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Yeasts used to delay browning in white wines

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Abstract

Commercial white wines of the Sherry type were subjected to accelerated browning at 35 °C in the absence and presence of yeasts at concentrations of 1, 1.5 and 2 g/l. Based on the results, the yeasts delayed browning in the wines, measured in terms of the absorbance at 420 nm, the effect increasing with increase in the yeast concentration. The addition of yeasts was also found to affect phenolic compounds, particularly decreasing the concentrations of brown compounds. Wines of the same type stored in stoppered bottles at 19 °C for 12 months exhibited a considerable delay of browning in the presence of yeasts with not-too-serious alteration of their sensory properties, which made them still acceptable for consumption.

Keywords: Browning; Yeasts; White Wines

1. Introduction

Browning is a serious problem during the storage of drinks, such as white wine, the colour of which becomes unstable, shortening its commercial life as a result. Essentially, the problem arises from phenolic compounds which can evolve via different chemical pathways to produce coloured compounds in the yellow-brown spectral region. One such pathway involves their oxidation and subsequent polymerization of the resulting products, the former process being catalysed by metals such as Fe and Cu (Benitez, Castro, & Garcia-Barroso, 2002; Cilliers & Singleton, 1989; Clark & Scollary, 2003; Clark & Scollary, 2002; Es-Safi, Cheynier, & Moutounet, 2003; Hocking & Intihar, 1986; Oszmianski, Cheynier, & Moutounet, 1996; Speier, 1986). Other pathways potentially increasing the colour of white wines involve polymerization reactions between phenols and other compounds in the wine, such as acetaldehyde (Es-Safi, Fulcrand, Cheynier, & Moutounet, 1999a; Fulcrand, Doco, Es-Safi, Cheynier, & Moutonet, 1996; Lopez-Toledano, Villano-Valencia, Mayen, Merida, & Medina, 2004; Saucier, Bourgeois, Vitry, Roux, & Glories, 1997), or with oxidation products of tartaric acid (Clark, Prenzler, & Scollary, 2003; Es-Safi, Guerneve, Fulcrand, Cheynier, & Moutounet, 1999b; Es-Safi et al., 1999c; Es-Safi, Guerneve, Cheynier, & Moutounet, 2000).

Several procedures to prevent wine browning have by now been developed. Some diminish the concentrations of polyphenols in wine, thereby making it less prone to colour changes. Particularly notable are the advances in recent years concerning grape pressing and the use of processes such as the hyperoxidation of must. This technique, based on the oxidation of phenols in grape must under the action of air or oxygen, can effectively reduce the tendency of the resulting wine to brown (Castro & Barroso, 2001; Ho et al., 1999; Schneider, 1998). However, the results have been found to depend on the particular grape variety used in winemaking (Cheynier, Rigaud, Souquet, Barillere, & Moutounet, 1989; Nagel

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& Graber, 1988), so this technique is difficult to standardize. Also, phenols are currently regarded as beneficial to health, so any techniques causing their destruction (e.g., hyperoxidation) will obviously diminish the nutritional value of the wine.

Other procedures have been applied to decrease the browning rate, resulting in increased colour stability. Some of them are based on the protection of wine from contact with atmospheric oxygen, storing in an inert atmosphere, improving the efficiency of stoppers, and using antioxidants such as ascorbic acid, complementary to the action of SO_2 (added as antibacterial). Other procedures attempt to decrease the catalytic activity of some metals on the oxidation of phenols through the use of complexing agents, such as citric acid, to reduce their active concentrations. Recently, the decrease in the concentration of metals in white wine by passing through ion-exchange resins has been proposed as a method for preventing browning (Benitez et al., 2002).

Finally, browning can also be corrected by using fining treatments to remove brown compounds already formed in the wine. This can be accomplished with fining agents such as active charcoal, PVPP or potassium caseinate, or with more recent alternatives, such as chitosan (a byproduct of the fishing industry), bovine scleroproteins or polylactic acid (Spagna, Barbagallo, & Pifferi, 2000).

Although the mechanism of interactions is unknown, some yeasts have been found to retain anthocyanins in wine (Augustin, 1986; Bourzeix & Heredia, 1976; Cuinier, 1988) and to interact with other polyphenols in model solutions (Salmon, Fornairon-Bonnefond, & Mazauric, 2002). In this respect, authors such as Bonilla, Mayen, Merida, and Medina (2001) have proposed the use of these microorganisms in wine fining treatments on account of the selectivity of their cell walls to brown polymers (Razmkhab et al., 2002). However, in addition to this retention ability, yeasts in contact with model solutions of some flavans have been found to delay browning, which has been ascribed to an inhibitory effect on the formation of coloured compounds (Lopez-Toledano, Mayen, Merida, & Medina, 2002). Such an effect is reflected in a marked drop in the rates of degradation of (+)-catechin and (−)-epicatechin that results in much slower production of brown compounds. Based on the foregoing, it is interesting to examine the behaviour of yeasts kept into contact with wine in order to detect potential changes in its sensitivity to browning.

This work aims to study delay in the browning of white wines caused by the presence of yeasts. With this objective, changes in colour and phenolic compounds during temperature-induced accelerated browning of Sherry white wines in the presence and absence of yeasts were examined. Likewise, in order to study the behaviour of the yeasts under real conditions, changes in colour and sensory properties were also examined after one year of wine storage.

2. Materials and methods

2.1. Samples and experiments

Commercially available white wines obtained by biological aging in the Montilla-Moriles winemaking region (Córdoba, southern Spain) with an ethanol content of 15% (v/v) were subjected to browning under variable conditions.

In a first experiment, commercial bottles (70 cl) were opened, the wine homogenized and the bottles refilled to half their capacity, in order to obtain an air chamber that would facilitate browning. The wine of the different bottles was supplied with dehydrated bakers' yeasts (Mauripan Fleischmann, Canada) at concentrations of 0, 1, 1.5 or 2 g/l. The bottles were stoppered and placed in a stove at a controlled temperature of 35 ± 1 °C to accelerate browning. The wines were sampled at intervals of three days and the experiment was finished when the value of the absorbance at 420 nm reached the accepted commercial maximum for consumption of this type of wine (0.180 au, is established in the winemaking of pale sherry wines). Because of the different yeast concentrations, this took 18, 33, 39 and 48 days, respectively. Prior to addition, the yeasts were inactivated with a 50:50 ethanol/water mixture that was subsequently heated to complete evaporation of the liquid.

In a second experiment, bottles from another batch of the same commercial wine were stored at a controlled temperature of 19 ± 1 °C for one year. The samples were homogenized and split into two batches that were supplied with inactive yeasts at a concentration of 0 and 2 g/l, respectively. The wines were sampled at intervals of three months. All treatments involved in the two experiments were conducted in triplicate.

2.2. Analytical procedures

Spectrophotometric measures at 280 nm (after dilution 1:10) and 420 nm were made in a Beckman spectrophotometer, DU 600 model, on 10 mm pathlength.

2.3. Extraction of phenolic compounds

A volume of 100 ml of wine was concentrated in a vacuum at 40 °C to 20 ml, which was adjusted to pH 7 with 0.1 M NaOH. The concentrate was passed through a Sep-Pak C18 cartridge, with 900 mg of filling (Long Body Sep-Pak Plus; Waters Associates; Milford, MA) that was previously activated with 8 ml methanol and washed with distilled water, which was adjusted to pH 7 with NaOH according to Jaworski and Lee

(1987). 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 (Oszmianski, Ramos, & Bourzeix, 1988). The two collected fractions were concentrated and passed through a filter of 0.45 μm pore size for injection into a Spectra-Physics (San Jose, CA) P4000 HPLC instrument.

2.4. Identification and HPLC analysis

2.4.1. General

The identification of the phenolic compounds was achieved by comparing with the retention times of the standards, UV spectra obtained by HPLC; Dyode Array (Spectra-Physics UV6000LP) and calculation of UV absorbance ratios after coinjection of samples and standards (Fabios, Lopez-Toledano, Mayen, Merida, & Medina, 2000). 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, Timberlake, and Lea (1978). Procyanidins were obtained from a grape seed extract according to Bourzeix, Weyland, and Heredia (1986). The standards' purity was 95–99%. Each compound was quantified by comparison with a calibration curve obtained with the corresponding standard, except for the procyanidins B3 and B4 that were quantified as procyanidin Bl.

Analyses were carried out on a LiChrospher 100 RP-18 column (250 mm × 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.

2.4.2. Phenolic acids 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, the following compounds were quantified: gallic, protocatechuic, vanillic, syringic, caffeic, *trans*-*p*-coumaric, ferulic, *trans*-caftaric and *cis*-coutaric acids.

2.4.3. 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 l0 min. In this fraction (+)-catechin, (−)-epicatechin and procyanidins Bl, B2, B3, and B4 were quantified.

2.4.4. HPLC direct injection

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

2.5. Sensory analyses

Sensory analyses were performed on freshly bottled commercial wines and the wines stored for one year in the presence and absence of yeasts. A first analysis involved a triangular test to identify significant overall differences (colour excepted) between the wines in accordance with ISO 4120–1983. In the second and third tests, samples were rated for flavour and colour, respectively, on the following scale: 1 = undesirable, 2 = acceptable and 3 = desirable (ISO 4121–1987). All tests were conducted by 25 tasters chosen in accordance with ISO 6658–1985.

3. Results and discussion

Fig. 1 shows the changes of the absorbance at 420 nm during accelerated browning of the wines at 35 °C in the

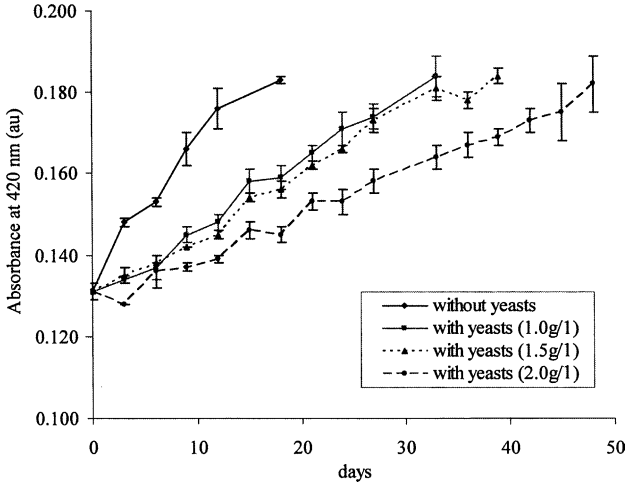

Fig. 1. Changes in the absorbances at 420 nm during accelerated browning of the wines in the presence and absence of yeasts.

presence and absence of yeasts. As can be seen, $A420$ for the wine containing no yeasts increased from 0.131 to the accepted commercial maximum for consumption of this type of wine (0.180 au) in 18 days. The best fit to the results obtained was a linear regression ($r^2 = 0.9435$, $p < 0.001$), so the browning rate was calculated to be 0.0029 au/day. In the presence of the different yeast concentrations (1, 1.5 and 2.0 g/l), $A420$ increased to a much lesser extent, so the wines took 33, 39 and 48 days, respectively, to reach the absorbance limit, showing the last to be the most efficient in delaying browning. The best fits of the colour–time plots for the wines browned in the presence of yeasts were also linear functions (r^2 values of 0.9914, 0.9837 and 0.9881, respectively, at $p < 0.001$). Their corresponding rates of $A420$ increase were lower than that for the non-treated wine (0.0017, 0.0014 and 0.0011 au/day, respectively). These results show that yeasts can effectively delay wine browning by a factor of 1.7–2.6, depending on their concentration. This can be explained because of the ability of yeasts to retain brown compounds (Bonilla et al., 2001; Razmkhab et al., 2002) as they are formed. Also, authors such as Lopez-Toledano et al. (2002) have suggested that yeasts could inhibit the browning of some flavans, so their presence would delay this alteration.

By comparison, similar changes for the $A280$ of the wines stored in the absence and presence of yeasts were observed (Fig. 2), showing in all the cases a trend to increase this absorbance with increasing storage time, without significant differences among treatments. These results can reasonably be explained because the only polymers coloured are those with a high degree of polymerization, but their smaller precursors are colourless compounds at 420 nm, although absorbing strongly in the UV spectral region. Such compounds, would not be retained (or their formation inhibited) by the yeasts, suggesting a more specific interaction of these microorganisms with the coloured polymers. This different retention ability of yeasts has been shown in model solutions of (+)-catechin and acetaldehyde (Lopez-Toledano et al., 2004).

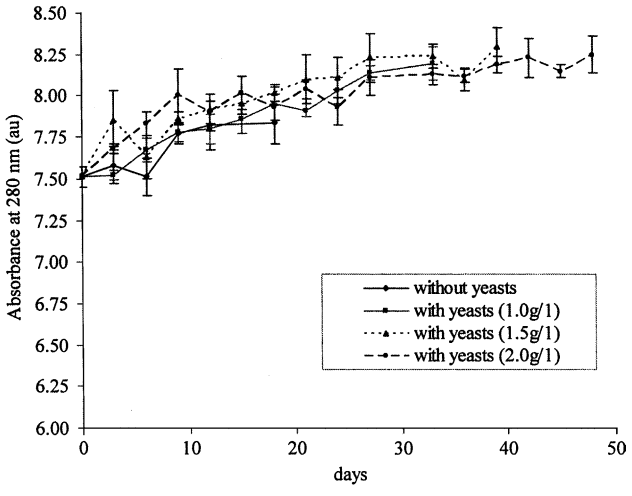

Fig. 2. Changes in the absorbances at 280 nm during accelerated browning of the wines in the presence and absence of yeasts.

Table 1 shows the contents of phenolic compounds of the initial wine, those browned for 18 days in the absence of yeasts or in their presence at the highest concentration studied, and all the wines at the end of the experiment (33, 39 and 48 days). An analysis of variance of the results ($p < 0.01$), for the initial wine and those not treated with yeasts at day 18, revealed an increase in the contents of protocatechuic acid, procyanidin B3 and the compounds named browning peaks (BP). At this point, however, the wines treated with a 2 g/l concentration of yeasts, which were much less markedly coloured, exhibited a lower BP content, and higher caftaric acid and (+)-catechin concentration, than the wines without yeasts. These results can be explained by taking into account of fact that the concentration of some phenolic compounds is dependent on a balance of reactions, some of these increasing their contents and others decreasing them. In fact, the flavan contents are known to be decreased by oxidation and polymerization reactions, and increased through hydrolysis of higher oligomers (Dallas, Ricardo-da-Silva, & Laureano, 1995; Haslam, 1980; Timberlake & Bridle, 1976). Because compounds in this family can undergo some of these reactions at different rates (Dallas, Hipolito-Reis, Ricardo-Da-Silva, & Laureano, 2003), the above-mentioned increase–decrease balance, at a given time, may be specific for each compound. This could explain how the contents of all the flavans studied, except procyanidin B3, remained constant in the absence of yeasts, because the decreases were similar in extent to the increases. However, the presence of yeasts could inhibit some degradation mechanisms (such as phenol oxidation or polymerization), prevailing in this way hydrolytic mechanisms. As a result, compounds such as (+)-catechin would increase their contents. Obviously, these arguments cannot be applied to those compounds that are formed exclusively through browning (BP). Such compounds are partially retained and/or their formation inhibited by the presence of yeasts, so they contribute to a lesser extent to the above-described colour increase.

Table 1 also shows the results of an analysis of variance carried out on all the wines at the end of experiment. Whether or not yeasts were present, and whatever their concentration, the wines exhibited no clear-cut trend for most of the compounds, perhaps with the exceptions of protocatechuic, vanillic and *cis*-coutaric acids, and (+)-catechin. Because the degree of browning was the same for all wines, the scarce differences encountered suggest that the variable time required to reach this degree of browning and the presence of yeasts are not factors too influential on the final phenolic composition.

Table 1
Contents of phenolic compounds (mg/l) of the initial wine and those browned after 18, 33, 39 and 48 days in absence and presence of yeasts

	0 days			18 days				33 days		39 days		48 days	
	*A*420 = 0.132			*A*420 = 0.183		*A*420 = 0.149		*A*420 = 0.180					
	Initial wine			Without yeasts		With yeasts (2.0 g/l)		With yeasts (1.0 g/l)		With yeasts (1.5 g/l)		With yeasts (2.0 g/l)	
		(1)	(2)		(1)		(1)		(2)		(2)		(2)
Gallic acid	4.43 ± 0.30	a	e	4.40 ± 0.09	a	3.86 ± 0.29	a	3.89 ± 0.32	de	3.77 ± 0.04	de	3.53 ± 0.13	d
Protocatechuic acid	5.85 ± 0.24	a	d	8.52 ± 0.14	b	7.54 ± 0.48	b	13.8 ± 0.7	e	14.2 ± 0.2	e	15.9 ± 0.1	f
Vanillic acid	0.978 ± 0.064	a	d	1.08 ± 0.00	a	1.13 ± 0.16	a	1.33 ± 0.03	de	1.38 ± 0.11	e	1.41 ± 0.03	e
Syringic acid	1.96 ± 0.08	a	d	1.88 ± 0.02	a	1.92 ± 0.33	a	2.26 ± 0.35	d	2.26 ± 0.08	d	2.15 ± 0.06	d
Caffeic acid	0.342 ± 0.023	a	d	0.405 ± 0.005	a	0.390 ± 0.055	a	0.538 ± 0.062	de	0.592 ± 0.046	e	0.574 ± 0.038	de
p-Coumaric acid	0.613 ± 0.078	a	d	0.591 ± 0.003	a	0.600 ± 0.113	a	0.616 ± 0.063	d	0.686 ± 0.057	d	0.78 ± 0.016	d
Ferulic acid	1.57 ± 0.05	a	d	1.45 ± 0.02	a	1.46 ± 0.24	a	1.35 ± 0.09	d	1.44 ± 0.04	d	1.53 ± 0.03	d
trans-Caftaric acid	17.5 ± 0.4	ab	d	15.1 ± 0.1	a	18.2 ± 0.8	b	16.2 ± 0.4	d	15.5 ± 0.5	d	16.0 ± 0.8	d
cis-Coutaric acid	10.7 ± 0.5	a	d	13.0 ± 0.2	a	13.4 ± 0.8	a	11.9 ± 1.0	c	12.3 ± 0.5	e	12.4 ± 0.3	e
(+)-Catechin	34.1 ± 0.6	a	d	31.9 ± 0.2	a	38.6 ± 0.8	b	35.3 ± 0.8	e	37.5 ± 0.6	e	35.9 ± 0.6	c
(−)-Epicatechin	14.8 ± 0.9	a	d	16.6 ± 0.3	ab	19.4 ± 1.0	b	16.8 ± 0.6	d	19.9 ± 0.9	e	16.6 ± 0.4	d
Procyanidin Bl	10.0 ± 0.5	a	e	10.3 ± 0.2	a	9.99 ± 0.74	a	9.17 ± 0.08	de	8.60 ± 0.55	d	9.43 ± 0.36	de
Procyanidin B2	7.06 ± 0.33	b	d	6.02 ± 0.20	ba	5.22 ± 0.46	a	6.58 ± 0.25	d	6.36 ± 0.60	d	5.71 ± 0.12	d
Procyanidin B3	13.8 ± 0.3	a	d	18.5 ± 0.4	b	18.7 ± 1.0	b	20.3 ± 0.6	dc	21.Q ± 0.6	e	18.9 ± 0.4	d
Procyanidin B4	5.91 ± 0.59	a	d	4.57 ± 0.04	a	5.01 ± 0.20	a	5.24 ± 0.60	d	5.36 ± 0.42	d	5.29 ± 0.19	d
Browning peaks	4.55 ± 0.18	a	d	9.49 ± 0.25	c	7.53 ± 0.53	b	8.15 ± 0.86	d	7.99 ± 0.82	d	8.37 ± 0.12	d

[a,b,c] Homogeneous groups obtained among initial wine and the wines after 18 days of browning (columns numbered (1)).
[d,e,f] Homogeneous groups obtained among initial wine and the wines after 33, 39 and 48 days of browning (columns numbered (2)).

The above-described results show to the ability of yeasts to interact with browning compounds and delay adverse colour changes in white wines. However, the composition of brown compounds in wine has been reported to depend on the particular method used to accelerate the process (Benitez, Castro, & Garcia-Barroso, 2003). In addition, increasing the temperature, to expedite browning, does not imply that all the reactions involved will be accelerated to the same extent. Taking into account that each mechanism possesses a differential kinetic at each step, the specific temperature used can affect the result in different ways. Therefore, the most accurate way to measure the efficiency of yeasts in delaying browning of white wine, in practice, is by using the habitual conditions for wine storage.

Fig. 3 shows the variation of the absorbance at 420 nm in commercial wines of the same type as the previous ones but stored at 19 ± 1 °C in the presence or absence and yeasts. As can be seen, the wines containing no yeasts reached an absorbance slightly above 0.180 au after 12 months, which is the typical commercial life of pale Sherry wines. In the presence of a yeast concentration of 2 g/l, however, the wines reached an absorbance of 0.157 au, largely during the last three months of storage. It is therefore difficult to extrapolate the results with a view to calculating the longest time of wine storage in the presence of yeasts. With a reasonable approximation, and taking into account that the absorbance changes the presence and absence of yeasts fitted to a linear function at $p < 0.001$, the maximum theoretical time needed for the wines containing yeasts to reach 0.185 au would be 22.3 months. Certainly, this calculation can be criticized because the rate of darkening can increase during storage by the effect of a gradual loss in the ability of yeasts to interact with browning compounds. In any case, it is remarkable that, in their presence, the absorbance increased by only 60% of the maximum commercially tolerated level after 12 months.

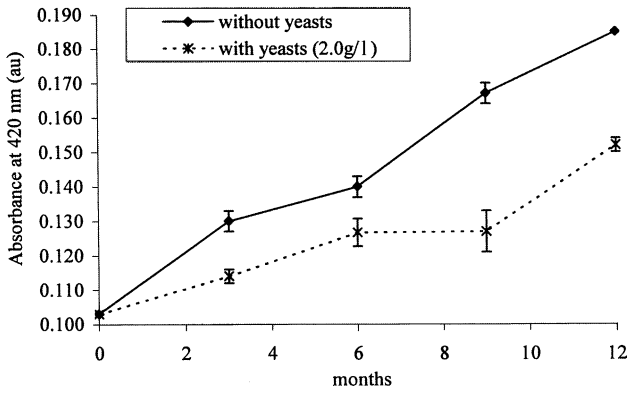

Fig. 3. Changes in the absorbances at 420 nm during 12 month of wine storage in the presence and absence of yeasts.

Taking into account that prolonged contact of the wines with the yeasts might alter their sensory properties, a triangular test was performed in accordance with ISO 4120–9183 in order to evaluate the overall quality (colour excluded, in opaque glasses). The wines stored in the presence of yeasts were compared with freshly bottled wines of the same type, ready for marketing. The results obtained at $p < 0.001$ revealed that 57% of the taster answers found differences, so the sensory properties of the wines supplied with yeasts were not

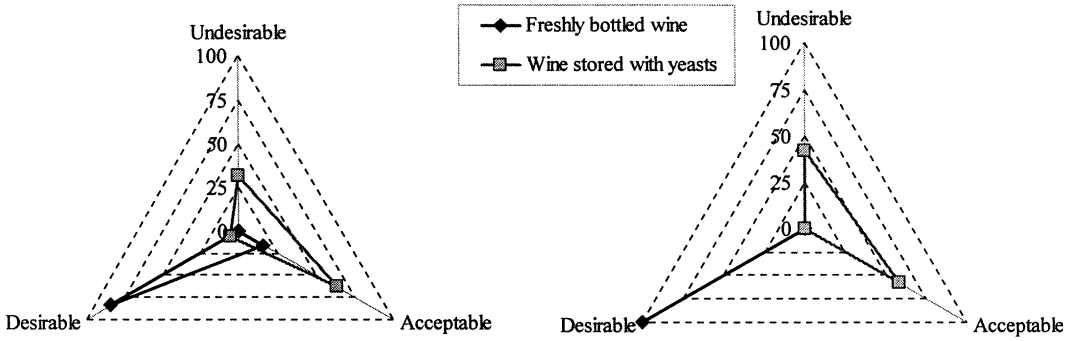

Fig. 4. Sensorial analyses of the wine stored for 12 months with yeasts and freshly bottled wine: (a) flavour and (b) colour.

too different from those of freshly bottled wines. In order to analyze these difference in depth, two additional tests (flavour and colour) in accordance with ISO 4121–1987 and using a scale of three scores (undesirable, acceptable and desirable) were carried out. Based on the results of these two sensory tests (Fig. 4), the wine containing yeasts was judged acceptable in terms of flavour, in 63.2% of the taster answers, and the freshly bottled wine in 84.2%. In terms of colour (100% desirable in the freshly bottled wine), the acceptable and undesirable scores were closer (57.9% and 42.1%, respectively). Overall, the sensory analyses revealed that wine stored in contact with yeasts for one year (and thereby partially browned) was obviously of lower quality than freshly bottled wine, but still considered acceptable by the tasters. This confirms the appreciations above-described in the study of the absorbances.

In conclusion, the contact of yeasts with the wine can appreciably delay, not only the increase in its colour, but also the deterioration of the sensory properties resulting from browning reactions. In practice, however, wine containing yeasts cannot be marketed, so the yeasts can only be used to inhibit browning at the industrial production stage. In this respect, it should be pointed out that some white wines are bottled in accordance with the market demands, specifically because of browning. Otherwise, a considerable volume of the wine marketed would brown considerably during bottled storage and be rejected by consumers. If the wine is stored in a cellar, then browning can be corrected prior to bottling, using a fining treatment involving active charcoal or PVPP, for example. In this way, the wine recovers its pale colour prior to bottling and its commercial life will be prolonged. However, the concentration of fining agent to be used will have to be proportional to the extent of browning of the wine and some authors have noted that too high concentrations can alter the sensory properties of the wine and consequently its quality (Mazzoleni, Testa, & Colagrande, 1986; Sims, Eastridge, & Bates, 1995; Singleton & Draper, 1962). However, if the wine is stored in cellar, in contact with yeasts, it will brown to a lesser extent and therefore require a lower concentration of fining agent. It is important to point out that, in this work, 12 months were used as the maximum reference of storage time under habitual conditions. This time, however, is too long as the typical industrial storage period, preceding bottling is realisticaly 3–4 months. Under these conditions, the wine may even not need a fining treatment, which would be especially interesting with a view to the production of organic wines.

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