft box diffuser, made of a plastic opaque box with tissue paper on the inner and outer surfaces, was mounted on the integrated camera flash device. The image was processed for colour using Adobe Photoshop CS for Windows XP. The region of interest was set to 5×5 pixels taking four replicates for each strain. Photoshop's red-green-blue colour mode assigned an intensity value to each region. In a colour image, the intensity values ranged from zero (black) to 255 (white) for each of the red, green, and blue components. Accordingly, low grape pigment adsorption matched the high red, green, and blue values, *i.e.* white yeasts; high grape pigment adsorption matched the low red, green, and blue values, *i.e.* dark brown yeasts (Fig. 1).

Fermentation trials

Microscale wine fermentation trials were performed using grapes of Magliocco cultivar. They were destemmed, crushed, cold soaked at 0 °C for 3 days, and punched down twice per day. The must obtained after pressing (23 °Bx) was adjusted to pH=3.5, divided in 60 aliquots of 100 mL and fermented at 20 °C with the 20 wine yeasts inoculated at 5 % in triplicate. Carbon dioxide was measured by mass loss to determine the end of fermentation. Wines were centrifuged at 4500 rpm for 5 min, and the absorbance was read at 420, 520 and 620 nm. The colour intensity was given by the sum of the three absorbances; the colour tint was expressed by the ratio of the absorbances at 420 and 520 nm. The total polyphenolic content was determined using the Folin-Ciocalteu's index (*28*).

Statistical analysis

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 ($p<0.05$). For biomass colour parameters, cluster analysis (nearest neighbour method based on the squared Euclidean distance) was performed.

Results

Tables 1–3 report mean values, standard deviations and homogeneous groups ($p<0.05$) among the 20 wine yeasts for red, green, and blue components of the yeast biomass grown on chromogenic medium and on YPD agar, as measured using Photoshop. Considering the red component (Table 1), the yeasts grown on the medium prepared using grape skins of Greco Nero cultivar were distributed in 14 homogeneous groups showing a mean value of 152, with a minimum of 4

and a maximum of 210. Yeasts grown on the medium prepared using grape skins of Magliocco cultivar were distributed in 13 homogeneous groups (mean 123, minimum 2, maximum 176). Yeasts grown on the medium prepared using grape skins of Nero d'Avola cultivar were distributed in 12 homogeneous groups (mean 153, minimum 5, maximum 194). Yeasts grown on the control medium, YPD agar, were distributed in 16 homogeneous groups (mean 210, minimum 166, maximum 253). Considering the green component (Table 2), the yeasts grown on the medium prepared using grape skins of Greco Nero cultivar were distributed in 14 homogeneous groups (mean 121, minimum 1, maximum 182). Yeasts grown on the medium prepared using grape skins of Magliocco cultivar were distributed in 15 homogeneous groups (mean 89, minimum 1, maximum 134). Yeasts grown on the medium prepared using grape skins of Nero d'Avola cultivar were distributed in 15 homogeneous groups (mean 124, minimum 1, maximum 168). Yeasts grown on the control medium, YPD agar, were distributed in 12 homogeneous groups (mean 195, minimum 141, maximum 254). Considering the blue component (Table 3), the yeasts grown on the medium prepared using grape skins of Greco Nero cultivar were distributed in 11 homogeneous groups (mean 132, minimum 9, maximum 178). Yeasts grown on the medium prepared using grape skins of Magliocco cultivar were distributed in 15 homogeneous groups (mean 108, minimum 6, maximum 153). Yeasts grown on the medium prepared using grape skins of Nero d'Avola cultivar were distributed in 10 homogeneous groups (mean 138, minimum 13, maximum 173). Yeasts grown on the control medium, YPD agar, were distributed in 12 homogeneous groups (mean 172, minimum 129, maximum 239).

Both after growth on chromogenic grape-skin-based medium and on the control YPD agar, the tested yeasts showed wide and significant differences in their colour components, above all in the medium containing grape skins. Although the results of the control medium were statistically significant, the differences were not as wide, and due to the absence of grape pigments are not of oenological interest. Statistical distribution of the yeasts in homogeneous groups – based on their red, green, and blue component – clearly confirms the presence of significant differences in their adsorption of grape skin pigments. In general, when the cultivar changes, the homogeneous group assigned by statistical analysis to each strain is not modified. Strains ICV D254, Sc1864, Sc2640, Sc2717 and TT254, which are always included in the last (high value) homogeneous groups according to the LSD analysis, can be described as low adsorbing yeasts. Strains 12233, Sc1304, Sc1483, Sc2489 and TT173, which are always included in the first (low value) homogeneous groups according to the LSD analysis, can be described as high adsorbing yeasts. This category exhibits an interesting phenomenon: the respective position of the strains in the homogeneous groups was somewhat different, above all for strains Sc1304, Sc1483 and TT173. This indicates a different adsorption of grape skin pigments by yeasts, depending on the grape cultivar. However, there was always a positive, highly significant ($p<0.001$) correlation between the red, green and blue component values observed for the three grape cultivars tested, as shown by the following correlation coefficients: (*i*) red component: Greco Nero×Magliocco=0.8687, Greco Nero×Nero d'Avola=0.9108, Magliocco×Nero d'Avola=0.7984; (*ii*) green component: Greco Nero×Magliocco=0.8122, Greco Nero×Nero d'Avola=0.8647, Magliocco×Nero d'Avola=0.7411; (*iii*) blue component: Greco Nero×Magliocco=0.8258, Greco Nero×Nero d'Avola=0.9027, Magliocco×Nero d'Avola=0.7544.

Table 4 shows the mean values and significant differences ($p<0.05$) among the 20 wine yeasts for the Folin-Ciocalteu's index, absorbance, intensity and tint parameters determined in the wines produced using grape must from Magliocco cultivar. Yeasts were distributed (*i*) for the Folin-Ciocalteu's index in 17 homogeneous groups showing a mean value of 12.1, with a minimum of 10.4 (strain Sc226) and a maximum of 13.6 (strain Sc2640), (*ii*) for the absorbance value at 420 nm in 17 homogeneous groups showing a mean value of 1.067, with a minimum of 0.948 (strain Sc226) and a maximum of 1.516 (strain Sc1483), (*iii*) for the absorbance value at 520 nm in 15 homogeneous groups showing a mean value of 1.167, with a minimum of 0.958 (strain Sc2659) and a maximum of 1.806 (strain Sc1483), (*iv*) for the absorbance value at 620 nm in 13 homogeneous groups showing a mean value of 0.230, with a minimum of 0.186 (strain Sc226) and a maximum of 0.446 (strain Sc1483), (*v*) for the colour intensity in 15 homogeneous groups showing a mean value of 2.463, with a minimum of 2.116 (strain Sc2659) and a maximum of 3.768 (strain Sc1483), and (*vi*) for the colour tint in 16 homogeneous groups showing a mean value of 0.918, with a minimum of 0.838 (strain Sc708) and a maximum of 0.998 (strain Sc2659). Strains Sc226, Sc560, and Sc1766, which are always included in the first (low value) homogeneous groups according to the LSD analysis for Folin-Ciocalteu's index and colour intensity values, produce lightly coloured wines with a low phenolic content; on the contrary, strains Sc1483, Sc2640, and TT254, which are always included in the last (high value) homogeneous groups according to the LSD analysis, produce highly coloured wines with high phenolic content.

To identify any significant correlation between the colony colour and oenological traits, all the available data on the 20 yeasts were statistically analysed. As expected, correlations between the red, green, and blue components of the yeast biomass, grown on chromogenic medium prepared using grape skins of Magliocco cultivar, and the related Folin-Ciocalteu's index, absorbance, intensity and tint parameters determined in the wines produced using grape must from the same cultivar were not statistically significant (Table 5). Effectively, yeasts can interact with the colour and the phenolic content of wine in different ways, and grape pigment adsorption *via* wine yeasts is only one of these. Nevertheless, negative correlation, very close to the statistical significance limit, was observed among the red component of the yeast biomass and the absorbances at 520 and 620 nm and the intensity values of the wines. Analogously, negative correlation, very close to the statistical significance limit, was observed among the blue component of the yeast biomass and the absorbances at 520 and 620 nm and the intensity values of the wines. To stress more clearly the existing differences in the pigment adsorption among yeast strains, it was useful to perform a multiparameter statistical analysis to cluster the strains. Fig. 2 shows notable differences for the red, green, and blue

biomass parameters among the 20 strains in the three lots of chromogenic medium. This approach may be helpful to select yeast strains by clustering them based on specific parameters.

Discussion

Grape pigments can adsorb on wine yeast mannoproteins, which are in the outermost layer of their cell walls (*29*,*30*). The adsorption process has important consequences for wine quality because large amounts of grape pigments are retained, due to a surface area of yeast cells greater than 10 m2/L of must (*19*). Pigment adsorption is a strain- (*20*) and time-dependent (*17*) trait. To assess the amount of grape pigments that wine yeasts can adsorb during winemaking, a consistent analytical method is needed. One approach is to perform winemaking trials using different wine yeasts and to analyse colour and phenolic content of the wines (*20–22*). This provides results hard to understand, since colour and phenolic content of wines only partially correlate with grape pigment adsorption (*23*). Another approach is to analyse the phenolic compounds adsorbed on the cell wall at the end of winemaking (*19*,*24*). The analysis of the phenolic compounds may be effective, but very complex and expensive (*25*). A further approach is to assess the colour of wine yeast grown in Petri dishes on media containing grape pigments. Until now, this approach was uncertain because the available methods were simple but not fully consistent. To assess the colour of yeasts grown in Petri dishes, researchers first attempted to inoculate wine yeasts on a medium of 1:1 red grape must and agar solution (40 g/L); then they incubated the yeasts at 28 °C for 5 days before observing their colour (*26*). This culturing technique was based on a qualitative visual inspection of the yeast biomass.

More recently, the colour of yeasts grown in Petri dishes was studied by inoculating the yeasts on grape-skin-based media, to increase their chromogenic power. Then, the colour was also qualitatively determined by visual inspection of the yeast biomass (*27*). The assumption was that biomass colour reflects the binding of grape pigments to the yeasts. Consequently, colour depends on the adsorption of grape skin pigments on yeasts: white is related to low adsorption, dark brown to high adsorption. Also this method, based on a qualitative visual inspection of the yeast biomass, has the following three limits: (*i*) subjectivity, (*ii*) semi-quantitative approach, and (*iii*) impossibility to perform statistical analysis of the data.

A more objective, quantitative approach to assess the colour of yeasts and to subject the colourimetric data to statistical analysis was needed. Thus a simple but consistent culturing technique was developed (*23*) that enables the colour of yeasts grown on chromogenic grape media in Petri dishes to be photographically processed, by measuring their red, green, and blue components using Photoshop and statistically analysing the colourimetric data. To confirm yeast biomass colour results, microscale wine fermentation trials using red must were performed, and the obtained wines were analysed. As expected, the relationship between the red, green and blue components of yeast biomass colour and the analytical parameters of wines was strong but not complete, *i.e.* not for all the strains, because colour and phenolic content of wines are influenced by a variety of yeast properties, other than grape pigment adsorption on cell walls.

The present research has improved the last culturing technique, standardizing it in some methodological details. Moreover, variability of the adsorption of grape skin pigments has been tested on a congruous number of yeast strains, when three different lots of the chromogenic medium, prepared using grape skins from different cultivars, were employed. Lastly, using the same cultivar both to prepare the chromogenic medium and to perform microscale wine fermentation trials, the correlation degree between adsorption of grape skin pigments from the medium on yeasts and the wine composition has been tested. According to previous observations (*23*), wine colour and phenolic content may also be influenced by yeast factors apart from adsorption of grape pigments. Since each strain reacts differently as a function of external factors, it would seem difficult to quantitatively predict an increase or decrease in adsorption of pigments by yeasts. Nevertheless, the present work shows an approach, based on microbial culturing techniques, to simplify the study of the adsorption phenomena in wine yeasts.

It is known that grape pigments, primarily anthocyanins, are sensitive to pH changes (*31*,*32*) and that parietal mannoproteins vary from strain to strain depending on the percentage of acidic oligosaccharides (*33*). Clearly, this influences the parietal pH and consequently grape pigment colour; a further investigation into this phenomenon is desirable.

One important implication of the chromogenic grape-skin-based medium is that, if compared to a similar medium prepared with commercial products using mixtures of anthocyanins, this medium enhances the possibility of investigation into each cultivar. In fact, the chromogenic medium is prepared using specific varieties with specific content of anthocyanins and derivatives. Considering that anthocyanin composition and concentration can significantly vary both among and within grape cultivars depending on environmental conditions, including water deficit, climate, soil, and vineyard management practices (*34*), and that this simple medium can be prepared using any grape cultivar (*35*), it is possible to conclude that this method allows the selection of the most suitable yeast strain for each grape must. It is useful to consider that the choice of a suitable strain for each grape variety cannot only ensure the production of quality wine, but also maintain the cultivar's individual characteristics (*36*). Yeast strain selection was demonstrated to be important in protecting the colour of wine during winemaking (*20*); consequently, strategies proposed to enhance the concentration of grape pigments in wine *via* the choice of yeast strain are welcome (*37*).

Conclusions

With the present study a low-cost and simple but consistent culturing technique to measure the adsorption of grape skin pigments on yeasts was improved, by allowing data to be subjected to statistical analysis, thus providing a useful tool in the enhancement of red wine colour. This method can allow the characterization and the selection of the most suitable wine starters to ferment musts from black grapes. The yeast distribution into homogeneous groups showed the presence of highly significant differences among the strains and the high reproducibility of the analysis when the grape skin cultivar was changed. Based on grape cultivar used for chromogenic grape-skin-based medium, a very different pigment adsorption was observed among yeasts. The proposed chromogenic medium can be prepared using any grape cultivar allowing the selection of the most suitable yeast strain for each grape must.

Acknowledgements

This research was supported by the Calabria Region, Research Fund APQ, Action 2, Laboratories QUASIORA and AGROMATER. The author would like to thank Dr Daniela Sidari for help in setting and optimizing the digital camera to photograph the yeast biomass.

References

1. A. Delcroix, Z. Günata, J.C. Sapis, J.M. Salmon, C. Bayonove, Glycosidase activities of three enological yeast strains during winemaking: Effect on the terpenol content of Muscat wine, *Am. J. Enol. Vitic. 45* (1994) 291–296.
2. P. Manzanares, V. Rojas, S. Genovés, S. Valles, A preliminary search for anthocyanin-b-D-glucosidase activity in non-*Saccharomyces* wine yeasts, *Int. J. Food Sci. Technol. 35* (2000) 95–103.
3. L.F. Hernández, J.C. Espinosa, M. Fernández-González, A. Briones, b-Glucosidase activity in a *Saccharomyces cerevisiae* wine strain, *Int. J. Food Microbiol. 80* (2003) 171–176.
4. M. Fernández, J.F. Úbeda, A.I. Briones, Typing of non-*Saccharomyces* yeasts with enzymatic activities of interest in wine-making, *Int. J. Food Microbiol. 59* (2000) 29–36.
5. P. Ribéreau-Gayon, D. Dubourdieu, B. Donèche, A. Lonvaud: *Handbook of Enology, Vol. 1: The Microbiology of Wine and Vinifications*, John Wiley & Sons, New York, NY, USA (2000).
6. M.A. Ducasse, R.M. Canal-Llauberes, M. de Lumley, P. Williams, J.M. Souquet, H. Fulcrand *et al.*, Effect of macerating enzyme treatment on the polyphenol and polysaccharide composition of red wines, *Food Chem. 118* (2010) 369–376.
7. H. Fulcrand, C. Benabdeljalil, J. Rigaud, V. Cheynier, M. Moutounet, A new class of wine pigments generated by reaction between pyruvic acid and grape anthocyanins, *Phytochemistry, 47* (1998) 1401–1407.
8. A. Morata, M.C. Gómez-Cordovés, B. Colomo, J.A. Suárez, Pyruvic acid and acetaldehyde production by different strains of *Saccharomyces cerevisiae*: Relationship with visitin A and B formation in red wines, *J. Agric. Food Chem. 51* (2003) 6475–6481.
9. C. Dallas, J.M. Ricardo da Silva, O. Laureano, Interactions of oligomeric procyanidins in model wine solutions containing malvidin-3-glucoside and acetaldehyde, *J. Sci. Food Agric. 70* (1996) 493–500.
10. S.Q. Liu, G.J. Pilone, An overview of formation and roles of acetaldehyde in winemaking with emphasis on microbiological implications, *Int. J. Food Sci. Technol. 35* (2000) 49–61.
11. Y. Hayasaka, M. Birse, J. Eglinton, M. Herderich, The effect of *Saccharomyces cerevisiae* and *Saccharomyces bayanus* yeast on colour properties and pigment profiles of a Cabernet Sauvignon red wine, *Aust. J. Grape Wine Res. 13* (2007) 176–185.
12. S. Escot, M. Feuillat, L. Dulau, C. Charpentier, Release of polysaccharides by yeasts and the influence of released polysaccharides on colour stability and wine astringency, *Aust. J. Grape Wine Res. 7* (2001) 153–159.
13. M. Feuillat, S. Escot, C. Charpentier, L. Dulou, Aging of red wines on lees. Interest in the interactions between yeast polysaccharides and wine polyphenols, *Rev. OEnologues, 98* (2001) 17–18 (in French).
14. K. Medina, E. Boido, E. Dellacassa, F. Carrau, Yeast interactions with anthocyanins during red wine fermentation, *Am. J. Enol. Vitic. 56* (2005) 104–109.
15. A. Morata, M.C. Gómez-Cordovés, F. Calderón, J.A. Suárez, Effects of pH, temperature and SO2 on the formation of pyranoanthocyanins during red wine fermentation with two species of *Saccharomyces*, *Int. J. Food Microbiol. 106* (2006) 123–129.
16. M. Bourzeix, N. Heredia, Study of anthocyanin dyes adsorbed by wine yeasts, *C.R. Acad. Agric. France*, *62* (1976) 750–753 (in French).
17. J.M. Salmon, C. Fornairon-Bonnefond, J.P. Mazauric, Interactions between wine lees and polyphenols: Influence on oxygen consumption capacity during simulation of wine aging, *J. Food Sci. 67* (2002) 1604–1609.
18. J. Merida, A. Lopez-Toledano, T. Marquez, C. Millan, J.M. Ortega, M. Medina, Retention of browning compounds by yeasts involved in the winemaking of sherry type wines, *Biotechnol. Lett. 27* (2005) 1565–1570.
19. A. Morata, M.C. Gómez-Cordovés, J. Suberviola, B. Bartolomé, B. Colomo, J.A. Suárez, Adsorption of anthocyanins by yeast cell walls during the fermentation of red wines, *J. Agric. Food Chem. 51* (2003) 4084–4088.
20. A. Caridi, A. Cufari, R. Lovino, R. Palumbo, I. Tedesco, Influence of yeast on polyphenol composition of wine, *Food Technol. Biotechnol. 42* (2004) 37–40.

21. G. Mazza, L. Fukumoto, P. Delaquis, B. Girard, B. Ewert, Anthocyanins, phenolics, and color of Cabernet Franc, Merlot, and Pinot Noir wines from British Columbia, *J. Agric. Food Chem. 47* (1999) 4009–4017.
22. P. Malacrinò, E. Tosi, G. Caramia, R. Prisco, G. Zapparoli, The vinification of partially dried grapes: A comparative fermentation study of *Saccharomyces cerevisiae* strains under high sugar stress, *Lett. Appl. Microbiol. 40* (2005) 466–472.
23. A. Caridi, R. Sidari, L. Solieri, A. Cufari, P. Giudici, Wine colour adsorption phenotype: An inheritable quantitative trait loci of yeasts, *J. Appl. Microbiol. 103* (2007) 735–742.
24. J.P. Mazauric, J.M. Salmon, Interactions between yeast lees and wine polyphenols during simulation of wine aging: II. Analysis of desorbed polyphenol compounds from yeast lees, *J. Agric. Food Chem. 54* (2006) 3876–3881.
25. M. Rizzo, D. Ventrice, M.A. Varone, R. Sidari, A. Caridi, HPLC determination of phenolics adsorbed on yeasts, *J. Pharm. Biomed. Anal. 42* (2006) 46–55.
26. C. Cuinier, Yeast strains and phenolic composition of red wines, *Vignevini*, *24* (1997) 39–42 (in Italian).
27. A. Caridi, A. Cufari, D. Ramondino, Isolation and clonal pre-selection of enological *Saccharomyces*, *J. Gen. Appl. Microbiol. 48* (2002) 261–268.
28. S.L. Singleton, J.A. Rossi, Colorimetry of total phenolics with phosphomolybdic-phosphotungstic acid reagents, *Am. J. Enol. Vitic. 16* (1965) 144–158.
29. Y. Vasserot, S. Caillet, A. Maujean, Study of anthocyanin adsorption by yeast lees. Effect of some physicochemical parameters, *Am. J. Enol. Vitic. 48* (1997) 433–437.
30. A. Caridi, Enological functions of parietal yeast mannoproteins, *Antonie van Leeuwenhoek*, *89* (2006) 417–422.
31. B. Girard, D. Yuksel, M.A. Cliff, P. Delaquis, A.G. Reynolds, Vinification effects on the sensory, colour and GC profiles of Pinot noir wines from British Columbia, *Food Res. Int. 34* (2001) 483–499.
32. K. Torskangerpoll, Ø.M. Andersen, Colour stability of anthocyanins in aqueous solutions at various pH values, *Food Chem. 89* (2005) 427–440.
33. Y. Jigami, T. Odani, Mannosylphosphate transfer to yeast mannan, *Biochim. Biophys. Acta*, *1426* (1999) 335–345.
34. R.B. Boulton, V.L. Singleton, L.F. Bisson, R.E. Kunkee: *Principles and Practices of Winemaking,* Chapman & Hall, New York, NY, USA (1996).
35. R. Sidari, S. Postorino, A. Caparello, A. Caridi, Evolution during wine aging of colour and tannin differences induced by wine starters, *Ann. Microbiol. 57* (2007) 197–201.
36. P. Romano, Metabolic characteristics of wine strains during spontaneous and inoculated fermentation, *Food Technol. Biotechnol. 35* (1997) 255–260.
37. C.S. Stockley, P.B. Høj, Better wine for better health: Fact or fiction?, *Aust. J. Grape Wine Res. 11* (2005) 127–138.

Table 1. Mean values, standard deviations and homogeneous groups ($p<0.05$) among 20 wine yeasts for the red component of the yeasts grown on chromogenic medium, prepared using grape skins from the black cultivars Greco Nero, Magliocco and Nero d'Avola, and on YPD agar, as measured using Photoshop

Strains	Greco Nero	Magliocco	Nero d'Avola	YPD agar
1042	(153±2)^{e}	(155±2)^{h}	(152±5)^{e}	(192±4)^{c}
12233	**(4±3)^{a}**	**(2±1)^{a}**	**(5±3)^{a}**	(183±4)^{b}
BM45	(171±4)^{f}	(139±2)^{f}	(161±2)^{f}	(196±6)^{c}
ICV D254	(198±4)^{k}	(164±4)^{i}	(177±6)^{ij}	(233±4)^{j}
Sc226	(192±3)^{j}	(141±6)^{f}	(166±3)^{fg}	(212±4)^{fg}
Sc560	(173±6)^{fg}	(147±2)^{g}	(177±2)^{ij}	(205±2)^{de}
Sc708	(181±2)^{hi}	(135±2)^{f}	(149±5)^{e}	(225±7)^{i}
Sc1304	(104±1)^{c}	(26±6)^{b}	(135±1)^{d}	(226±8)^{ij}
Sc1483	(104±5)^{c}	**(3±2)^{a}**	(114±5)^{c}	(209±2)^{def}
Sc1661	(175±4)^{fg}	(140±6)^{f}	(168±5)^{gh}	(183±5)^{b}
Sc1766	(111±5)^{d}	(125±2)^{e}	(165±3)^{fg}	(211±5)^{efg}
Sc1864	(183±2)^{i}	**(176±4)^{k}**	(180±6)^{j}	**(253±1)^{l}**
Sc2489	(62±4)^{b}	(68±6)^{d}	(66±5)^{b}	**(166±5)^{a}**
Sc2621	(171±3)^{fg}	(162±4)^{i}	**(194±5)^{k}**	(203±3)^{d}
Sc2640	(181±4)^{hi}	(167±6)^{ij}	**(189±1)^{k}**	(220±6)^{hi}
Sc2659	(176±4)^{fgh}	(150±6)^{gh}	(174±6)^{hi}	(210±5)^{efg}
Sc2717	(190±6)^{j}	(163±2)^{i}	(180±4)^{j}	(216±3)^{gh}
TT77	(177±1)^{gh}	(153±5)^{gh}	(170±2)^{gh}	(245±6)^{k}
TT173	(113±3)^{d}	(56±3)^{c}	(153±4)^{e}	(210±6)^{defg}
TT254	**(210±7)^{l}**	(171±3)^{jk}	**(189±5)^{k}**	(194±2)^{c}
average	152	123	153	210
CV	34	47	30	10

CV=coefficient of variation
In the same column, different letters in superscript indicate significant differences at 95 % of confidence level; bold are the values included in the first (low value) and in the last (high value) homogeneous group

Table 2. Mean values, standard deviations and homogeneous groups ($p<0.05$) among 20 wine yeasts for the green component of the yeasts grown on chromogenic medium, prepared using grape skins from the black cultivars Greco Nero, Magliocco and Nero d'Avola, and on YPD agar, as measured using Photoshop

Strains	Greco Nero	Magliocco	Nero d'Avola	YPD agar
1042	$(114\pm2)^{f}$	$(120\pm2)^{hi}$	$(118\pm7)^{ef}$	$(180\pm4)^{cd}$
12233	$\mathbf{(1\pm1)^{a}}$	$\mathbf{(1\pm1)^{a}}$	$\mathbf{(1\pm1)^{a}}$	$(159\pm3)^{b}$
BM45	$(140\pm5)^{h}$	$(100\pm2)^{ef}$	$(117\pm2)^{e}$	$(186\pm5)^{de}$
ICV D254	$(169\pm3)^{l}$	$\mathbf{(131\pm4)^{l}}$	$(157\pm6)^{kl}$	$(223\pm5)^{i}$
Sc226	$(161\pm4)^{k}$	$(103\pm5)^{fg}$	$(133\pm3)^{gh}$	$(200\pm5)^{g}$
Sc560	$(136\pm7)^{h}$	$(109\pm1)^{g}$	$(139\pm3)^{hi}$	$(189\pm3)^{ef}$
Sc708	$(141\pm4)^{hi}$	$(95\pm5)^{e}$	$(109\pm3)^{d}$	$(216\pm7)^{h}$
Sc1304	$(79\pm3)^{d}$	$\mathbf{(2\pm1)^{a}}$	$(103\pm1)^{cd}$	$(179\pm8)^{cd}$
Sc1483	$(88\pm4)^{e}$	$\mathbf{(3\pm1)^{a}}$	$(98\pm5)^{c}$	$(201\pm4)^{g}$
Sc1661	$(146\pm5)^{i}$	$(107\pm5)^{g}$	$(143\pm8)^{ij}$	$(174\pm5)^{c}$
Sc1766	$(69\pm3)^{c}$	$(86\pm2)^{d}$	$(126\pm2)^{fg}$	$(200\pm7)^{g}$
Sc1864	$(138\pm3)^{h}$	$(129\pm5)^{kl}$	$(136\pm10)^{hi}$	$\mathbf{(254\pm1)^{j}}$
Sc2489	$(58\pm3)^{b}$	$(61\pm4)^{c}$	$(53\pm3)^{b}$	$\mathbf{(141\pm3)^{a}}$
Sc2621	$(128\pm4)^{g}$	$(124\pm6)^{ijk}$	$(163\pm6)^{lm}$	$(186\pm4)^{de}$
Sc2640	$(152\pm4)^{j}$	$\mathbf{(134\pm7)^{l}}$	$\mathbf{(168\pm5)^{m}}$	$(201\pm4)^{g}$
Sc2659	$(154\pm4)^{j}$	$(115\pm6)^{h}$	$(150\pm9)^{jk}$	$(200\pm6)^{g}$
Sc2717	$(163\pm3)^{k}$	$(123\pm1)^{ij}$	$(157\pm8)^{kl}$	$(212\pm3)^{h}$
TT77	$(125\pm3)^{g}$	$(89\pm8)^{d}$	$(117\pm4)^{e}$	$(225\pm7)^{i}$
TT173	$(76\pm2)^{d}$	$(10\pm6)^{b}$	$(117\pm4)^{e}$	$(195\pm7)^{fg}$
TT254	$\mathbf{(182\pm3)^{m}}$	$(129\pm3)^{jkl}$	$(164\pm2)^{lm}$	$(181\pm3)^{d}$
average	121	89	124	195
CV	38	53	32	13

CV=coefficient of variation
In the same column, different letters in superscript indicate significant differences at 95 % of confidence level; bold are the values included in the first (low value) and in the last (high value) homogeneous group

Table 3. Mean values, standard deviations and homogeneous groups ($p<0.05$) among 20 wine yeasts for the blue component of the yeasts grown on chromogenic medium, prepared using grape skins from the black cultivars Greco Nero, Magliocco and Nero d'Avola, and on YPD agar, as measured using Photoshop

Strains	Greco Nero	Magliocco	Nero d'Avola	YPD agar
1042	(131±4)^{d}	(141±5)^{hij}	(135±6)^{d}	(152±8)^{bc}
12233	**(9±5)^{a}**	(12±7)^{ab}	**(13±6)^{a}**	(151±3)^{b}
BM45	(151±4)^{f}	(118±2)^{f}	(140±5)^{de}	(161±5)^{cd}
ICV D254	(161±3)^{gh}	(143±3)^{hij}	(160±6)^{g}	(210±10)^{i}
Sc226	(165±3)^{h}	(128±7)^{g}	(151±5)^{f}	(165±7)^{de}
Sc560	(161±7)^{gh}	(130±3)^{g}	(163±3)^{g}	(168±5)^{de}
Sc708	(159±3)^{g}	(117±3)^{f}	(134±7)^{d}	(189±7)^{h}
Sc1304	(96±4)^{c}	(18±6)^{b}	(116±2)^{c}	(150±5)^{b}
Sc1483	(97±9)^{c}	**(6±4)^{a}**	(111±3)^{c}	(179±2)^{fg}
Sc1661	(149±3)^{ef}	(130±6)^{g}	149±5)^{f}	(155±6)^{bc}
Sc1766	(95±5)^{c}	(108±6)^{e}	(152±3)^{f}	(166±7)^{de}
Sc1864	(161±4)^{gh}	**(153±7)^{k}**	(160±7)^{g}	**(239±9)^{j}**
Sc2489	(61±5)^{b}	(69±4)^{d}	(58±6)^{b}	**(129±2)^{a}**
Sc2621	(143±2)^{e}	(145±6)^{hijk}	(166±6)^{gh}	(161±4)^{cd}
Sc2640	(160±3)^{gh}	(149±4)^{ijk}	**(172±6)^{h}**	(169±4)^{de}
Sc2659	(161±3)^{gh}	(139±8)^{h}	(162±9)^{g}	(170±8)^{e}
Sc2717	(158±5)^{g}	(140±2)^{hi}	(164±6)^{g}	(182±9)^{gh}
TT77	(152±3)^{f}	(120±4)^{f}	(147±3)^{ef}	(191±7)^{h}
TT173	(99±4)^{c}	(43±7)^{c}	(140±5)^{de}	(172±4)^{ef}
TT254	**(178±3)^{i}**	(147±6)^{ijk}	**(173±7)^{h}**	(167±2)^{de}
average	132	108	138	172
CV	32	46	29	14

CV=coefficient of variation
In the same column, different letters in superscript indicate significant differences at 95 % of confidence level; bold are the values included in the first (low value) and in the last (high value) homogeneous group

Table 4. Mean values, standard deviations and homogeneous groups ($p<0.05$) among 20 wine yeasts for the Folin-Ciocalteu's index, absorbance, intensity and tint parameters determined in the wines produced using the grape must of Magliocco cultivar

Strains	Folin-Ciocalteu's index	$A_{420\ nm}$	$A_{520\ nm}$	$A_{620\ nm}$	Intensity	Tint
1042	(11.4±0.3)^{cd}	(1.001±0.004)^{ef}	(1.097±0.003)^{f}	(0.205±0.001)^{d}	(2.303±0.007)^{d}	(0.913±0.001)^{f}
12233	(11.8±0.3)^{fg}	(1.045±0.004)^{i}	(1.199±0.003)^{l}	(0.213±0.001)^{e}	(2.457±0.008)^{i}	(0.872±0.001)^{c}
BM45	(12.1±0.0)^{ij}	(1.093±0.002)^{l}	(1.217±0.001)^{m}	(0.236±0.003)^{h}	(2.546±0.002)^{k}	(0.898±0.002)^{d}
ICV D254	(11.7±0.1)^{efg}	(1.065±0.003)^{j}	(1.103±0.003)^{g}	(0.218±0.002)^{ef}	(2.386±0.008)^{f}	(0.966±0.002)^{l}
Sc226	**(10.4±0.0)^{a}**	**(0.948±0.002)^{a}**	(0.988±0.004)^{b}	**(0.186±0.002)^{a}**	**(2.122±0.006)^{a}**	(0.960±0.003)^{k}
Sc560	(11.5±0.1)^{cde}	(0.992±0.003)^{d}	(1.053±0.003)^{d}	(0.195±0.004)^{b}	(2.240±0.010)^{c}	(0.942±0.002)^{i}
Sc708	(11.4±0.1)^{c}	(0.977±0.003)^{c}	(1.165±0.005)^{i}	(0.245±0.002)^{i}	(2.388±0.009)^{f}	**(0.838±0.001)^{a}**
Sc1304	(12.3±0.1)^{jk}	(0.989±0.002)^{d}	(1.041±0.003)^{c}	(0.195±0.003)^{b}	(2.224±0.007)^{b}	(0.950±0.001)^{j}
Sc1483	(12.8±0.0)^{m}	**(1.516±0.007)^{p}**	**(1.806±0.006)^{o}**	**(0.446±0.005)^{k}**	**(3.768±0.017)^{n}**	**(0.840±0.001)^{a}**
Sc1661	(11.6±0.1)^{def}	(1.083±0.002)^{k}	(1.150±0.001)^{h}	(0.222±0.002)^{fg}	(2.455±0.004)^{i}	(0.941±0.001)^{i}
Sc1766	(11.1±0.1)^{b}	(1.007±0.004)^{fg}	(1.083±0.005)^{e}	(0.199±0.004)^{bc}	(2.289±0.012)^{d}	(0.930±0.001)^{h}
Sc1864	(12.0±0.1)^{hi}	(1.000±0.004)^{e}	(1.105±0.003)^{g}	(0.219±0.005)^{ef}	(2.323±0.009)^{e}	(0.905±0.003)^{e}
Sc2489	(12.5±0.1)^{kl}	(1.138±0.005)^{n}	(1.174±0.003)^{j}	(0.245±0.003)^{i}	(2.557±0.004)^{k}	(0.969±0.006)^{m}
Sc2621	(11.8±0.1)^{gh}	(1.045±0.004)^{i}	(1.160±0.003)^{i}	(0.218±0.004)^{ef}	(2.422±0.010)^{h}	(0.901±0.002)^{de}
Sc2640	**(13.6±0.1)^{o}**	(1.195±0.004)^{o}	(1.295±0.004)^{n}	(0.254±0.002)^{j}	(2.745±0.010)^{m}	(0.923±0.001)^{g}
Sc2659	(12.4±0.1)^{kl}	(0.956±0.002)^{b}	**(0.958±0.001)^{a}**	(0.203±0.004)^{cd}	**(2.116±0.005)^{a}**	**(0.998±0.003)^{n}**
Sc2717	(12.5±0.1)^{l}	(1.009±0.004)^{g}	(1.163±0.008)^{i}	(0.225±0.009)^{g}	(2.397±0.021)^{fg}	(0.867±0.002)^{b}
TT77	(13.3±0.1)^{n}	(1.115±0.002)^{m}	(1.185±0.001)^{k}	(0.227±0.004)^{g}	(2.527±0.005)^{j}	(0.941±0.002)^{i}
TT173	(13.3±0.0)^{n}	(1.021±0.001)^{h}	(1.173±0.002)^{j}	(0.218±0.003)^{ef}	(2.412±0.004)^{gh}	(0.870±0.002)^{bc}
TT254	(12.9±0.1)^{m}	(1.143±0.005)^{n}	(1.216±0.003)^{m}	(0.227±0.002)^{g}	(2.587±0.009)^{l}	(0.940±0.002)^{i}
average	12.1	1.067	1.167	0.23	2.463	0.918
CV	7	11	14	23	14	5

CV=coefficient of variation

In the same column, different letters in superscript indicate significant differences at 95 % of confidence level; bold are the values included in the first (low value) and in the last (high value) homogeneous group

Table 5. Correlation coefficients and their statistical significance among the red, green, and blue components of the yeasts, grown on chromogenic medium prepared using grape skins from Magliocco cultivar, as measured using Photoshop, and the related Folin-Ciocalteu's index, absorbance, intensity and tint parameters determined in the wines produced using grape must from the same cultivar

	Folin-Ciocalteu's index		$A_{420\ nm}$		$A_{520\ nm}$		$A_{620\ nm}$		Intensity		Tint	
	CC	S	CC	S	CC	S	CC	S	CC	S	CC	S
red	−0.1392	0.5582	−0.3675	0.1109	−0.4309	0.0578	−0.4150	0.0688	−0.4120	0.0711	0.3164	0.1741
green	−0.1884	0.4264	−0.2918	0.2119	−0.3724	0.1059	−0.3454	0.1358	−0.3446	0.1368	0.3373	0.1458
blue	−0.1876	0.4284	−0.3704	0.1080	−0.4405	0.0519	−0.4175	0.0670	−0.4182	0.0665	0.3420	0.1399

CC=correlation coefficient, S=significance

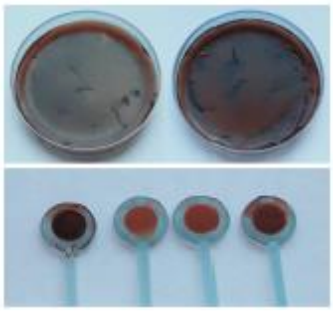

Fig. 1. Biomass of a yeast strain with low grape pigment adsorption ability (top left) and of a yeast strain with high grape pigment adsorption ability (top right) grown on the chromogenic medium prepared using grape skins. Yeast biomass of four yeast strains spread on calibrated loops (bottom). The images were processed for colour using Adobe Photoshop CS for Windows XP

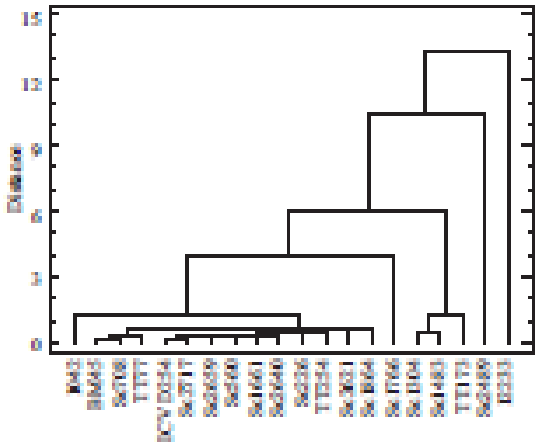

Fig. 2. Dendrogram showing the clusters of 20 wine yeast strains obtained using the nearest neighbour method based on the squared Euclidean distance. The clustering was based on red, green, and blue components of the yeast biomass grown on chromogenic medium prepared using grape skins from the black cultivars Greco Nero, Magliocco and Nero d'Avola, as measured using Photoshop