

Optimization of the Operating Conditions for Color Correction in White Wines Based on the Use of Yeast as Fining Agent

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ABSTRACT: Sherry-type pale white wines were subjected under different stirring times to color correction treatments based on the use of variable concentrations of free yeast and a single concentration of yeast immobilized on carrageenan gel. At all concentrations tested, the free yeasts decreased the A420 of the initial wine. The immobilized yeast showed slightly lower effectiveness to retain browning compounds of the wine and exhibited no significant differences among the treatment times assayed. Choosing the most unfavorable conditions (24 h), significant decreases in the concentrations of low-molecular-weight phenolic compounds were observed. The sensory properties of the wines treated with the immobilized yeast were shown to be similar to those of wines treated with activated charcoal for flavor, better for color, and slightly worse for aroma.

Keywords: browning, immobilized yeast, white wines, fining agents

Introduction

Browning is a well-known alteration process that changes not only the color of white wine, but also its flavor. The process originates from phenolic compounds in the wine, which partly can undergo oxidation followed by polymerization, but also direct condensation with other substances such as acetaldehyde or glyoxylic acid to give yellowish-brown compounds (Fulcrand and others 1996; Es-Safi and others 1999, 2000, 2002, 2003; Clark and Scollary 2002; Clark and others 2003). Oxidation of the phenols is a slow process through which wine gradually loses its characteristics after bottling and is then rejected by consumers. The time that wine takes to become unfit for consumption depends on its type, and for wines with a distinctive pale color, browning is especially unfavorable. For example, a degree of browning measured as absorbance at 420 nm above 0.170 to 0.180 au usually causes rejection of sherry fino type wines.

Because no definitely effective procedure for avoiding browning after bottling of wine exists, it is very important to ensure that the wine is pale enough before it is bottled (for example with an A420 value about 0.100 au for fino sherry wines). In this way, a wine's time of life in the market can be expanded. This is usually accomplished by using fining agents to correct browning of the beverage prior to its bottling. The 2 most widely used agents for this purpose are activated charcoal and polyvinylpyrrolidone (PVPP) (Faldes 1989; Zoecklein and others 1990; Gorinstein and others 1993; Baron and others 1997). However, at the present time, the food industry tends to reduce or avoid the use of synthetic or chemically treated compounds as food additives or technological aids for industrial treatments. Finding natural compounds that will effectively replace those traditionally used to avoid wine browning is of interest.

Yeasts can effectively retain phenols (Bourzeix and Heredia

1976; Augustin 1986; Cuinier 1988; Salmon and others 2002) and some colored compounds such as those involved in the pinking of white wines (Dubourdieu 1995) or their browning (Razmkhab and others 2002). Based on this last ability of yeasts, Bonilla and others (2001) developed a procedure that uses them in the fining treatment for sherry fino type white wines. These authors found yeast to be similarly effective to activated charcoal and even more effective than PVPP. However, the operating conditions of the treatment with yeast require some improvement to avoid problems. The yeast-wine contact time could be reduced from the proposed 24 h to allow a shorter operation (which ideally would be continuous), which is always desirable on an industrial level. Somewhat more problematic is removing the yeast after treatment. Certainly, winemakers usually filter the wine to ensure its biological stability after bottling. In theory, this practice dispenses with the need for additional treatments to eliminate the yeast used as fining agents. However, the filtration of much yeast leads to a fast clogging of filters with less than 1 μm of pore size, which then must be replaced, resulting in added costs. With traditional fining agents, this problem can be resolved by using larger particle sizes, but with microorganisms such as yeasts, this solution is not possible.

Immobilized yeasts have been used in winemaking with the main aim of improving control over alcoholic fermentation (Kourkoutas and others 2003). One advantage of immobilized yeasts is that they can be used on a variety of supports in regard to their nature and final particle size. Therefore, it would be interesting to examine their ability to retain wine browning compounds, while using particle sizes that allow for easy removal.

In this work, the ability of free and immobilized yeast cells to retain browning compounds in white wines was studied. In addition, the optimum conditions were determined regarding concentration and wine-yeast contact time with a view to their use as fining agents.

Materials and Methods

Experimental

Unclarified white wines, obtained by biological aging in the

MS 20040120 Submitted 2/27/04, Revised 3/25/04, Accepted 6/22/04. Authors are with Dept. of Agricultural Chemistry, Faculty of Sciences, Univ. of Cordoba, Edificio C3, Campus de Rabanales, E-14014, Cordoba, Spain. Direct inquiries to author Medina (E-mail: vitenol@uco.es).

Montilla-Moriles region (southern Spain) and containing $15 \pm 0.3\%$ v/v ethanol, were subjected to different fining treatments with yeasts to correct browning.

In a 1st experiment, 1-L samples of wine were treated with dehydrated bakers' yeast (Mauripan Fleischmann, Montreal, Canada) at 6 different concentrations (0.5, 1, 2, 3, 4, and 5 g/L) in 14.5×11 cm cylindrical containers. Treatments were performed in triplicate, using a magnetic stirrer at 100 rpm and 1 h of wine-yeast contact.

In a 2nd experiment, 1-L samples of the same type of wine, but with a higher degree of browning, were treated with a 1 g/L concentration of bakers' yeast. The treatments were also carried out in triplicate, using the same stirring speed but variable wine-yeast contact times (15, 30, 60, and 120 min).

In a 3rd experiment, yeast was immobilized on carrageenan gels in the form of cylindrical beads 2 mm thick and 4.75 mm in dia (Medina and others 2002). One-liter samples in triplicate of the same type of wine, with a similar degree of browning as the ones used for the 1st experiment, were treated with the gel beads at a concentration of 2 g yeast/L and different stirring times (1, 2, 4, 6, and 24 h).

Analytical procedures

Spectrophotometric measurements at 280 nm (after 1:10 dilution) and 420 nm were made with a Beckman spectrophotometer, DU 600 model (Beckman Coulter Inc., Fullerton, Calif., U.S.A.), on 10 mm pathlength.

Extraction of phenolic compounds

A volume of 100 mL of wine was concentrated in vacuum at 40 °C up to 20 mL, which was adjusted to pH 7 with 0.1 M NaOH. The concentrate was passed through a Sep-Pak C_{18} cartridge, with 900 mg of filling (Long Body Sep-Pak Plus; Waters Associates; Milford, Mass., U.S.A.) that was previously activated with 8 mL methanol and washed with distilled water that was adjusted to pH 7 with NaOH according to Jaworski and Lee (1987). The cartridge was eluted with 8 mL water at pH 7. This volume, in addition to the volume obtained as a result of the sample run-through prior to the elution, was used for the determination of the phenolic acid fraction. After preconditioning of the cartridge with 2 mL of water at pH 2, the flavan-3-ol fraction was eluted with 8 mL of 16% acetonitrile in water at pH 2 (Oszmianski and others 1988). The 2 collected fractions were concentrated and passed through a filter of 0.45 μ m pore size for injection into a Spectra-Physics (San Jose, Calif., U.S.A.) P4000 HPLC instrument.

Identification and HPLC analysis

The identification of the phenolic compounds was achieved by comparing with the retention times of the standards, UV spectra obtained by high-performance liquid chromatography Dyode Array (Spectra-Physics UV6000LP), and calculation of UV absorbance ratios after co-injection of samples and standards (Fabios and others 2000). Commercial standards were purchased from Sigma-Aldrich Chemical Co. (Madrid, Spain) and Sarsynthese Co. (Genay, France). Caftaric and coutaric acids were isolated by the method described by Singleton and others (1978). Procyanidins were obtained from a grape seed extract according to Bourzeix and others (1986). The standards' purity was 95% to 99%. Each compound was quantified by comparison with a calibration curve obtained with the corresponding standard, except the procyanidins B3 and B4, which were quantified as procyanidin B1.

Analyses were carried out on a LiChrospher 100 RP-18 column (250 mm \times 4.6 mm, 5- μ m particle size) by using 2% aqueous acetic acid and acetonitrile as mobile phases at a flow rate of 2 mL/min and detection at 280 nm.

Phenolic acid fraction. The elution phases for this fraction were as follows: gradient elution from 0% to 5% CH_3CN in 5 min, isocratic elution for 10 min, gradient elution up to 15% CH_3CN in 5 min, isocratic elution for 10 min, gradient elution up to 100% CH_3CN in 10 min, and isocratic elution for 10 min. In this fraction were quantified the following compounds: gallic, protocatechuic, vanillic, syringic, caffeic, *trans p*-coumaric, ferulic, *trans*-caftaric, *cis*-coutaric acids, and tyrosol.

Flavan-3-ol fraction. The elution phases for this fraction were as follows: gradient elution from 0% to 15% CH_3CN in 5 min, isocratic elution for 10 min, gradient elution up to 20% CH_3CN in 5 min, gradient elution up to 30% CH_3CN in 10 min, gradient elution up to 100% CH_3CN in 10 min, and isocratic elution for 10 min. In this fraction were quantified: (+)-catechin, (-)-epicatechin, and procyanidins B1, B2, B3, and B4.

HPLC direct injection. To avoid possible retention by the Sep-Pak cartridge affecting the browning products, all the wine samples were subjected to a direct injection after filtration through a 0.45- μ m-pore-size filter. The chromatograms registered at 420 nm showed a group of overlapped peaks eluted at high retention times and absorbing at this wavelength, thereby corresponding to colored compounds (named "browning peaks"). The elution conditions were the same as the ones used for the phenolic acid fraction. Because these peaks showed a higher absorbance at 280 nm than at 420 nm, the former wavelength was used for their quantification (expressed as gallic acid).

Sensory tests

Wine samples were subjected to 3 sensory tests. In the 1st, the samples were subjected to a triangle test (ISO 4120-1983) for aroma and flavor. The 2nd test was a paired comparison (ISO 5495-1983) to evaluate the color. In the 3rd (ISO 4121-1987), the wines were scored for color, aroma, and flavor on a scale from 1 to 6 (6 = perfect, 5 = typical, 4 = typical with slight deviations, 3 = perceptible deviations, 2 = with important defects, 1 = entirely faulty). According to the ISO 6658-1985 standard, 37 tasters were chosen for the realization of the 1st two tests and 4 expert tasters for the last.

Statistical procedures

Variance analyses were performed on the replicated samples by using Statgraphics Statistical Computer Package (Statistical Graphics Corp., Rockville, Md., U.S.A.)

Results and Discussion

Figure 1 shows the percent decrease in the absorbances at 280 and 420 nm of wine samples treated with variable concentrations (0.5, 1, 2, 3, 4, and 5 g/L) of the free yeast for a stirring time of 60 min. The absorbance values for the initial wine ($A_{280} = 8.196$ 0.01 au and $A_{420} = 0.166$ 0.001 au) were both decreased, albeit in a nonlinear way, with all the yeast concentrations used. For total polyphenols, measured as A_{280} , a yeast concentration of 2 g/L provided maximal retention, with no significant differences ($P < 0.01$) from the higher doses. However, the color due to browning products (measured as A_{420}) was decreased in a more proportional way. From a practical view, the concentration of 1 g/L proved to be the most desirable, because it exhibited no significant differences from the 2-g/L concentration. The concentration of 1 g/L showed significant differences with the higher doses, but the cost of the increase in the yeast concentration is not compensated by the higher retention of colored compounds (for the 5 g/L concentration used the retention increased by only 10%).

These results are better than those reported by Bonilla and others (2001) who used the same yeast concentrations but without

stirring during the treatment. Thus, the ability of yeast to retain brown-colored polymers increased from 23% to 33% as a result of stirring, reflecting the favorable effect of better wine-yeast contact. In this respect, it should be pointed out that in the absence of stirring all the fining agents tend to decant and to get lumpy at the bottom of the container (such as the treatment in the static experiment used by the above-mentioned authors). As a result, the wine-finishing agent contact is hindered to an extent dependent on the particle size of the agent. On the other hand, the use of yeast in a dynamic environment (under stirring) provides a 2nd advantage, because it allows the treatment to be expedited, reducing the 24 h in the static regimen to much shorter times.

To optimize the treatment duration, a 2nd experiment with variable stirring times for the yeasts in contact with the wine (15, 30, 60, and 120 min) was carried out. For the development of this experiment the same type of wine, but with different absorbance values (namely, $A_{280} = 9.29 \pm 0.01$ au and $A_{420} = 0.199 \pm 0.001$ au), was used. This was expected because in this 2nd experiment we used the yeast concentration that provided the best result in the 1st experiment (1 g/L). In addition, using wine with a higher degree of browning is useful with a view to comparing the ability of the yeast to retain different concentrations of the browning compounds. Figure 2 shows the percent decrease, with respect to the initial wine, in the absorbances measured at the different stirring times used. As can be seen, only the shortest contact time (15 min) was inadequate to reach a degree of retention of colored compounds similar to that provided by a yeast concentration of 1 g/L in the 1st experiment. Also, there were no significant differences ($P < 0.01$) in A_{420} beyond 30 min of stirring. In terms of absolute values of absorbance, however, the yeast exhibited an increased retention capacity with the most severely browned wine. Thus, with a yeast concentration of 1 g/L and a stirring time of 60 min, A_{420} decreased from 0.166 ± 0.001 au to 0.111 ± 0.004 au (0.055 au) in the 1st experiment and from 0.199 ± 0.001 au to 0.130 ± 0.001 au (0.069 au) in the most severely browned wine. These results confirm previous findings by other authors (Razmkhab and others 2002), who suggested that yeast exhibits some specificity for browning compounds at a higher degree of polymerization, the concentration of which increases as browning develops.

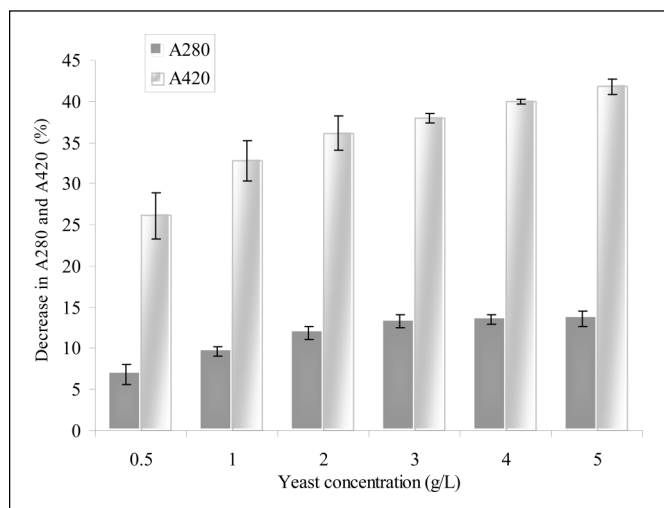


Figure 1—Decrease in the absorbances measured at 280 and 420 nm of the wines treated with different concentrations of free yeast. Stirring time 1 h.

With regard to total polyphenols (measured as A_{280}), it should be pointed out that for all the stirring times assayed, the percent retention of UV-absorbing compounds was 1.5 to 2 times higher in the more browned wine than in the less browned one treated with 1 g/L of yeast. This differential behavior can reasonably be explained by considering the hypothesis that highly polymerized brown compounds may have a higher molar absorptivity coefficient than less polymerized ones. This has been pointed out by authors such as Ortega and others (2003) who examined oxidative aging in sherry wines, as characterized by a darkening of color as a result of a marked increase in the concentration of brown polymers. Based on the above-mentioned hypothesis, a potentially higher retention of the most polymerized compounds by yeast should be better observed at the wavelength where they exhibit the highest molar absorptivity coefficient, this is at 280 nm and on the most browned wine.

On the whole, the previous experiments showed that bakers' yeast can be used as an effective agent to correct browning in white wine under conditions close to those of a continuous treatment, with its inherent advantages from an industrial-scale viewpoint. However, for its application, the problem of yeast removal after the treatment must be resolved. Certainly, before bottling, it is a common winemaking practice to use a microbiological stabilization process by filtering the wine across a membrane of small pore size (0.45 to 1.2 μm). However, this operation is only affordable when the number of microorganisms to be removed is fairly small. Otherwise the filtration membrane is quickly clogged and must be replaced. This considerably raises the treatment costs because of the high price of this type of membrane. Obviously, a 1-g/L concentration is high enough to discard direct removal of the yeast by filtration.

To avoid this problem, a 3rd experiment in which yeasts were immobilized on a polysaccharide gel with a bead size of 2 mm was performed. In this way, yeast removal through industrial filters of a relatively large pore size is simple. Because immobilizing the yeast might to some extent hinder the interaction with browning compounds in the wine. In this experiment we used a yeast concentration of 2 g/L and different stirring times to better observe the

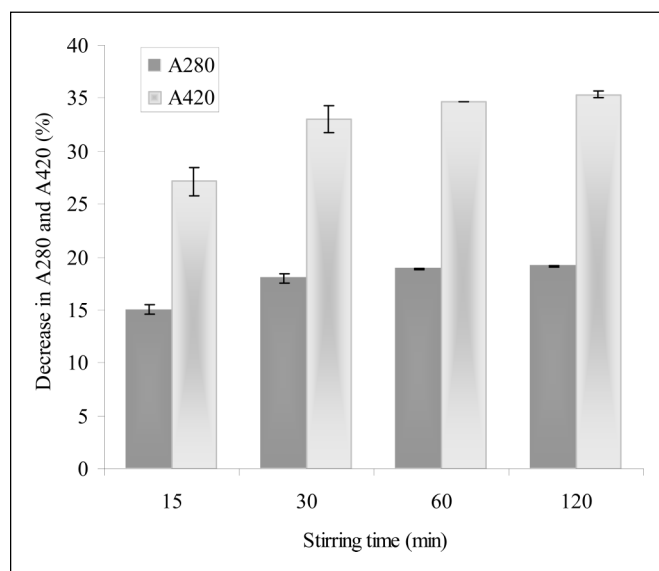


Figure 2—Decrease in the absorbances measured at 280 and 420 nm of the wines treated with 1 g/L of free yeast and different stirring times

changes. The A280 and A420 for the initial wine used in this experiment were 8.90 ± 0.04 au and 0.170 ± 0.001 au respectively. Figure 3 shows the percent decrease in the 2 absorbances measured at the times studied (1, 2, 4, 6, and 24 h). Overall, immobilizing the yeast slightly reduced its ability to retain browning compounds absorbing in the visible spectral region. Thus, while a 2-g/L concentration of free yeast decreased A420 about 36% (Figure 1), an identical concentration of immobilized yeast reduced it by only 27%, both with 1 h of stirring. On the other hand, taking into account the standard errors, the use of a longer treatment time (up to 6 h) had no effect on the retention capacity of the yeast, showing the results obtained with 1 h of stirring to be only significantly different one from those provided by 24 h of treatment.

It is well known that the treatments affecting wine color cause the retention of variable amounts of phenolic compounds. Because these compounds have beneficial effects on health, it is interesting to examine their behavior before and after application of a fining agent. Taking into account that the retention of these compounds would increase with increasing wine-yeast contact time, for this study the wine stirred with immobilized yeast for 24 h (representing the most unfavorable situation) was chosen.

Table 1 lists the contents of hydroxybenzoic acids, hydroxycinnamic acids, and esters; flavan-3-ol monomers and dimers; and tyrosol, before and after treatment with immobilized yeast at a concentration of 2 g/L. Table 1 also shows the combined area (expressed as gallic acid) of a group of overlapped peaks with high retention times corresponding to browning products (BPs) absorbing in the visible region, as well as the significant differences at 3 different *P* levels revealed by a one-way analysis of variance performed for each compound. As can be seen, with the exception of *p*-coumaric acid, all the compounds studied exhibited decreases in their contents with variable significance. It should be pointed out that the contents of many phenolic compounds of white wine are very low, so even a marked decrease will have no substantial effect on the entire phenolic fraction. However, a small decrease in some major phenols can have a strong effect on the overall presence of these compounds in wine. Such was the case with tyrosol, (+)-catechin, hydroxycinnamic esters, some procyanidins (B1 and B3), and BPs, although the decreases of these are interesting because they are colored compounds.

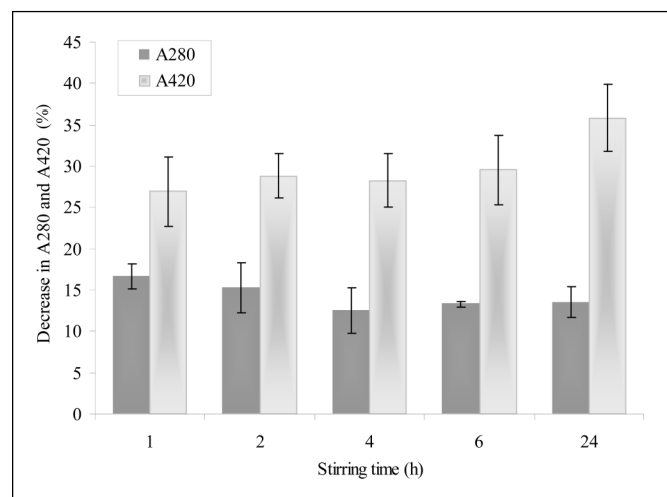


Figure 3—Decrease in the absorbances measured at 280 and 420 nm of the wines treated with 2 g/L of immobilized yeast and different stirring times

Table 1—Phenolic compounds (mg/L) in the wines before and after treatment with immobilized yeast (2 g/L, 24 h)

	Before treatment	After treatment	
Gallic acid	4.64 ± 0.606	3.62 ± 0.036	**
Protocatechuic acid	4.17 ± 0.49	1.98 ± 0.076	**
Vanillic acid	1.21 ± 0.051	0.720 ± 0.117	**
Syringic acid	1.11 ± 0.121	0.837 ± 0.104	*
Caffeic acid	0.348 ± 0.008	0.227 ± 0.001	***
<i>p</i> -Coumaric acid	0.31 ± 0.038	0.356 ± 0.035	—
Ferulic acid	0.563 ± 0.078	0.208 ± 0.023	**
<i>trans</i> -Caftaric acid	16.8 ± 0.65	11.6 ± 1.28	**
<i>cis</i> -Coutaric acid	16.9 ± 1.65	11.2 ± 1.45	*
Tyrosol	90.8 ± 1.65	63.7 ± 10.6	*
(+)-Catechin	38.0 ± 1.61	26.3 ± 4.90	*
(-)-Epicatechin	11.5 ± 0.63	8.21 ± 1.29	*
Procyanidin B1	17.2 ± 0.41	13.5 ± 0.73	***
Procyanidin B2	5.03 ± 0.705	3.39 ± 0.098	**
Procyanidin B3	25.0 ± 2.75	16.5 ± 1.67	**
Procyanidin B4	8.88 ± 1.13	4.65 ± 0.476	**
Browning peaks	13.7 ± 0.26	11.6 ± 0.36	***

****P* < 0.001; ***P* < 0.01; **P* < 0.05

On the other hand, the potential impact of the treatment with immobilized yeast on the color, flavor, and aroma of the wine was also examined by taste. To this end, two 1st sensory tests according to ISO 4120-1983 (aroma and flavor) and ISO 5495-1983 (color) were performed. The wines were tasted before and after treatment with gel beads for 24 h by a panel of 37 tasters who performed a triangle test for aroma and flavor (identifying the different sample), and a paired comparison test for color. The tasters found the treated wine to differ in aroma (81.2%), flavor (56.8%), and color (100%) from the untreated wine, with these differences significant at the *P* < 0.001 level.

To evaluate quality, the wines treated with immobilized yeast were compared with wines clarified by using traditional fining agents, such as activated charcoal and PVPP in their typical doses for sherry wines (Bonilla and others 2001). To this end, a 3rd sensory test (ISO 4121-1987) was conducted by 4 expert tasters who used a scale from 1 to 6 for the color, aroma, and flavor of the wines.

As can be seen from Figure 4, the treatment of 24 h with yeast gel beads provided slightly better results than the traditional treatments regarding wine color. On the other hand, the tasters found no differences in flavor between the wines treated with the gel and those treated with activated charcoal, although the former received slightly lower aroma scores. The wines treated with PVPP received the lowest scores for the 3 sensory properties evaluated, reflecting a decreased efficiency in reducing color, and in altering flavor and, particularly, aroma.

Conclusions

The use of immobilized yeast as a fining agent to correct color in white wines was found to be clearly advantageous over that of free yeast because of easy removal after the treatment. However, immobilized yeast is slightly less effective in removing browning compounds and the need to use a support raises the costs of the process. It is therefore difficult to assess, in economic terms, the suitability of using free or immobilized yeast. In our opinion, the decision would depend on the degree of browning of the wine to be treated. As browning increases, the yeast concentration to be used will increase, also quickly clogging the necessary microbiological filters for yeast removal; therefore, the use of immobilized yeast may be preferable under these conditions. On the other hand, the phenolic compounds were quantified and sensory properties as-

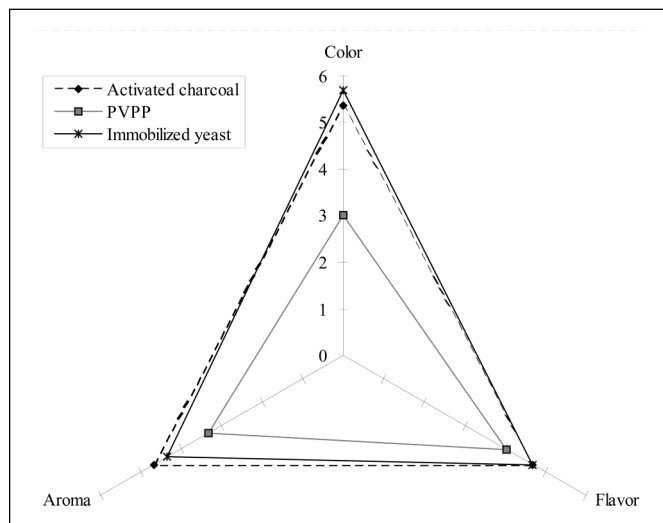


Figure 4—Scores by expert tasters for the color, flavor, and aroma of the treated wines with activated charcoal, PVPP, and immobilized yeast

sessed in terms of the treatment involving the longest wine-finishing agent contact time. Therefore, one can reasonably think that the removal of some of the phenolic compounds and the effect on the sensory properties of the wine might be negligible with shorter treatments. In any case, further research is needed with a view to improving the efficiency of immobilized yeast gels, particularly in relation to their characteristics, such as the type of polysaccharide used, bead size, and polysaccharide-yeast ratio. In this respect, one can reasonably assume that altering some or all of these characteristics may result in improved contact of the yeasts with the browning products and, hence, in more efficient retention.

Acknowledgments

The authors gratefully acknowledge financial support from the Spanish Government, Dept. of Science and Technology (AGL-2002-04154-CO2-02), for the realization of this work.

References

Augustin MM. 1986. Étude de l'influence de certains facteurs sur les composés phénoliques du raisin et du vin [Ph.D. thesis]. France: Univ. Bordeaux. Available from: Univ. Bordeaux Bibliothèque Sciences et Techniques. 120 p.

Baron R, Mayen M, Merida J, Medina M. 1997. Changes in phenolic compounds and color in pale sherry wines subjected to fining treatments. *Z Lebensm Unters Forsch A* 205:474–8.

Bonilla F, Mayen M, Merida J, Medina M. 2001. Yeast used as fining treatment to correct browning in white wines. *J Agric Food Chem* 49:1928–33.

Bourzeix M, Heredia N. 1976. Étude des colorants anthocyaniques fixés par les levures de vinification. *CR Acad Agric* 750–3.

Bourzeix M, Weyland D, Heredia N. 1986. Étude des catéchines et des procianidols de la grappe de raisin, du vin et d'autres dérivés de la vigne. *Bull OIV* 59:1171–254.

Clark AC, Scollary GR. 2002. Copper (II)-mediated oxidation of (+)-catechin in a model white wine system. *Aus J Grape Wine Res* 8:186–95.

Clark AC, Prenzler PD, Scollary GR. 2003. The role of copper(II) in the bridging reactions of (+)-catechin by glyoxylic acid in a model white wine. *J Agric Food Chem* 51:6204–10.

Cuinier C. 1988. Influence of yeasts on the phenolic compounds of wine. *Bull OIV* 61:596–601.

Dubourdieu D. 1995. Intérêts œnologiques et risques associés à l'élevage des vins blancs sur lies en barriques. *Rev Fr OEnol* 155:30–5.

Es-Safi NE, Fulcrand H, Cheynier V, Moutounet M. 1999. Competition between (+)-catechin and (-)-epicatechin in acetaldehyde-induced polymerization of flavanols. *J Agric Food Chem* 47:2088–95.

Es-Safi NE, Guerneve C, Cheynier V, Moutounet M. 2000. New phenolic compounds formed by evolution of (+)-catechin and glyoxylic acid in hydroalcoholic solution and their implication in color changes of grape-derived foods. *J Agric Food Chem* 48:4233–40.

Es-Safi NE, Cheynier V, Moutounet M. 2002. Role of aldehydic derivatives in the condensation of phenolic compounds with emphasis on the sensorial properties of fruit-derived foods. *J Agric Food Chem* 50:5571–85.

Es-Safi NE, Cheynier V, Moutounet M. 2003. Effect of copper on oxidation of (+)-catechin in a model solution system. *Int J Food Sci Tech* 38:153–63.

Fabios M, Lopez-Toledano A, Mayen M, Merida J, Medina M. 2000. Phenolic compounds and browning in sherry wines subjected to oxidative and biological aging. *J Agric Food Chem* 48:2155–9.

Fialdes E. 1989. Les charbons actifs en Œnologie. *Revue des Oenologues* 54:19–22.

Fulcrand H, Doco T, Es-Safi NE, Cheynier V, Moutounet M. 1996. Study of the acetaldehyde-induced polymerisation of flavan-3-ols by liquid chromatography-spray mass spectrometry. *J Chromatogr* 752:85–91.

Gorinstein S, Weisz M, Zenser M, Tilis K, Stiller A, Flam I, Gat Y. 1993. Spectroscopic analysis of polyphenols in white wines. *J Ferment Bioeng* 75:115–20.

Jaworski A, Lee CY. 1987. Fractionation and HPLC determination of grape phenolics. *J Agric Food Chem* 35:257–9.

Kourkoutas Y, Kanellaki M, Koutinas AA, Banat IM, Marchant R. 2003. Storage of immobilized yeast cells for use in wine-making at ambient temperature. *J Agric Food Chem* 51:654–8.

Medina M, Merida J, Mayen M, Lopez-Toledano A, inventors. 2002. New uses of gels of immobilized yeasts. European patent P200200448.

Ortega AF, Lopez-Toledano A, Mayen M, Merida J, Medina M. 2003. Changes in color and phenolic compounds during oxidative aging of sherry white wine. *J Food Sci* 68:2461–8.

Oszmianski J, Ramos T, Bourzeix M. 1988. Fractionation of phenolic compounds in red wines. *Am J Enol Vitic* 39:259–62.

Razmkhab S, Lopez-Toledano A, Ortega JM, Mayen M, Merida J, Medina M. 2002. Adsorption of phenolic compounds and browning product in white wines by yeasts and their cell walls. *J Agric Food Chem* 50:7432–7.

Salmon JM, Fornairon-Bonnefond C, Mazaucic JP. 2002. Interactions between wine lees and polyphenols: Influence on oxygen consumption capacity during simulation of wine aging. *J Food Sci* 67:1604–9.

Singleton VL, Timberlake CF, Lea AGH. 1978. The phenolic cinnamates of white grapes and wine. *J Sci Food Agric* 29:403–10.

Zoecklein B, Fugelsang KC, Gump BH, Nury FS. 1990. Production wine analysis. New York: Van Nostrand Reinhold.