11318 J. Agric. Food Chem. 2010, 58, 11318–11324
DOI:10.1021/jf101931j

JOURNAL OF
AGRICULTURAL AND
FOOD CHEMISTRY
ARTICLE

Delaying Effect of a Wine *Lactobacillus plantarum* Strain on the Coloration and Xanthylium Pigment Formation Occurring in (+)-Catechin and (−)-Epicatechin Wine Model Solutions

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This article reports for the first time on the capacity of a wine *Lactobacillus plantarum* strain to alter the oxidative coloration of (+)-catechin and (−)-epicatechin hydroethanolic wine model solutions in the presence of Fe^{2+} as catalyst. The time course of color development and pigment formation in the solutions was tracked over 42 days. The pigments formed were characterized as xanthylium structures regardless of the flavanol isomer present in the solution. The solutions supplied with *Lactobacillus plantarum* RM71 were oxidized at a slower rate, and consequently, its final color was less than that in the controls. The formation of both (+)-catechin and (−)-epicatechin-derived xanthylium pigments was also delayed over time in the presence of the bacterium compared to their respective cell-free controls. The delaying effects provided by *L. plantarum* on the oxidative coloration and the generation of xanthylium-derived pigments were more pronounced for the (−)-epicatechin than for the (+)-catechin model solutions. In view of these results and given that *L. plantarum* is naturally present in winemaking and generally recognized as a safe microorganism, the potential application of this bacterium as an antibrowning agent for wine is now opened.

KEYWORDS: ***Lactobacillus plantarum*; (+)-catechin; (−)-epicatechin; xanthylium cation; model solution; browning**

1. INTRODUCTION

The management of browning in certain plant foods and beverages is of crucial interest for its commercialization. In the case of wine, progressive browning is a natural process that begins once the grape is pressed, and it is one of the most obvious changes during aging. In white wines, browning is considered an outstanding defect that reduces its quality and market value. In red wines the intense bright color observed after fermentation progressively changes toward a ruby and then a tawny or brick-like shade, which is considered, if accompanied by an appreciated aged bouquet, initially favorable. The browning of grape must and wine mainly results from reactions involving grape phenolic compounds which give rise to new polymeric compounds not originally present in the grape (*1*). In a previous review (*2*), the main known chemical reactions involving phenolic compounds during winemaking have been classified as follows: (i) oxidation reactions that produce quinones of different degree of polymerization, (ii) direct or acetaldehyde-mediated condensation reactions involving phenols, and (iii) cycloaddition reactions leading to the production of pyroanthocyanins. Flavanol−flavanol condensation reactions also occur in wine in the presence of glyoxylic acid, which is produced by the oxidation of tartaric acid with iron as catalyst in the reaction (*3*). Although all of these reactions contribute to the browning of grape must or wine during winemaking or aging, in white wine the flavanol−flavanol condensation reactions are of prime importance to the browning process as they give rise to xanthylium pigments, which have been recently detected in a white wine and shown to mainly contribute to the color increase in this beverage (*4*).

A factor that may influence the above-mentioned chemical reactions during winemaking is the performance of the microorganisms involved in this fermentative process. During alcoholic fermentation, yeast produce metabolites, i.e., acetaldehyde and pyruvic acid, which are known to act as reactants in some of the above-mentioned reactions leading to the production of compounds involved in color changes (*2*). The known interaction of yeasts with wine polyphenols is another way by which yeast may influence the browning process. This ability has been attributed to cell walls which are able to directly adsorb brown polymers (*5*, *6*) or slow the production of brown products (*7*) by adsorption of intermediate colorless products (*8*, *9*). On the basis of this capacity, several authors have developed antibrowning biological strategies (*10*) that, by using yeasts, effectively reduce or slow wine browning.

Wine lactic acid bacteria (LAB) belonging to the genera *Lactobacillus*, *Pediococcus*, and *Oenococcus* are integral components in the ecology of winemaking (*11*); however, little is known on the involvement of this group of microorganisms in the browning of wine. Among wine LAB, *Lactobacillus plantarum* has been isolated with the highest frequency in grape must, white, and red wines from several areas (*12*−*15*). In some recognized

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 Published on Web 10/06/2010 © 2010 American Chemical Society

white wines, like Albariño (northwestern Spain), *Lactobacillus plantarum* was predominantly isolated among wine LAB at earlier, end of alcoholic, and malolactic fermentation stages of the winemaking process (*16*, *17*).

Since oxidative polymerization leading to the formation of brown pigments depends on the nature and relative concentration of the phenolic present (*18*), the interaction between phenolic compounds and LAB in wine could influence the browning process. In view of this, the aim of this work is to test the influence of *Lactobacillus plantarum* on the browning of wine model solutions. With this goal, we have studied changes in color, flavanol oxidation, and pigment development in (+)-catechin and (−)-epicatechin wine model solutions developed in presence or absence of a *Lactobacillus plantarum* strain of wine origin.

2. MATERIALS AND METHODS

2.1. Strain and Culture Medium. *Lactobacillus plantarum* RM71, originally isolated from wine at the Instituto de Fermentaciones Industriales (CSIC) (*19*), was used throughout this study. *L. plantarum* RM71 was routinely grown on de Man, Rogosa, and Sharpe (MRS) agar medium (*20*) (Difco Laboratories) at 30 °C.

2.2. Preparation of Wine Model Solutions for Browning Simulation. Two wine model solutions containing either 400 mg/L (+)-catechin or (−)-epicatechin (Sigma-Aldrich Chemical, S.A. Madrid, Spain) were prepared in a wine-like medium containing 5 g/L tartaric acid, 0.6 g/L acetic acid, and 1.75 g/L malic acid in 12% ethanol. The flavanol concentrations exceed largely those found normally in wine in order to facilitate the examination of changes during their degradation. The wine acids and ethanol were added at a concentration similar to the one previously used by Ugliano et al. (*21*) for a synthetic wine designed to reproduce the composition of wine. The solutions were adjusted to pH 3.5 with NaOH. Ferrous sulfate was added as catalyst to the model solutions to bring their final concentration up to 10 mg/L, which was previously demonstrated to accelerate the rate of (+)-catechin degradation (*22*).

Lactobacillus plantarum RM71 colonies from MRS plates were inoculated into MRS broth and incubated for 24 h at 30 °C. Ten milliliters of fully grown cells were centrifuged, washed three times with distilled water, and frozen at −40 °C; then the biomass was lyophilized (Virtis. 6KBTEL −85). The lyophilized biomass of *Lactobacillus plantarum* RM71 was used to inoculate the wine model solutions and bring their final inoculum size to 2×10^9 lyophilized cells/mL. Ten milliliters of the inoculated wine model solutions, their respective cell free controls, or wine model solutions devoid of flavanols (the last used to subtract the absorbance due to the medium) was incubated in stoppered glass tubes (16 × 150 mm) under microaerobic conditions in darkness at 25 °C in a BioRad incubator. Samples were periodically collected and filtered (pore size 0.45 μm, Millipore) over a 42 day time period for spectrophotometric and analytical determinations. Experiments were carried out in triplicate.

2.3. Analytical Methods. *2.3.1. Spectrophotometric Measurements.* Measurements at 420 nm of model solutions were carried out in a Shimadzu UV−visible 1601 spectrophotometer in 10 mm path length cells.

2.3.2. HPLC-DAD Analysis. For HPLC-DAD analysis, (+)-catechin, (−)-epicatechin, and its degradation products were extracted twice from one milliliter filtered aliquots with one-third (370 μL) of the reaction volume of ethyl acetate (Lab-Scan, Ireland). Analysis of the injected samples (50 μL) was carried out as previously described (*23*) in a Thermo (Thermo Electron Corporation, Waltham, MA) chromatographic system equipped with a P400 SpectraSystem pump, an AS3000 autosampler, and a UV6000LP photodiode array detector. A gradient of solvent A (water/acetic acid, 98:2, v/v) and solvent B (water/acetonitrile/acetic acid, 78:20:2, v/v/v) was applied to a reverse phase Nova-pack C_{18} column (25 cm × 4.0 mm i.d.; 4.6 μm particle size) at room temperature. The elution program was as follows: 0−55 min, 80% B linear, 1.1 mL/min; 55−57 min, 90% B linear; 1.2 mL/min; 57−70 min, 90% B isocratic, 1.2 mL/min; 70−80 min, 95% B linear, 1.2 mL/min; 80−90 min, 100% linear, 1.2 mL/min; 100−120 min, washing at 1.0 mL/min. Detection was performed by scanning from 220 to 500 nm. Samples were injected in duplicate onto the column after being filtered through a 0.45 μm polyvinylidene difluoride (PVDF) filter (Teknokroma, Spain). The chromatograms were recorded at 280 nm for quantification and 440 to monitor the formation of colored products resulting from the degradation of the flavanols during the oxidation of wine model solutions.

2.3.3. HPLC/ESI-MS Analysis. MS measurements were performed in a Hewlett−Packard series 1100 (Palo Alto, CA) chromatography system equipped with a diode array detector (DAD) and a quadrupole mass spectrometer (Hewlett−Packard series 1100 MSD) with an electrospray interface. Chromatographic separations were performed with the same column and solvent conditions as those used in LC-DAD experiments. DAD detection was performed from 210 to 500 nm, with a flow of 0.7 mL/min. For MS, nitrogen was used as the nebulizing and drying gas, and the ion source parameters were as follows: nebulizer pressure, 55 psi; drying gas, 11 L/min; and dry temperature, 350 °C. The capillary voltage was −4 kV. A fragmentation voltage of −90 V was used. Spectra were recorded in the negative ion mode over the m/z range of 100 to 1,500.

3. RESULTS AND DISCUSSION

3.1. Effect of *L. plantarum* RM 71 on the Color Development of Wine Model Solutions. To mimic part of the wine oxidation process, hydroethanolic model solutions containing a mixture of the wine tartaric, acetic, and malic acids, supplied with either (+)-catechin or (−)-epicatechin, were used. Ferrous ions were added to these solutions given its effectiveness as a catalyst for flavanol oxidation (*24*). The browning of drinks is usually monitored by variations in absorbance at 420 nm ($A_{420\ nm}$) since brown products resulting from the oxidation of phenols present in beverages increase the color in the yellow−brown region (*23*). Hence, in order to test the potential of *L. plantarum* RM71 to influence the browning process, the variations in $A_{420\ nm}$ displayed by the (+)-catechin or (−)-epicatechin wine model solutions were tracked as a function of the presence of this microorganism.

Figure 1 shows how (+)-catechin or (−)-epicatechin model solutions were oxidized over the incubation period in the presence of ferrous sulfate at 10 mg/L. As can be observed the $A_{420\ nm}$ of control solutions lacking *L. plantarum* RM71 markedly increased with time, revealing the formation of colored compounds absorbing in the visible region. The incubation of (+)-catechin or (−)-epicatechin model solutions in the presence of *L. plantarum* RM71, which was added at the onset of the experiments, resulted in a partial inhibition of the browning process as observed by the final $A_{420\ nm}$ and the rate of formation of brown compounds (**Figure 1A** and **B**). As calculated by the percentage of decrease in the final $A_{420\ nm}$ compared to controls lacking *L. plantarum* RM71, the percentage of browning inhibition provided by the presence of *L. plantarum* RM71 was 21.5% in the (+)-catechin and 29.2% in the (−)-epicatechin solutions. The observation of higher $A_{420\ nm}$ values in the solutions containing (−)-epicatechin than in the solutions containing (+)-catechin should not be surprising to judge by the results of Labrouche et al. (*25*) who reported that (−)-epicatechin has the ability to generate pigments with greater absorbance in the visible region (440 nm) than those found for (+) catechin. According to previous reports, one of the pathways that give rise to the formation of colored compounds is the oxidation of the flavanols catalyzed by copper and/or iron, and the subsequent polymerization of the oxidized products (*22*, *26*, *27*). However, when tartaric acid is present in the medium, as is the case in our wine model solutions and in wine, the oxidation process does not occur directly on the flavanols but on the tartaric acid which is converted into glyoxylic acid. The glyoxylic acid then reacts with two flavanol units to give rise to a colorless dimer which may undergo dehydration and subsequent oxidation to form yellow pigments (*3*).

3.2. Identification of Brown Reaction Products in (+)-Catechin Wine Model Solutions. To gain more information on the color changes observed, chemical changes were investigated by recording

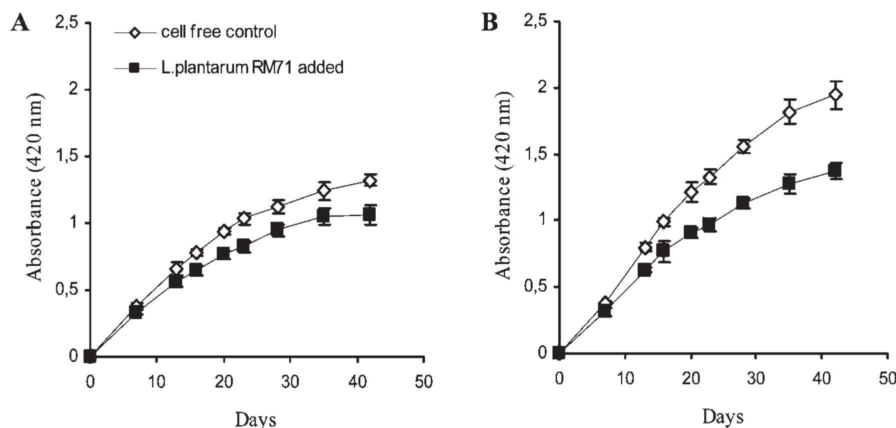

Figure 1. Delaying effect of *Lactobacillus plantarum* RM71 of the browning of (+)-catechin (**A**) and (−)-epicatechin (**B**) wine model solutions [12% ethanol (v/v), pH 3.5] as measured at 420 nm over a 42 day time period at 25 °C. Each point represents the average value of triplicates. Standard deviations are represented by error bars.

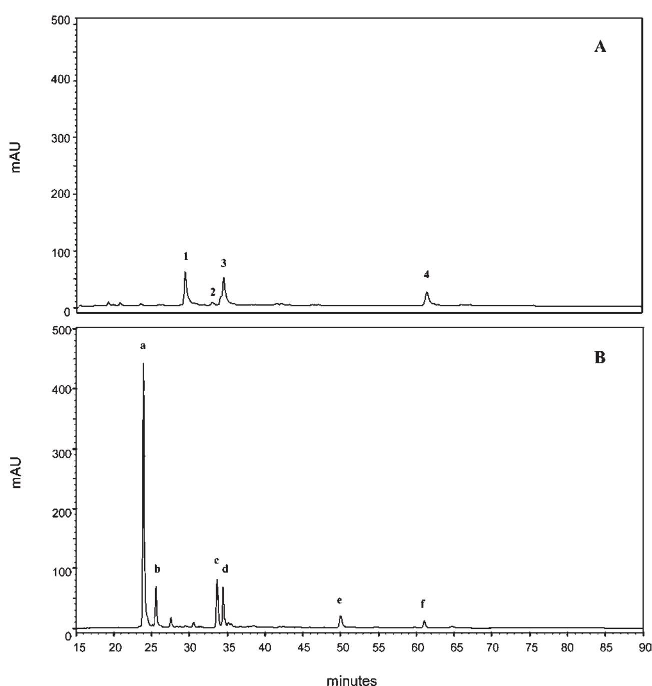

Figure 2. LC-DAD chromatograms recorded at 440 nm for cell free (+)-catechin (**A**) and (−)-epicatechin (**B**) wine model solutions after 42 days of incubation at 25 °C.

the HPLC-DAD chromatograms for the (+)-catechin and (−)-epicatechin wine model solutions at 280 and 440 nm. After 42 days of incubation, the chromatograms for the (+)-catechin wine model solutions recorded at 280 nm revealed that the major metabolite present in the solutions was (+)-catechin (retention time 18.17 min.), with other peaks appearing as result of its degradation. *L. plantarum* RM71 was apparently not involved in determining the degradation profile of this flavanol as the peaks observed in the solutions containing this bacterium, though with different intensity, were the same as those found in the cell free controls (not shown). **Figure 2A** shows the HPLC-DAD (440 nm) chromatogram of the (+)-catechin model system lacking *L. plantarum* RM71 recorded after 42 days of incubation. This chromatogram displays four peaks (*1−4*) that reveal the formation of new compounds from (+)-catechin not observed at the onset of the experiment. The HPLC-DAD chromatograms recorded at 440 nm of the solutions containing or lacking *L. plantarum* RM71 also had the same profile (not shown).

Browning of wine model solutions usually begins with the formation of colorless dimeric adducts composed by two flavanol units. These adducts might result from the condensation with either acetaldehyde (*28*), which arises from ethanol oxidation (also from yeast metabolism in wine), or with glyoxylic acid (*29−33*), which arises from the oxidation of tartaric acid. It has been demonstrated that (+)-catechin reacts three times faster with glyoxylic acid than with acetaldehyde (*34*). Also, a previous report using model solutions containing (+)-catechin, tartaric acid, and ethanol showed that the colorless and yellow compounds detected were derived from flavanol−flavanol condensation mediated by glyoxylic acid (*35*). Despite the different chromatography conditions utilized in this study, the peak elution pattern and relative peak intensities observed for peaks 1−4 are similar to those observed for the catechin degradation products formed in a (+)-catechin/glyoxylic acid system identified in past studies (*25*). It has been previously established that the brown products formed in (+)-catechin/glyoxylic acid model solutions are xanthylium pigments (*30−33*). These xanthylium cations arise from the oxidation of xanthenes formed by the dehydration of the first generated colorless dimers. Since these type of pigments display a characteristic UV−visible spectrum with a maximum absorbance at 440 nm, a shoulder at 310 nm, and a peak at 278 nm (*31*), the UV−visible scans of each (+)-catechin degradation peak were examined. For instance, the UV−visible spectral characteristics of peaks 1 and 3 extracted from the HPLC-DAD chromatograms are shown in **Figure 3A**, and they match (as does the peak 2; not shown) with the characteristics described above, that is, a peak maximum at 440 nm, a shoulder at 310 nm, and a peak at 278 nm. Regarding peak 4, it shows the spectral characteristic of the esterified xanthylium pigment, in particular, the ethyl ester, that is, the peak absorbance maxima at 460 nm instead of 440 nm and a shoulder at 310 nm, which have been already described by Es-Safi (*32*). The spectral characteristics displayed by peaks 1 to 4 provide a first clue that the products responsible for the increase of absorbance in the visible region for the wine-like (+)-catechin model solutions used here could be xanthylium pigments.

HPLC/ESI-MS experiments with induced fragmentation of the compounds responsible for peaks 1 to 4 were performed to provide further evidence that they represent xanthylium pigments formed from (+)-catechin. The mass spectra for peaks 1, 2, and 3 in the negative ion mode displayed prominent m/z 615 signals,

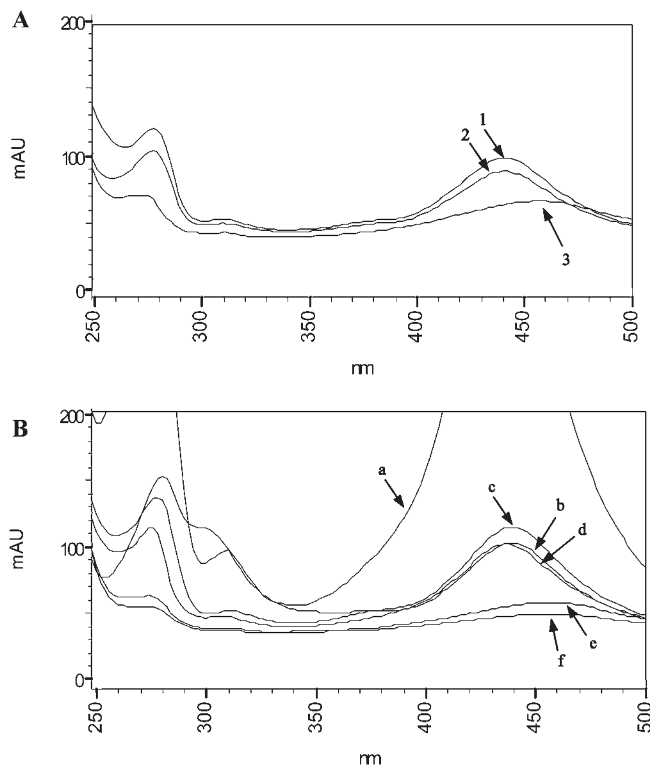

Figure 3. UV−visible spectra of the (+)-catechin (peaks 1, 3, and 4) (**A**) and (−)-epicatechin (peaks a−f) (**B**) degradation products in cell free wine model solutions after 42 days of incubation at 25 °C.

which released two main fragment ions at *m*/*z* 571 (−44 amu, decarboxylation) and *m*/*z* 419 (−152 amu, further retro Diels−Alder fission of a catechin moeity). These peak data are consistent with their assignment as xanthylium cations (*30*, *33*, *25*). As an example, the mass spectrum of peak 1 from a sample extracted after 42 days of incubation of the (+)-catechin model solution is shown in **Figure 4A**. The mass spectrum of peak 4 showed a signal in the negative ion mode at *m*/*z* 643 with a major fragment ion at *m*/*z* 597 (data not shown), which would correspond to the xanthylium cation ethyl ester previously described (*25*).

3.3. Identification of Brown Reaction Products in (−)-Epicatechin Wine Model Solutions. **Figure 2B** shows the HPLC-DAD chromatogram recorded at 440 nm of the cell free control (−)-epicatechin model solutions after 42 days of incubation at 25 °C. Peaks a−f in this chromatogram represent yellowish pigments from (−)-epicatechin. *L. plantarum* apparently did not influence the type of yellow pigments formed from (−)-epicatechin as the chromatogram of the model solutions incubated in the presence of *L. plantarum* RM71 rendered the same peak profile observed for the uninoculated controls (not shown). To get the first evidence that these peaks represented xanthylium pigments, the UV−visible spectrum of each peak was examined. Peaks a−d showed the spectral characteristics typical of xanthylium pigments, that is, λ_{max} at 440 nm, shoulder at 310 nm, and peak at 278 nm (**Figure 3B**). Peaks e and f had a λ_{max} at 460 nm and a shoulder at 310 nm, which are spectral characteristics typical of the ethyl ester of the xanthylium pigment (**Figure 3B**).

To further confirm that peaks a−f represent xanthylium pigments formed from (−)-epicatechin, the mass spectra for each peak formed was recorded in the negative ion mode. The xanthylium pigments produced in the (−)-epicatechin wine model system represented in peaks a−d were analogous to those represented in peaks 1−3 formed in the (+)-catechin system, as all gave signals at *m*/*z* 615. These xanthylium pigments exhibited nearly the same fragmentation patterns as those observed in the (+)-catechin system with the main fragment ions at *m*/*z* 571 and 419. Since (+)-catechin and (−)-epicatechin are diastereoisomers, their xanthylium derivatives keep a diastereoisomeric relationship, thus explaining that, though analogous, both type of cations display different retention times in their chromatograms (*25*). However, the observation of six instead of four degradation peaks in the (−)-epicatechin model solution with respect to the (+)-catechin model solution could be the result of coelution of the different xanthylium pigment isomers or the preferential formation of certain isomers, as previously proposed (*25*). These isomers showed slightly different fragmentation patterns; for instance, in peak b the fragmentation peak preferentially formed was the decarboxylated ion of the xanthylium cation (*m*/*z* 571), while in peak c it was that corresponding to the further retro-Diels−Alder fission of the decarboxylated cation (*m*/*z* 419). Despite these differences, further analysis on the degradation products of (−)-epicatechin will be necessary to investigate the above-mentioned hypothesis. In peak d, besides these ions, other intermediate ions (*m*/*z* at 560 and 512) were much less apparent. As an example, the mass spectrum of peak c from a sample extracted after 42 days of incubation of the (−)-epicatechin model solution is shown in **Figure 4B**. The mass spectra of peaks e and f showed signals at *m*/*z* 643 with the main fragment ion at *m*/*z* 597 which, as mentioned above for the (+)-catechin wine model solutions, would correspond to the xanthylium ethyl esters previously described. These results confirm that the xanthylium cations formed in both the (−)-epicatechin and (+)-catechin wine-like model solutions used in this study were analogous.

3.4. Delaying Effect of *Lactobacillus plantarum* RM71 on the Formation of Brown Pigments in the (+)-Catechin and (−)-Epicatechin Wine Model Solutions. To determine if the lower browning rates displayed by the wine-like (+)-catechin and (−)-epicatechin model solutions in the presence of *L. plantarum* RM71 (**Figure 1A** and **B**) were caused by the inhibition of the corresponding polymerization reactions leading to the xanthylium pigments identified above, the generation of these pigments was comparatively studied over time in model solutions lacking or supplemented with *L. plantarum*. For this goal, the peak areas that represent the xanthylium pigments in the HPLC-DAD 440 nm chromatograms were monitored over the 42 day incubation period. The total areas of peaks 1−4 were combined into one and monitored over the incubation period to track the formation of (+)-catechin-derived xanthylium pigments (**Figure 5A**). The variation in the combined areas of peaks a−f was monitored for the same length of time in order to track the formation of (−)-epicatechin-derived xanthylium pigments (**Figure 5B**). By comparing **Figure 5A** and **B**, it could be observed that the total peak areas for the xanthylium cations formed from (−)-epicatechin were about double those generated from (+)-catechin. To test if this difference was due to a faster loss of (−)-epicatechin than (+)-catechin, the decrease of their respective peak areas at 280 nm (retention times 25.19 and 18.17 min, respectively) was tracked and compared. By monitoring the loss of both flavanols over the 42 day incubation period, it was observed that the lost of (−)-epicatechin (**Figure 6B**) was 1.26 faster than that for (+)-catechin (**Figure 6A**). This observation is in agreement with previous works reporting the same trend for these flavanols in reactions with acetaldehyde (*36*) or glyoxylic acid (*25*). The discord between the rate of loss of (−)-epicatechin to (+)-catechin (1.26) and the difference in total 440 nm absorbance displayed by their respective xanthylium cations (2.0) can be explained by the higher collective molar absorptivity of the pigments formed from (−)-epicatechin than from (+)-catechin (*25*).

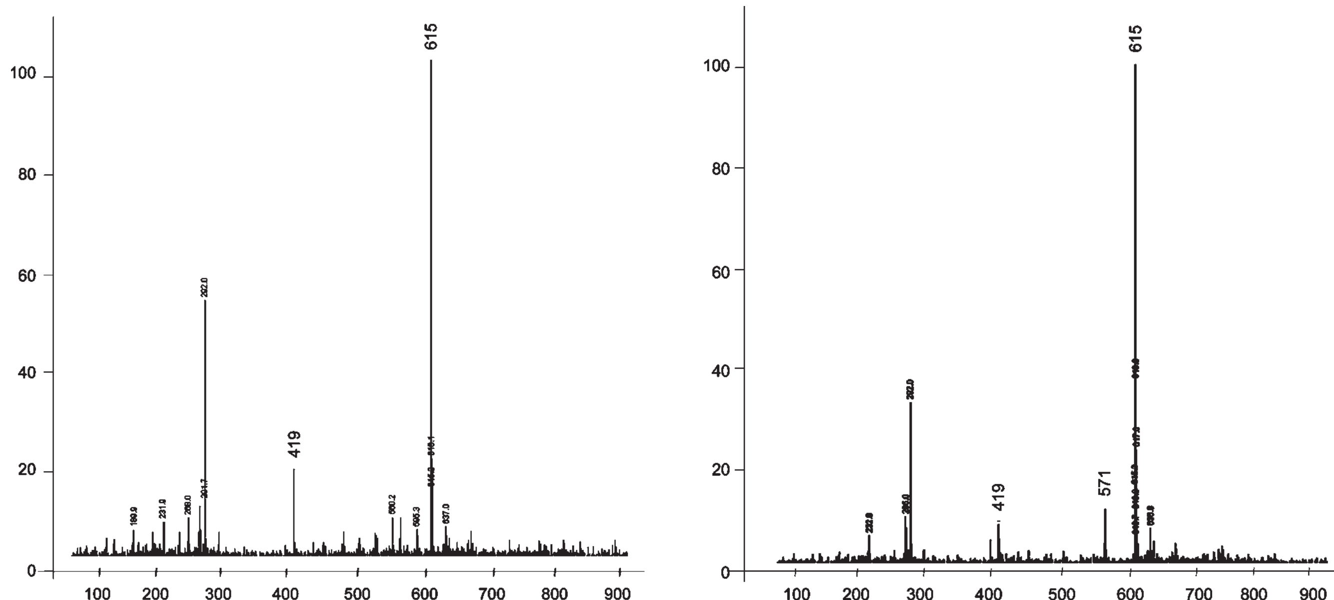

Figure 4. Mass spectra of (+)-catechin degradation peak 1 (**A**) and (−)-epicatechin degradation peak c (**B**) identified in cell free wine model solutions after 42 days of incubation at 25 °C.

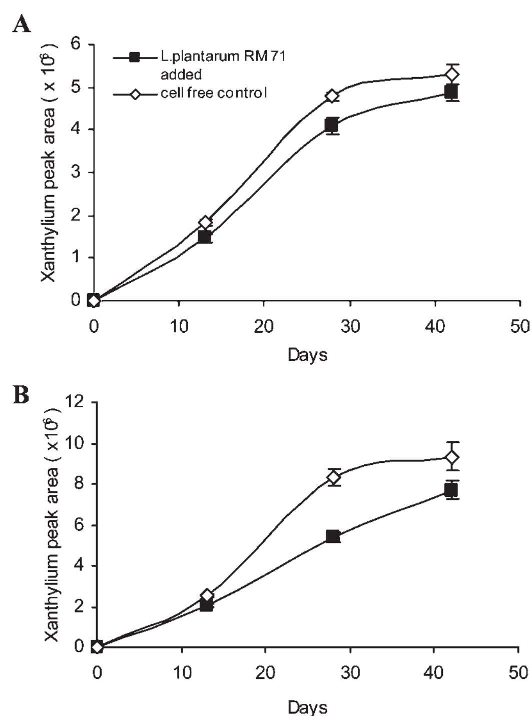

Figure 5. Delaying effect of *Lactobacillus plantarum* RM71 on the formation of xanthylium pigments formed in (+)-catechin (**A**) and (−)-epicatechin (**B**) wine model solutions. Each point represents the combined peak areas (LC-DAD 440 nm) of the xanthylium cations formed in the respective solutions (peaks 1−4 in the (+)-catechin or peaks a−f in the (−)-epicatechin model solutions, respectively) at the indicated times. Each point represents the average value of triplicates. Standard deviations are represented by error bars.

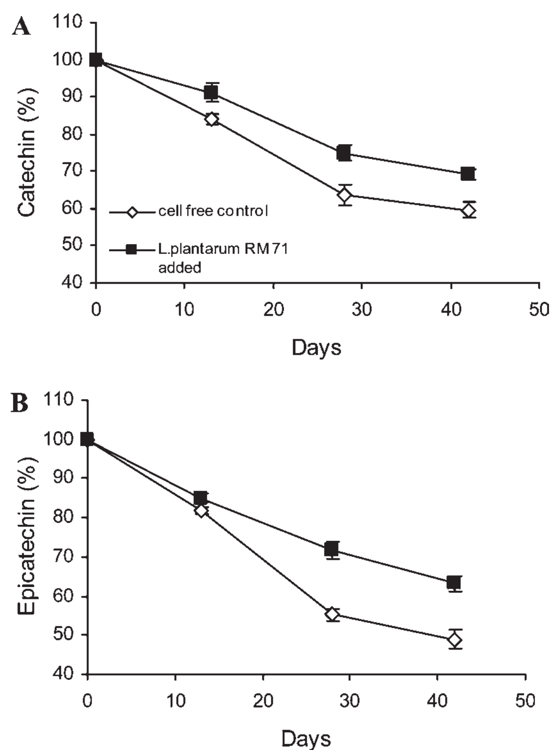

Figure 6. Preventive effect of the oxidative degradation of (+)-catechin (**A**) and (−)-epicatechin (**B**) by *Lactobacillus plantarum* RM71 in wine model solutions. Variations in the concentrations of both flavans over a 42 day time period at 25 °C are shown. Each point represents the average value of triplicates. Standard deviations are represented by error bars.

As shown in **Figure 6A** and **B**, the presence of *L. plantarum* RM71 delayed the oxidative degradation of (+)-catechin and (−)-epicatechin. Accordingly, the generation of xanthylium pigments derived from (+)-catechin (**Figure 5A**) and (−)-epicatechin (**Figure 5B**) were also delayed in the wine model solutions supplemented with this bacterium. The increase in the total peak areas representing the (+)-catechin and (−)-epicatechin derived xanthylium cations that were formed in the cell free controls as well as in the cultures supplemented with *L. plantarum* RM71 showed the same trend as the increases of $A_{420\ nm}$ observed in **Figure 1A** and **B**. Although the impact of *L. plantarum* RM71 on $A_{420\ nm}$ values (**Figure 1**) and peak areas representing the xanthylium cations differs slightly (probably due to unavoidable variations of the xanthylium cation concentration caused by the

extraction process), the slower increase in the total peak areas representing the (+)-catechin or (−)-epicatechin xanthylium cations in the solutions supplemented with *L. plantarum* reflected the delay of browning ($A_{420\ nm}$) in their respective model systems. These results confirmed that the xanthylium pigments were the main pigments responsible for the browning of the model solutions and that *L. plantarum* RM71 delayed its formation. The inhibitory effect provided by *L. plantarum* on the generation of xanthylium pigments was, however, more pronounced for (−)-epicatechin than for the (+)-catechin-derived pigments. In the samples extracted on day 28 (time point after which the browning rate of all solutions began to decrease), *L. plantarum* inhibited by 35.2% the formation of the (−)-epicatechin and by 14.1% the (+)-catechin-derived xanthylium pigments compared to their respective controls. The mechanism involved in preventing the formation of xanthylium salts by *L. plantarum* is now under study in our group. It has recently been shown that yeast membrane sterols are able to interact strongly with some polymerized compounds and colorless intermediates of browning reactions (*37*), thus explaining its ability to prevent this defect. Nevertheless, sterol biosynthesis is viewed as a eukaryotic process, and it is almost absent in prokaryotes. As can be seen in **Figure 6**, *Lactobacillus plantarum* RM71 is able to prevent the oxidative degradation of (+)-catechin and (−)-epicatechin. Since the presence of sterols is a very rare event in prokaryotes, we hypothesize that other bacterial membrane or cell wall components with a role similar to that of sterols, for instance hopanoids, could be present in *L. plantarum* RM71 membranes and interact with the compounds resulting from the browning reactions. However, this hypothesis requires further investigation. It has been reported that there exists a high correlation between browning and the (−)-epicatechin concentration in wine (*38*), which has been ascribed to the ability of this flavanol to form xanthylium pigments with greater absorbance at 440 nm than that found for (+)-catechin (*25*). Hence, the capacity of *L. plantarum* to preferentially prevent the formation of (−)-epicatechin over (+)-catechin-derived xanthylium pigments would be a valuable trait when using this microorganism to prevent the browning of wine. However, the capacity of this bacterium to delay the formation of both types of pigments would be of utility regardless of the seasonal growing conditions, grape, or winemaking practices, which are known to influence the content of these flavanols in wine (*39*). Nevertheless, due to the fact that (i) real wine display a more complex phenolic composition than the solutions used in this study and (ii) both the flavanol concentrations used as well as the bacterial inoculum size used largely exceed those found under real wine conditions, it is still necessary to confirm the impact of *L. plantarum* RM71 to delay the browning of a real wine.

This study expands our insight on the dynamics of wine browning as it ascribes for the first time a role to a wine lactic acid bacteria in the prevention of this oxidative process. The flavanol xanthylium salts are pigments that (i) occur in white (*4*) as well as in red wines (*33*) and (ii) may act as transitory species in the formation of colored quinonoidal derivative compounds that contribute to the color changes of grape-derived foods (*32*). In view of this, the preventive inhibition of the pathways leading to the xanthylium pigment will contribute to delay the browning processes associated with these pigments. In previous reports, the ability of fermentative yeast to prevent the browning of (+)-catechin and (−)-epicatechin model solutions (*7*), (+)-catechin/acetaldehyde and (+)-catechin/glyoxylic acid model solutions (*40*), and wine (*41*) was demonstrated. Yeast's ability to delay browning is notorious in pale white wines of the Sherry type, which are hardly browned because of the protective effect of the flor yeast growing in its surface (*42*). As an alternative to yeasts, the ability of *L. plantarum* to prevent the formation of xanthylium salts could be used to delay the browning process associated with these pigments, more so when this bacterium is naturally present after alcoholic fermentation (*16*); but contrary to other wine lactic acid bacteria such as *Oneococcus oeni*, this bacterium does not usually proliferate at this stage (*43*). This ability could therefore be of utility to prevent browning in organic wine and to relieve antibrowning strategies for wine based on the use of chemicals. However, the successful adaptation of *L. plantarum* to plant niches and its status as generally recognized as a safe microorganism now open, supported by the findings of this study, the potential application of this bacterium as an antibrowning agent in plant-derived foods.

ACKNOWLEDGMENT

We thank T. Herraiz for helpful comments.

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Received for review May 20, 2010. Revised manuscript received September 23, 2010. Accepted September 23, 2010. This work was supported by grants AGL2005-000470, AGL2008-001052, and Consolider INGENIO 2010 CSD2007-00063 FUN-C-FOOD (CICYT), and 200670M058 and S2009/AGR-1469 (ALIBIRD) (CAM). J.A.C. is a recipient of a fellowship from the Ministerio de Ciencia e Innovación (MICINN).