Yeasts Used As Fining Treatment To Correct Browning in White Wines

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White wine was subjected to several fining treatments using baker's yeast at concentrations of 0.5, 1, 2, 3, 4, and 5 g/L. At all these concentration levels, the yeasts decreased the color of the wine in different degrees. The wine samples treated with the higher yeast concentration were subjected to analysis of phenolic compounds by HPLC and found to exhibit significantly decreased contents of vanillic, syringic and *c*-coutaric acids, and procyanidins B2 and B4, and colored compounds eluted at high retention times. The efficiency of the yeast-based fining treatment (1 g/L) was compared with traditional treatments such as those involving the use of activated charcoal or PVPP, which were employed at the usual concentrations in Sherry winemaking. This yeast treatment was found to provide results similar to those of the activated charcoal treatment in terms of A_{420}. Likewise, significant differences in the degree of retention of various phenols were observed among the three treatments compared. Finally, the wine samples obtained with the different treatments were subjected to a sensory panel. All the wines were found to exhibit improved color, aroma, and flavor with respect to the untreated samples, although the treatment using yeast at 1 g/L provided the best results in terms of aroma.

Keywords: *Fining treatments; browning; white wine; yeast*

INTRODUCTION

Phenolic compounds are known to be responsible for wine browning. Specifically, flavan-3-ol derivatives have been pointed out as effective precursors for the browning reactions (*1*−*5*). This color alteration is particularly undesirable in white wine. In fact, it shortens its life in the market after bottling. However, significant advances in this topic in recent years have been reached, mainly acting from two views: decreasing the phenolic content in the must and protecting the wine from the action of atmospheric oxygen.

Techniques as hyperoxidation decrease the content in phenolic compounds in must, thereby giving rise to wines more resistant to browning (*6*). In practice, however, the results depend on the particular grape variety used in winemaking (*7*, *8*). Likewise, phenolic compounds have been found to be healthy in several aspects (*9*, *10*−*13*), so decreasing their contents in wine might be regarded as lowering its nutritional quality. On the other hand, protecting the wine from atmospheric oxygen by using improved grape pressing procedures, maintaining it under a nitrogen atmosphere, and using stoppers of better quality, also delay browning.

In industrial practice, the above-mentioned procedures are taken as a complement to fining treatments because of the need to bottle white wine with a pale color to extend its life in the market. Activated charcoal and PVPP are two fining agents typically used to correct browning in white wine (*14*−*17*). However, they have been found to alter its flavor to an extent that increases in parallel with the concentration of the agent used (*18*−*20*).

Yeast membranes possess the ability to retain some compounds present in the wine (*21*). Particularly, fermentation yeasts have been shown to retain anthocyanins in a variable extent in red wine (*22*−*24*). Likewise, very pale Sherry wine exhibits no browning for several years during its biological aging. In this aging type, a flor yeast film grows on the wine surface, developing an aerobic metabolism that endows its distinctive sensorial properties. Traditionally, no browning of the wine in this aging has been ascribed to a protective effect of the film yeast to the atmospheric oxygen. However, this explanation is partially questionable because of the wine is periodically aerated during the transfer operations typical of this aging. This suggests that film yeast could retain brown compounds (*25*).

In this work, a treatment based on the use of baker's yeasts is studied, with a view to developing an alternative fining treatment for white wines by using natural products.

MATERIAL AND METHODS

Unclarified white wines from the Montilla-Moriles region (southern Spain), with an ethanol contents of 15.5% (v/v), obtained by biological aging were subjected to different fining treatments to correct browning.

A first experiment was carried out on 1-L wine samples that were treated in triplicate in cylindrical containers (45 cm high × 6 cm wide) with dehydrated baker's yeasts (Mauripan Fleischmann, Canada) at concentrations of 0.5, 1, 2, 3, 4, and 5 g/L. After 24 h in contact with the yeasts, the wines were passed through a filter of 0.45 μm pore size.

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10.1021/jf0006269 CCC: $20.00
Published on Web 03/03/2001

In a second experiment, the same above wine, slightly browner as a result of the time elapsed during the achievement of the first experiment, was subjected to treatments with yeast (1 g/L), activated charcoal (BET surface area 900−1100 m^2/g, particle diameter 10−100 Å, Camel Chemicals, Barcelona, Spain), and PVPP (Polyclar VT, diameter exceeding 37 μm in over 90% of particles, GAF Comp., Gipuzcoa, Spain). The latter two were used at a concentration of 0.06 and 0.10 g/L, respectively, both typical in Sherry wine winemaking without modification of its sensory properties. Three batches of the same wine were treated in triplicate in containers identical to the above-mentioned cylindrical vessels. The fining agents were maintained into contact with the wines for 24, 48, and 72 h, after which they were passed through a filter of 0.45 μm pore size.

The same wine used for the second experiment was treated with 1, 3, and 5 g/L of yeast, activated charcoal, and PVPP. The resulting wines were tasted by a sensory panel of experts in Sherry wine. The tasters scored the wines for color, flavor, and aroma on a relative scale with respect to the untreated wine.

Extraction of Phenolic Compounds. A volume of 100 mL of wine was concentrated in a vacuum at 40 °C up to 20 mL, which was adjusted to pH 7 with 0.1 N NaOH. The concentrate was passed through a Sep-Pak C_{18} cartridge, with 900 mg of filling (Long Body Sep-Pak Plus, Water Associates) that was previously activated with 8 mL of methanol and washed with distilled water, which was adjusted to pH 7 with NaOH according to Jaworski and Lee (*26*). The cartridge was eluted with 8 mL of 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 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 (*27*).

The two collected fractions were concentrated and passed through a filter of 0.45 μm pore size for injection into a Spectra-Physics SP880 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 HPLC Rapid Scanning detector (Spectra-Physics mod. Focus) and calculation of UV absorbance ratios after co-injection of samples and standards (*28*). Commercial standards were purchased from Sigma-Aldrich Chem. Co. (Madrid, Spain) and Sarsynthese Co. (Genay, France). Caftaric and Coutaric acids were isolated by the method described by Singleton et al. (*29*). Procyanidins were obtained from a grape seed extract according to Bourzeix et al. (*30*). The standards purity was 95−99%. Each compound was quantified by comparison with a calibration curve obtained with the corresponding standard, except the procyanidins that were quantified as catechin.

Analyses were carried out on a C_{18} column (250 × 4.6 mm, 5 μm 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 Acids Fraction. The elution phases for this fraction were as follows: gradient elution from 0.1 to 5% CH_3CN in 5 min, isocratic elution for 10 min, gradient elution up to 15% CH_3CN in 5 min, and isocratic elution for 10 min. In this fraction were quantified the following compounds: gallic, protocatechuic, *m*-hydroxybenzoic, vanillic, syringic, caffeic, *trans-p*-coumaric, ferulic, *trans*-caftaric, *cis*- and *trans*-coutaric acids and tyrosol.

Flavan-3-ol Fraction. The elution phases for this fraction were as follows: gradient elution from 0.1 to 15% CH_3CN in 15 min, isocratic elution for 5 min, gradient elution up to 20% CH_3CN in 5 min, and gradient elution up to 30% CH_3CN in 5 min. In this fraction were quantified catechin, epicatechin, and procyanidins B1, B2, B3, and B4.

HPLC Direct Injection. To avoid possible retentions by the Sep-Pak cartridge affecting the condensed products, all the wine samples were subjected to a direct injection. The elution conditions were the same as those used for the phenolic acids fraction, and detection was carried out at 280 nm. The unknown peaks were quantified as gallic acid.

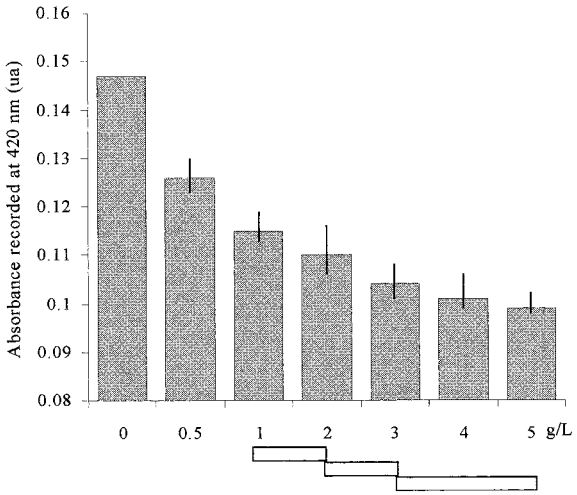

Figure 1. Absorbances at 420 nm for the untreated and treated wines with different concentrations of yeasts. Horizontal bars show homogeneous groups obtained for the different doses used.

Statistical Procedures. Variance and principal component analyses were performed on the replicated samples by using Statgraphics Statistical Computer Package (Statistical Graphics Corp.).

RESULTS AND DISCUSSION

Figure 1 shows the absorbance at 420 nm for the untreated wine and the samples treated with a yeast concentration of 0.5, 1, 2, 3, 4, and 5 g/L. To reproduce the usual conditions where the fining treatments are applied in cellars, the starting wine exhibited some browning, which however was within the commercially acceptable limits for Sherry wines ($A_{420} < 0.180$). As can be seen, all the yeast treatments tested decreased wine color, to an extent that increased with increasing yeast concentration. However, an analysis of variance of the absorbances revealed the absence of significant differences between the concentrations 1−2 and 2−3 g/L, and also among 3−4−5 g/L. Three uniform groups were thus established where A_{420} decreased by 22−25, 25−29, and 29−32%, respectively, in relation to the untreated wine.

Figure 2 shows the total phenol contents, measured as the absorbance at 280 nm, for the wine samples studied. It should be noted that the decrease, calculated as a percentage with respect to the untreated wine, was much smaller than that for A_{420} (as low as 4.4% with the highest yeast concentration, 5 g/L). This differential behavior of the yeasts suggests a preferential retention of the compounds absorbing at 420 nm. Taking into account that the highest yeast concentration (5 g/L) was that resulting in the greatest decrease in A_{280} and A_{420}, an HPLC analysis was carried out to determine the effect of this yeast treatment on the levels of phenolic compounds in the wine.

Table 1 lists the phenol contents in the wine samples prior to and after treatment with the above-mentioned yeast concentration, as well as the homogeneous groups obtained for each compound as a result of the variance analysis carried out. Only for vanillic, syringic and c-coutaric acids, as well as for procyanidins B2 and B4, was a statistically significant decrease ($p < 0.05$) observed by effect of the fining treatment. At this point, it should be noted that, at about 35 min of retention

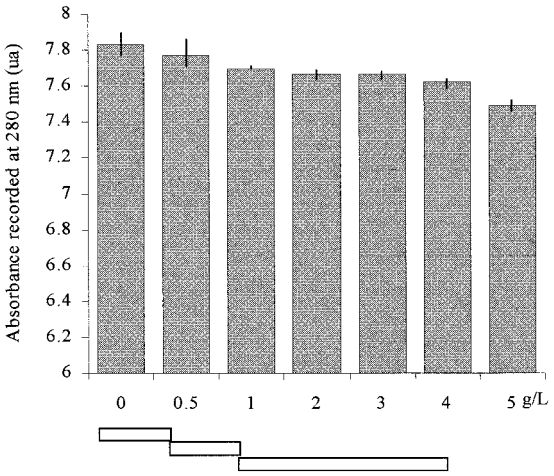

Figure 2. Absorbances at 280 nm for the untreated and treated wines with different concentrations of yeasts. Horizontal bars show homogeneous groups obtained for the different doses used.

Table 1. Phenolic Compounds Contents (mg/L) in Untreated and Treated Wines with a Dose of 5 g/L of Yeast

	untreated wine	treated wine	homogeneous groups[a]
gallic acid	7.84 ± 0.566	7.87 ± 0.786	a a
protocatechuic acid	4.33 ± 0.670	4.00 ± 0.670	a a
m-hydroxybenzoic acid	13.8 ± 2.39	11.1 ± 0.795	a a
vanillic acid	0.939 ± 0.028	0.655 ± 0.036	a b
syringic acid	1.55 ± 0.305	0.657 ± 0.079	a b
caffeic acid	0.715 ± 0.050	0.674 ± 0.078	a a
p-coumaric acid	<0.001	<0.001	a a
ferulic acid	0.603 ± 0.081	0.749 ± 0.044	a a
t-caftaric acid	22.2 ± 3.26	21.2 ± 1.52	a a
c-coutaric acid	23.7 ± 3.75	16.9 ± 1.66	a b
t-coutaric acid	14.4 ± 1.30	12.9 ± 1.29	a a
tyrosol	74.7 ± 9.77	67.8 ± 0.393	a a
catechin	29.9 ± 1.47	31.0 ± 3.22	a a
epicatechin	13.2 ± 2.46	9.77 ± 1.68	a a
procyanidin B1	17.4 ± 1.00	18.7 ± 1.78	a a
procyanidin B2	9.26 ± 1.14	6.55 ± 0.947	a b
procyanidin B3	14.6 ± 1.35	14.5 ± 1.03	a a
procyanidin B4	3.52 ± 0.240	2.48 ± 0.452	a b
grouped peaks	16.9 ± 2.19	12.6 ± 1.06	a b

[a] Homogeneous groups obtained at $p < 0.05$.

time, the chromatograms recorded at 280 nm showed a group of overlapped peaks, reasonably of low polarity, which must correspond to products resulting from the oxidation and polymerization of phenol compounds to a variable extent. The added areas of these compounds, named as grouped peaks and quantified as gallic acid, also exhibited significant differences prior to and after treatment with the yeast. This region of the chromatogram can be especially interesting because the compounds eluting in it absorb at 420 nm in Sherry wines under oxidative aging (*31*). The wine studied in this work, obtained by biological aging, exhibited no appreciable absorbance at 420 nm, probably because the degree of browning of this type of wine is much less than that observed in wines aged under oxidizing conditions over long periods.

To evaluate the performance of the yeast used as fining agent, the wine was treated, and its result was compared with those of other traditional treatments, such as the use of activated charcoal and PVPP. The concentrations used were those typically employed in industrial practice for this type of wine. Taking into account that, in terms of A_{420}, the lowest yeast concentration that resulted in no significant differences with the next was 1 g/L, it was chosen for the comparative study. Also, because the efficiency of the fining agents tested might depend on the length of the treatment, three fixed wine contact times were used (24, 48, and 72 h).

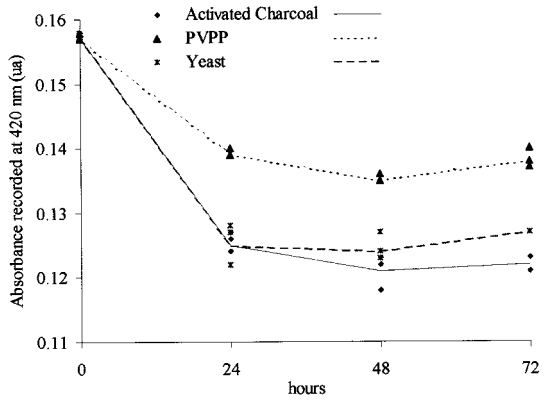

Figure 3. Changes in A_{420} during 24, 48, and 72 h of contact time for the wines treated with yeast, activated charcoal, and PVPP as fining agents.

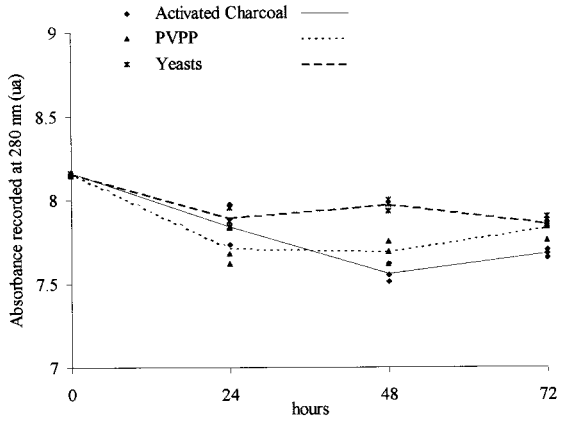

Figure 4. Changes in A_{280} during 24, 48, and 72 h of contact time for the wines treated with yeast, activated charcoal, and PVPP as fining agents.

Figure 3 shows the changes in A_{420} for the wine samples in contact with the fining agents at the abovementioned times. The results were subjected to an analysis of variance among fining agents for each contact time and also among different times for the same agent. The three treatments tested decreased the wine color, although those based on the yeast and activated charcoal proved to be more efficient than the PVPP treatment at the three times considered, with no significant differences between them at 24 or 48 h. The slight increase observed between 48 and 72 h can be ascribed to slight browning resulting from the maintenance of the wines in contact with the atmosphere.

Figure 4 shows the A_{280} values for the wine samples subjected to the different fining treatments. At 24 h, the three treatments decreased A_{280} to a similar extent, with no significant differences among them. Activated charcoal continued to decrease this absorbance after 48 h of contact, although again with no significant differences to the PVPP treatment in this point. On the other hand, from 24 h the yeast provided a constant absorbance during the remainder studied period. In any case, it should be noted that A_{280} values were measured at a 1:50 dilution, so the differences in the absorbances really

Table 2. Phenolic Compounds Contents (mg/L) in Untreated and Treated Wines with Yeast (1 g/L), Activate Charcoal, and PVPP

		treatment		
	untreated wine	activate charcoal	PVPP	yeast
gallic acid	6.85 ± 0.628	6.05 ± 1.07	5.98 ± 0.343	7.81 ± 1.11
protocatechuic acid	6.73 ± 1.10	5.59 ± 1.06	5.45 ± 0.867	5.42 ± 0.318
m-hydroxybenzoic acid	7.61 ± 0.457	4.49 ± 0.476	4.10 ± 0.178	8.36 ± 0.196
vanillic acid	1.49 ± 0.237	1.16 ± 0.077	1.27 ± 0.120	1.35 ± 0.115
syringic acid	1.57 ± 0.110	0.643 ± 0.022	0.820 ± 0.104	0.717 ± 0.046
caffeic acid	2.90 ± 0.528	3.67 ± 0.470	3.44 ± 0.538	2.14 ± 0.228
p-coumaric acid	<0.001	<0.001	<0.001	<0.001
ferulic acid	0.822 ± 0.140	0.626 ± 0.111	0.422 ± 0.082	0.297 ± 0.040
t-caftaric acid	28.1 ± 3.42	22.9 ± 0.305	20.6 ± 1.31	32.2 ± 3.89
c-coutaric acid	19.8 ± 2.87	19.1 ± 1.19	18.5 ± 2.60	18.2 ± 0.763
t-coutaric acid	10.3 ± 1.10	9.59 ± 0.585	9.85 ± 1.52	9.60 ± 0.984
tyrosol	45.6 ± 2.74	42.9 ± 2.12	46.1 ± 6.01	45.6 ± 2.52
catechin	32.4 ± 6.28	21.9 ± 2.91	24.1 ± 2.98	24.2 ± 1.68
epicatechin	6.27 ± 0.981	7.52 ± 0.584	6.65 ± 1.21	4.89 ± 0.223
procyanidin B1	20.3 ± 3.52	18.2 ± 1.96	17.8 ± 2.87	20.5 ± 1.36
procyanidin B2	4.21 ± 0.195	4.01 ± 0.249	4.94 ± 0.289	5.23 ± 0.351
procyanidin B3	8.82 ± 1.61	8.33 ± 1.48	9.22 ± 1.17	9.23 ± 1.44
procyanidin B4	0.996 ± 0.118	1.32 ± 0.026	1.81 ± 0.360	1.76 ± 0.325
grouped peaks	17.6 ± 0.750	13.4 ± 0.642	13.4 ± 0.269	14.8 ± 0.265

Table 3. Statistical Weights of the Selected Phenolic Compounds on the Two Principal Components

	component 1	component 2
m-hydroxybenzoic acid	0.36	−0.31
syringic acid	0.41	0.16
caffeic acid	−0.22	0.42
ferulic acid	0.28	0.4
t-caftaric acid	0.31	−0.32
catechin	0.39	0.11
epicatechin	−0.13	0.41
procyanidin B2	−0.08	−0.41
procyanidin B4	−0.32	−0.3
grouped peaks	0.45	0.02

measured in a spectrophotometric scale were very low. Thereby, the differences observed at this wavelength must be taken cautiously.

Table 2 lists the contents in phenolic compounds in the untreated wine and after subjection to the treatments with activated charcoal, PVPP, and yeast. A variance analysis was carried out on the results; it showed only significant differences at $p < 0.05$ for the *m*-hydroxybenzoic, syringic, caffeic, ferulic, and *t*-caftaric acids, as well as for the flavan-3-ol derivatives catechin and epicatechin, procyanidins B2 and B4, and the so-called grouped peaks. To examine the individual performance of each fining agent, a principal component analysis was carried out using only the above-mentioned phenols as variables.

The first two principal components (with eigenvalues greater than unity) account for 80% of the overall variance. Table 3 lists the statistical weights of the variables and Figure 5 shows the scores of each sample in the plane defined by these two principal components (PCs). The selected phenols resulted in a different distribution for the untreated and treated wines. The activated charcoal and PVPP treatments provided results similar to those for the untreated wine with regard to PC 2 but different in relation to PC1, with no significant differences between them. These results are consistent with those found by Baron et al. (*17*) for different types of fining agents applied to white wines. The yeast treatment exhibited differences with the untreated wine as well as with the activated charcoal and PVPP treatments. The results suggest a lower retention of the yeast to the compounds with the greater weights on PC1 but higher to those with a greater influence on PC2. Notwithstanding the statistical results, it should noted that none of the treatments resulted in no especially strong retention on the selected phenols, which suggests preferential retention of colored browning compounds.

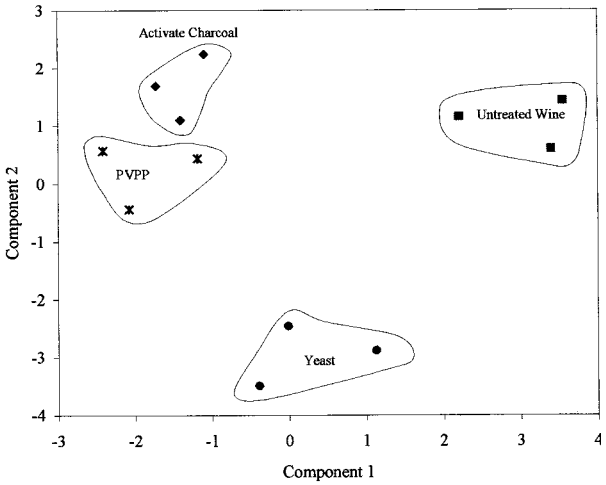

Figure 5. Principal component analysis. Score values of the untreated and treated wines and their grouping in the plane defined by the first two components.

Taking into account that the fining treatments using yeast might alter the sensory properties of the wine, the treated samples were subjected to a sensory panel of experts in Sherry wine. The tasters scored the wines treated with activated charcoal, PVPP and 1, 3, or 5 g/L yeasts for color, flavor, and aroma in relation to the untreated wine. A relative scale for each above-mentioned sensory property was used for this purpose (Figure 6). All the treatments were judged to improve color with respect to the untreated wine, with no appreciable differences among them.

Regarding aroma, the sample treated with 1 g/L yeast received the highest scores, followed by those resulting from the treatments with activated charcoal and PVPP, with no significant differences between them. It should be noted that the wine samples treated with 3 and 5 g/L yeasts were given much worse scores than the previous ones as a result of their odor of "overaged" wine, it reasonably due to the excessive amounts of yeasts used. Finally, the wine samples treated with 1

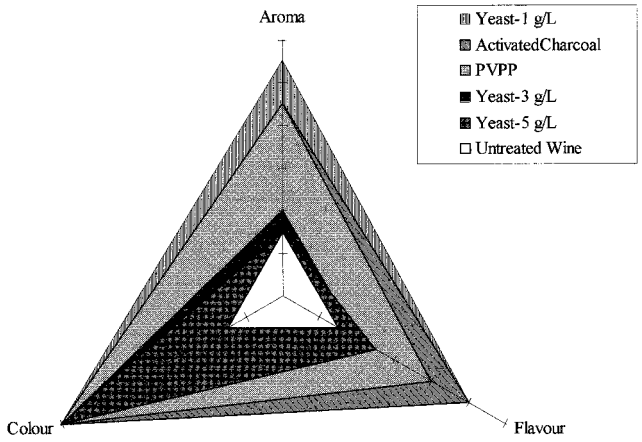

Figure 6. Scores of the tasters for the color, flavor, and aroma of the activated charcoal, PVPP, and yeasts (1, 3, and 5 g/L) treated wines relative to the untreated wine.

g/L yeast and activated charcoal were given better flavor scores than the others.

On the whole, the use of yeast at a 1 g/L concentration as a fining treatment to correct browning in white wines, measured in terms of A_{420}, showed a similar efficiency that the traditional treatment based in activated charcoal used at a concentration not altering the sensory properties of Sherry wine. On the other hand, these two treatments performed similarly with regard to wine flavor, even though the yeast treatment was found to improve the aroma to a slightly greater extent than did the activated charcoal treatment. One additional advantage of using the yeast is that it represents a green technology, of increasing interest to the food industry. By contrast, it has the disadvantage that this treatment must be followed by a microbiological sterilization to ensure that any yeasts remaining in the wine are removed before they can grow and alter its transparency and/or sensory properties. This problem is more likely to arise in wine with a low alcohol content. However, in industrial practice it is not a great problem since, in most cases, fining treatments for correction of browning are applied immediately before microbiological sterilization and bottling of the wine. Further research is required, basically with regard to the way the yeast treatment is to be applied, to optimize the length of the treatment in relation to the yeast concentration used.

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Received for review May 19, 2000. Revised manuscript received January 9, 2001. The authors gratefully acknowledge financial support from the Spanish CICYT (ALI98-1047-C02-01) for the realization of this work.

JF0006269