Yeast-Induced Inhibition of (+)-Catechin and (−)-Epicatechin Degradation in Model Solutions

Azahara Lopez-Toledano, Manuel Mayen, Julieta Merida, and
Manuel Medina*

Department of Agricultural Chemistry, Faculty of Sciences, University of Cordoba, Edificio C3,
Campus de Rabanales, E-14014 Cordoba, Spain

(+)-Catechin and (−)-epicatechin degradation in water−alcohol solutions containing Fe^{2+} and tartaric acid was studied in the presence and absence of yeasts. On the basis of the results, yeast partially inhibited the degradation of both flavans, with much slower formation of browning products absorbing at 420 and 520 nm. In comparative terms, yeast was found to be more efficient toward the degradation products of (+)-catechin absorbing at the latter wavelength. Likewise, the presence of yeast decreased the yield of a group of colored compounds eluting at high retention times in HPLC and indicated these as important contributors to color darkening in white wines. This inhibitory effect may in part account for the resistance to browning observed over periods of several years in sherry wines subjected to biological aging under flor yeast.

KEYWORDS: Browning; (+)-catechin; (−)-epicatechin; model solutions; yeast; sherry wine

INTRODUCTION

Browning resulting from reactions of phenolic compounds is an important alteration during storage of vegetable foods and beverages. In white wines, such alteration is probably the main source of economic losses, because consumers tend to reject the drink as it darkens, a change that is accompanied by others in the sensory properties of the wine.

One origin of browning is the enzymatic and nonenzymatic oxidation of phenolic compounds. The former usually takes place at the early stages of the wine-making process, whereas the latter is more typical of the storage period. In an acid medium such as wine the nonenzymatic pathway is slow, although it can be catalytically accelerated by the presence of traces of some metals (1−4). Also, the composition of the polyphenol fraction is quite influential; in fact, flavan-3-ol derivatives are proposed to be effective substrates for nonenzymatic browning reactions (5−8). In particular, the catalytic oxidation of (+)-catechin in an acid medium containing Fe^{2+} has been studied by authors such as Oszmianski et al. (4), who observed the formation of compounds similar to those generated by the enzymatic reaction in addition to other colorless and yellowish compounds. Subsequent analysis of such products revealed that the above-mentioned oxidation pathway may be accompanied by other reactions involving phenolic compounds, contributing to an increase in the absorption in the yellow-brown region. In this respect, the formation of colorless oligomers of (+)-catechin in model solutions containing tartaric acid and Fe^{2+} has been reported (9). These oligomers are formed by oxidation of tartaric acid to glyoxylic acid, a compound that acts as a

bridge between units of the flavan. The subsequent dehydration and oxidation of the colorless dimers yields yellowish compounds (10−12). Once glyoxylic acid has been formed, it condenses with flavans, in a similar way as found for other compounds such as acetaldehyde, which may thus be a potential competitor (13).

Because all of the previously mentioned compounds are present in wine, its browning may be the result of the different above-described reactions, even though the contribution of each individual process can only be estimated from a kinetic study. In any case, color change in sherry white wine subjected to biological aging is quite striking. Certainly, browning in these wines is similar to that in other white wines after bottling, so the proposed mechanisms should also apply. However, sherry wines exhibit no browning for years while aging under flor yeast (14, 15). Traditionally, this different behavior has been ascribed to a protective effect on the action of atmospheric oxygen exerted by the flor film yeast in two ways. On the one hand, the film hinders diffusion of oxygen from the atmosphere to the wine, thereby partially isolating it. On the other, flor yeasts consume oxygen to develop their typical aerobic metabolism, thereby becoming competitors for oxidizable compounds such as phenols. As a result, aging wine exhibits very low levels of dissolved oxygen, so that it preserves its pale color. This explanation is somewhat questionable if one takes into account that sherry wines are usually subjected to periodic transfers (so-called rocios), by which a fraction of the younger wines is mixed with the older ones during biological aging. In such transfers (typically three or four per year), the wine saturates with oxygen, which could result in cumulative progress of browning; this, however, is not the case. Baron et al. (16) ascribe the absence of browning in these conditions to the retention of brown

* Author to whom correspondence should be addressed (fax 957-218606; e-mail qe1mecam@uco.es).

polymers by flor yeast, which thus act as scavengers for these compounds. On the other hand, Bonilla et al. (*17*) use baker's yeast as a clarificant agent in the treatment of white wines containing browning compounds. These authors suggest that the ability of yeast to retain the brown polymers might be a result of these compounds interacting with their cell walls.

Even if the previous hypothesis is accepted, the resistance of sherry wines to browning during aging cannot be accounted for in full. In fact, for the brown compounds to be removed, they must have been previously formed; this requires the flavan concentrations to decrease during aging, which is not the case (*16*). Therefore, it is reasonable to consider some inhibition of the mechanisms for the formation of the yellow-brown compounds, an effect that may overlap with the above-described retention and protective effects. In this study, we examined changes in (+)-catechin and (−)-epicatechin model solutions containing yeast in order to identify potential inhibitory effects on some of the mechanisms causing darkening in white wines.

MATERIALS AND METHODS

Model Solutions. (+)-Catechin and (−)-epicatechin, supplied by Sigma-Aldrich Chemical, S.A. (Madrid, Spain), were used to prepare two different model solutions, each containing 500 mg/L of (+)-catechin or (−)-epicatechin and ethanol (15% v/v). This concentration of the flavans was chosen to better quantify the products obtained as a result of their degradation. The pH of the solutions was adjusted to 3.5 with tartaric acid. To increase the degradation rate of the flavans, all of the solutions were supplied with ferrous sulfate at a level of 20 mg of Fe^{2+}/L, as an oxidative catalyst. At this concentration of metal a high degradation of (+)-catechin has been found by some authors (*4*). Each solution of flavan was divided into three batches that were incubated for 8 weeks at 20 °C. The composition of each batch was as follows:

batch 1	batch 2	batch 3
catechin + Fe^{2+}	catechin + Fe^{2+} + yeast	catechin + Fe^{2+} + yeast after 3 weeks
epicatechin + Fe^{2+}	epicatechin + Fe^{2+} + yeast	epicatechin + Fe^{2+} + yeast after 3 weeks

Batches 2 and 3 were inoculated with 2 g/L of *Saccharomyces cerevisiae* dehydrated baker's yeast (Mauripan, Fleischmann, Canada). Because the model solutions did not contain the usual nutrients of a culture medium, the yeast cells did not grow during the 8 weeks of incubation, and they were accumulated at the bottom of the flask. All experiments were carried out in triplicate.

Analytical Procedures. For the analytical determinations, samples at 1, 2, 3, 4, 5, 6, and 8 weeks of incubation were taken. Spectrophotometric measurements at 420 and 520 nm were made with a Beckman spectrophotometer, model DU 600, in 10 mm path length cells, after filtration through 0.45 μm pore size.

After filtration (Millipore, 0.45 μm pore size), samples were analyzed by direct injection on HPLC (ThermoFinnigan, Spectra System P4000) in order to quantify the two flavans studied and their degradation products. The HPLC method has been described for the determination of flavans fraction in previous works (*16, 18*), with an estimated time of analysis of 40 min. Analyses were carried out on a C18 column (250 mm × 4.6 mm i.d., 5 μm particle size) by using 2% aqueous acetic acid and acetonitrile as mobile phases at a flow rate of 2 mL/min. The elution solvent was 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. The chromatograms were recorded at 280 and 420 nm by using a diode array detector (ThermoFinnigan UV6000LP). The former wavelength was used for quantification and the latter to observe the colored products of degradation of the flavans. These compounds were quantified as catechin and epicatechin, respectively.
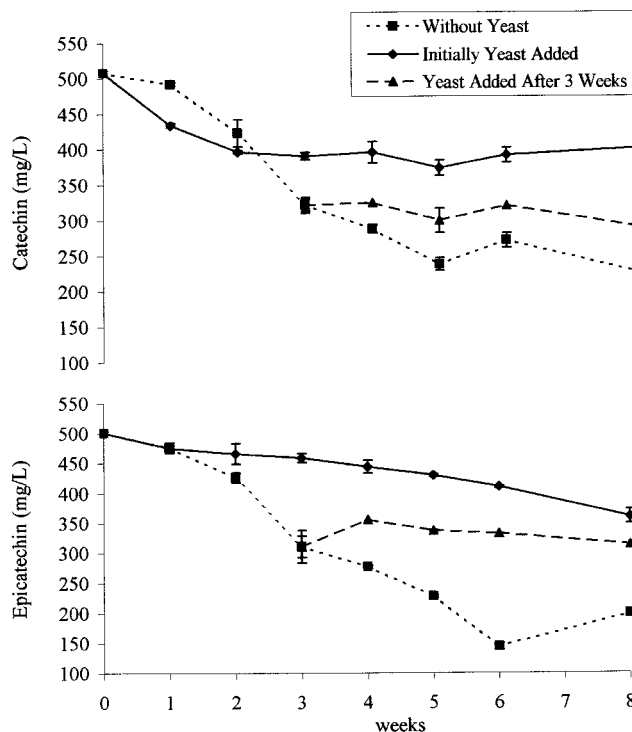

Figure 1. Means and standard deviations of the (+)-catechin and (−)-epicatechin concentrations during 8 weeks of incubation in the presence and absence of yeast.

RESULTS AND DISCUSSION

Figure 1 shows the changes of the (+)-catechin and (−)-epicatechin contents in the model solutions studied. As can be seen, in the absence of yeast (control solution), the content of both flavans decreased markedly during the experiments, with final concentrations about half of the initial levels for (+)-catechin and even lower for (−)-epicatechin. However, the flavan solutions that were initially supplied with yeast exhibited much stronger resistance to degradation, revealing a protective effect of the yeast. This effect cannot generally be ascribed to retention of the phenols by the yeast because their concentrations would have strongly decreased immediately after its addition and therefore would have been observed at the next sampling (1 week). To determine the potential relationship between the degradation products of (+)-catechin and (−)-epicatechin and the above-mentioned protective effect of the yeast, a third experiment in which yeast was added to solutions of the flavans after 3 weeks of degradation was carried out. As can be seen, this addition decreased the rate of degradation of the flavanes, the model solutions subsequently behaving similarly to those containing yeast from the beginning.

Absorbance at 420 nm is generally used as a measurement of browning in drinks such as wines, because the brown products resulting from the oxidation of phenolic compounds exhibit an absorption maximum in this zone of the visible spectral region. It should be noted that, under the conditions used in this work, colored compounds could have formed through two different pathways, either by autoxidation of the flavans (*4*) or by formation of oligomers of them in the presence of oxidation products from tartaric acid (*9, 12*). **Figure 2** shows the absorbance at 420 nm of the different (+)-catechin and (−)-epicatechin model solutions studied. In the absence of yeast, both flavan solutions exhibited marked increases at this wavelength due to the formation of colored degradation products. However, the solutions that were supplied with the yeast at the
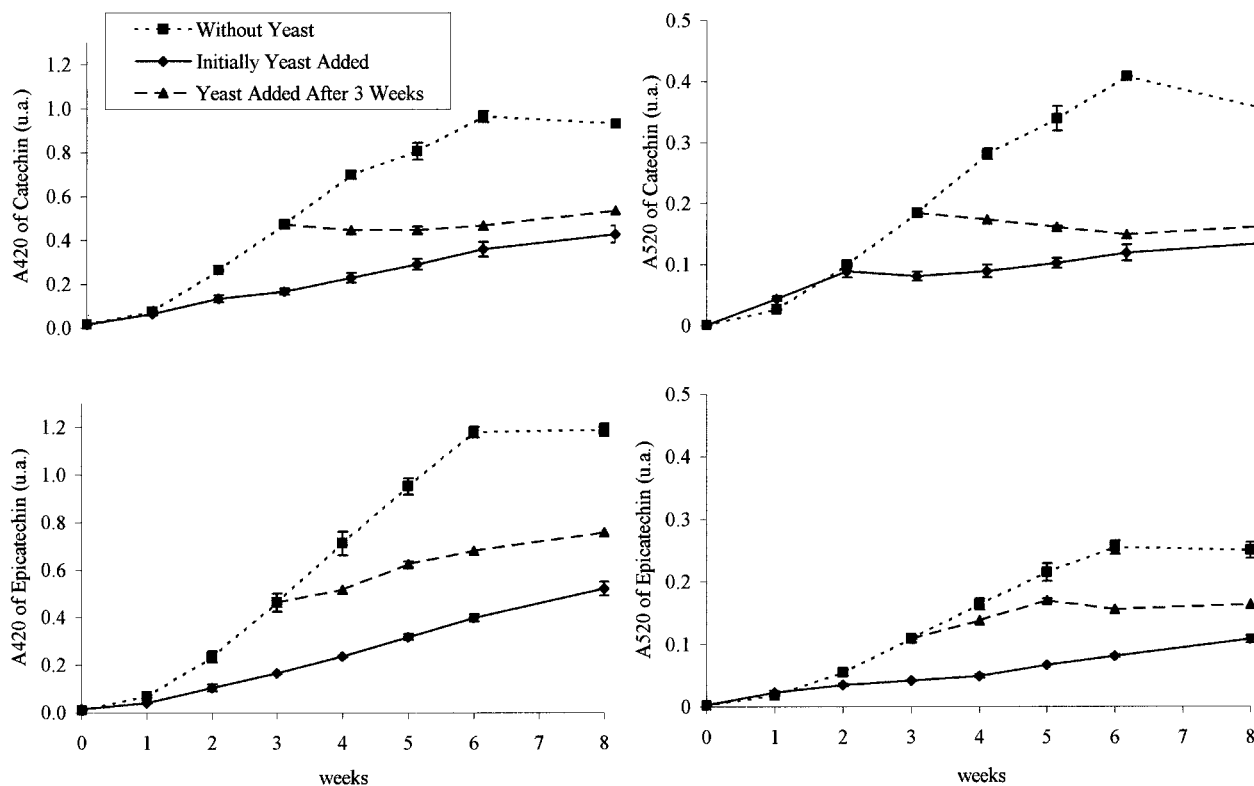
Figure 2. Means and standard deviations of the absorbances at 420 and 520 nm of the model solutions of flavans in the presence and absence of yeast.

beginning of the experiment showed a much more modest increase in absorbance at 420 nm, thereby reflecting the presence of lower concentrations of the brown compounds in solution.

Figure 2 shows the absorbance at 520 nm for the model solutions studied. This wavelength is an usual measurement of the red color in beverages such as the wines, and it was used because the degradation products of the flavans show a reddish hue. The results were similar to those obtained at 420 nm, although in the absence of yeast the compounds formed from the (+)-catechin solution were more reddish than those encountered in the (–)-epicatechin solution. Taking into account that the flavans studied are isomers and thereby possess the same molecular mass, the molar amounts of (–)-epicatechin degraded during the experiments involving no yeast exceeded those of (+)-catechin (**Figure 1**). However, because (+)-catechin exhibited greater 520 nm absorbance values in the absence of yeast, it is reasonable to suggest that the colored compounds formed from this flavan possess higher molar absorptivity coefficients at this wavelength than do those resulting from (–)-epicatechin. Also, the different behavior of the solutions of both flavans that were supplied with yeast after 3 weeks of incubation should be pointed out. Because the (+)-catechin solutions exhibited 520 nm absorbance values roughly twice those of the (–)-epicatechin solutions at the time the yeast was added, the former should have exhibited greater absorbances than the latter beyond that point, assuming an identical efficiency of the yeast on all types of colored compounds formed. However, the absorbance for the (+)-catechin solution failed to increase, which suggests that the yeast was more efficient toward the degradation products of this flavan.

Figures 3 and **4** show the chromatograms recorded at 280 nm for the model solutions after 8 weeks of incubation and in the absence of yeast. As can be seen, the peaks for the flavans are accompanied by a series of compounds resulting from their degradation (named C1–C8, E1–E5, and grouped peaks). To determine their contribution to the color of the solutions,

appropriate UV–vis scans for each peak were performed. All of the peaks showed a maximum in the UV region between 270 and 280 nm, most absorbing also in the visible spectral region, although very weakly. As an exception, the higher absorbance of a group of peaks eluting at 31–35 min should be noted. In fact, the chromatograms recorded at 420 nm exhibited appreciable absorbances only at these retention times. This suggests that these compounds are largely responsible for the increased color of the solutions, consistent with the findings of other authors (*17, 18*). From their retention times, it is reasonable that these compounds possess a high molecular mass and/or a low polarity, as previously reported (*4, 19, 20*) for the enzymatic and nonenzymatic oxidation of flavans. Also, the potential contribution to this zone of the chromatogram of yellowish oligomers resulting from the above-mentioned flavan condensation in the presence of glyoxylic acid should be considered.

To examine the changes in these compounds in the different experiments, and because their chromatographic resolution was poor, the areas of the overlapping peaks at retention times from 31 to 35 min were added and expressed as the corresponding flavan. **Figure 5** shows the variation of these grouped peaks for the (+)-catechin and (–)-epicatechin solutions during the studied period. As can be seen, in the absence of yeast, the concentrations of the catechin grouped peaks and epicatechin grouped peaks increased with time, thereby confirming that these compounds contribute to the above-described increase in color. The solutions initially containing yeast exhibited low increases in the concentrations of both grouped peaks, with a similar trend to those observed for the absorbances at 420 and 520 nm. On the other hand, the experiments in which the yeast was added after the third week again revealed a different behavior of the two flavans. Thus, the (–)-epicatechin solution exhibited moderately increased grouped peak values and a trend similar to those of the solution initially containing yeast; by contrast, the (+)-catechin solutions exhibited a decrease in their grouped
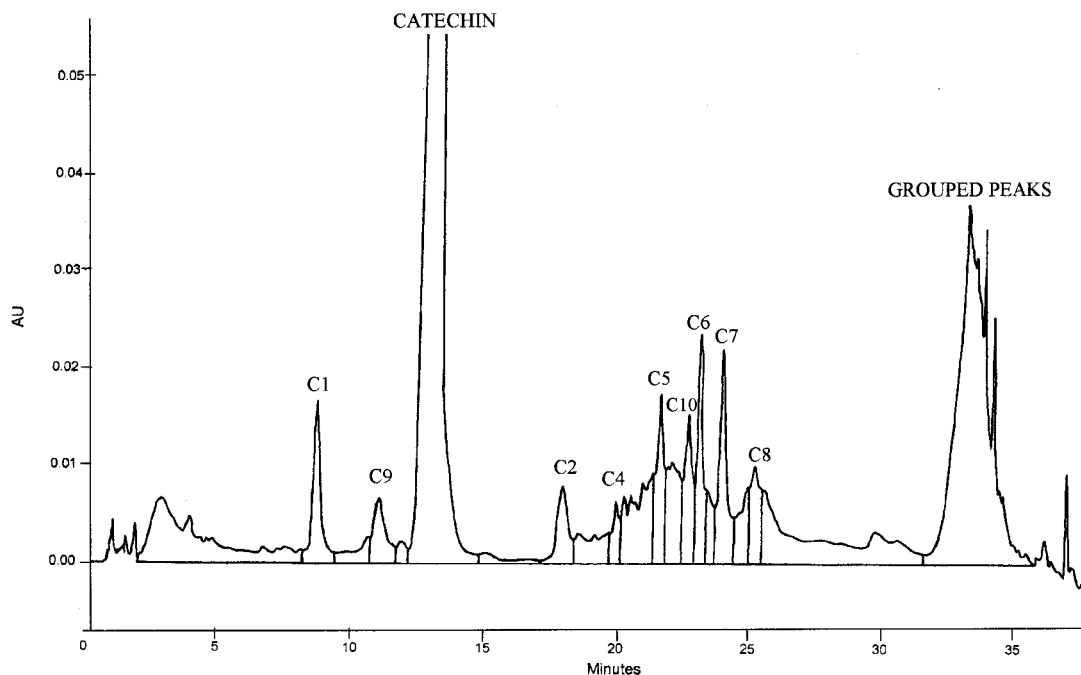
Figure 3. Chromatogram recorded at 280 nm of the (+)-catechin solution after 8 weeks of incubation. Degradation products: C1–C8 and grouped peaks.
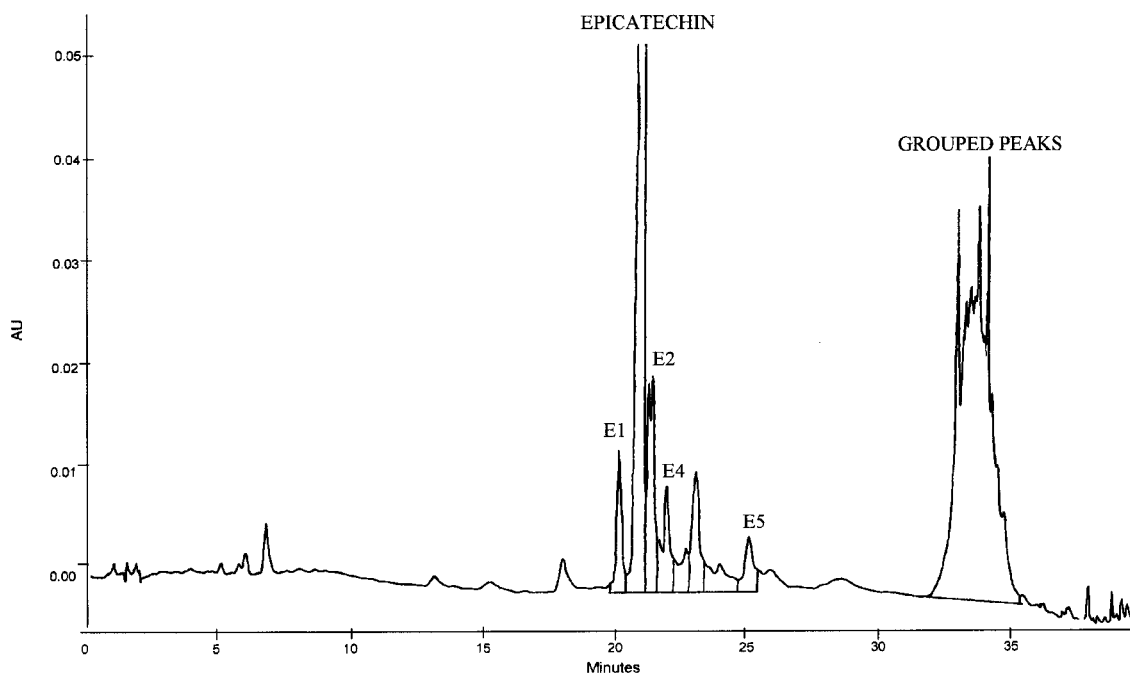

Figure 4. Chromatogram recorded at 280 nm of the (–)-epicatechin solution after 8 weeks of incubation. Degradation products: E1–E5 and grouped peaks.

peak levels. The fact that the decrease was especially marked between the third and fourth weeks suggests a retention of previously formed browning compounds by the yeast. This type of retention has been observed in middle-chain fatty acids (*21*), anthocyanins (*22−24*), and browning products (*17*).

Experiments involving the addition of yeast to (+)-catechin and (−)-epicatechin aqueous alcoholic model solutions containing tartaric acid and Fe^{2+} revealed partial inhibition in the degradation of these flavanes and much slower formation of browning compounds. In terms of color control in the solutions, the yeast was found to be more efficient toward the degradation products of (+)-catechin, particularly those absorbing at 520 nm. These results may partially explain the resistance to

darkening of some wines subjected to biological aging under flor yeast such as sherry wines.

It should be pointed out that this resistance has traditionally been ascribed to a protective effect on the action of atmospheric oxygen exerted by flor yeast growing on the wine surface. However, our results suggest that the mechanism by which the flavans are degraded is inhibited, either in the catalytic oxidation pathway or in the condensation with oxidation products of tartaric acid. This inhibitory effect does not exclude the above-mentioned protective effect, so the two may act in combination, resulting in more efficient preservation of wine color. On the other hand, the inhibition cannot be ascribed to a metabolic pathway involving the yeasts, because their cells did not grow
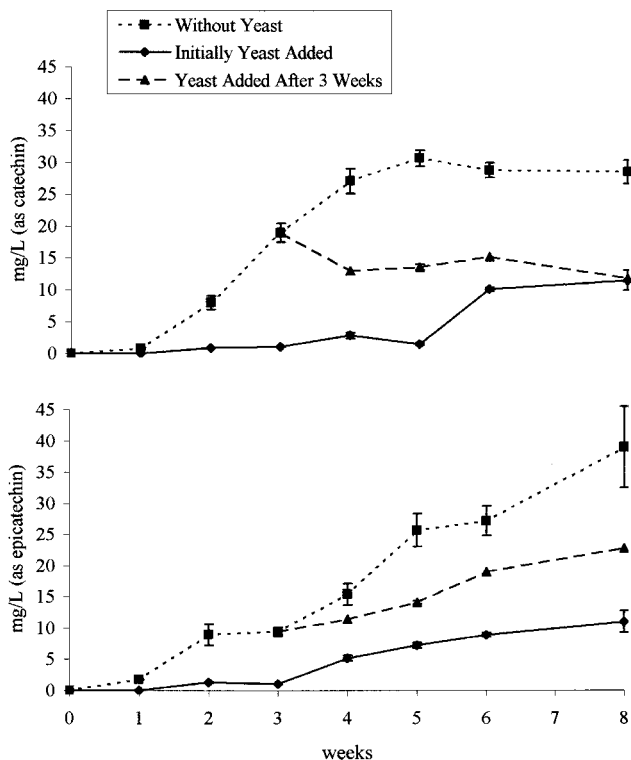
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Figure 5. Means and standard deviations of the grouped peak concentrations of the solutions of (+)-catechin and (−)-epicatechin during 8 weeks of incubation in the presence and absence of yeast.

in a model solution containing abundant ethanol and none of the typical components of a culture medium. Therefore, one can reasonably ascribe the inhibitory effect to some compound or fraction released by the yeast cells under conditions of autolysis. These compounds could inhibit the catalytic effect of Fe^{2+}, in this way decreasing the rate of browning. This hypothesis requires investigation, although it is supported by the fact that the wooden casks, in which sherry wine is biologically aged, simultaneously contain flor yeast growing on the wine surface and dead yeast in the cask bottom.

The (+)-catechin and (−)-epicatechin concentrations used in this work were in fact much higher than those usually present in wine so that any changes during their degradation could be readily observed. Therefore, yeast present in the aging of sherry wine containing normal concentrations of the flavans should be even more efficient in inhibiting their degradation, explaining at least in part the preservation of the pale color of this wine for years.

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