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Antioxidant Activity and Diffusion of Catechin and Epicatechin from Antioxidant Active Films Made of Poly(L-lactic acid)

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ABSTRACT: Active membranes and food packaging containing antioxidants like catechin and epicatechin, combined with the use of materials made of biopolymers obtained from renewable sources, could create a novel alternative to reduce oxidation in food, pharmaceutical, and cosmetic products. Poly(94% L-lactic acid) films containing 1.28% catechin and 1.50% epicatechin were extruded in a pilot plant-scale extrusion machine. The diffusion kinetics of catechin and epicatechin into 95% ethanol at 20, 30, 40, and 50 °C and 50% ethanol at 40 °C displayed Fickian release behavior and diffusion coefficients between 0.5 and 50 × 10^{-11} cm^2/s. According to the Arrhenius equation, the energy of activation for the diffusion of catechin and epicatechin in the films was 110.43 and 98.92 kJ/mol, respectively. The antioxidant activity of the films was measured in methanol extracts containing 46.42 μg/mL of catechin and 57.52 μg/mL of epicatechin as 32.90 and 36.68% of scavenging the 2,2-diphenyl-1-picrylhydrazyl radical, respectively.

KEYWORDS: *active packaging, poly(lactic acid), catechin, epicatechin, migration, diffusion*

INTRODUCTION

Poly(lactic acid) (PLLA) is one of the biopolymers that have gained major interest to replace the use of polymers from nonrenewable sources. PLLA is an aliphatic polyester, in which lactic acid as its precursor is obtained from the fermentation of corn sugars, potato, and sugar cane.[1,2] It can be processed by injection molding, sheet extrusion, blow molding, thermoforming, and film forming.[3] Nowadays, the world capacity for production of PLLA is 140 000 t per year, and it is expected to increase due to the constant trend of increase in the demand in the last 2 years (25−30%).[4] This biopolymer has achieved the greatest market penetration in the packaging sector, with extended application as a food packaging polymer for short shelf life products.[5] Some properties have limited its application, including its brittle character and poor impact strength.[6] Therefore, additives are incorporated into PLLA during processing to enhance its properties and/or to facilitate its processing. Additives not only maintain or improve the properties of polymers, but they can also be used to apply the material as a delivery device for antioxidants, antimicrobial agents, drugs, and so forth.

In the case of plastic materials in contact with food, there is always an interaction between the food and the polymer matrix in which small molecules diffuse from one part of the system to the other. This phenomenon can be used positively by the incorporation of antioxidants as additives in the plastic intended to migrate to the food in which it is in contact. The result is an antioxidant active packaging.[7] Migration is a mass transfer process that occurs in this kind of packaging, by which low molecular mass substances initially present in the package are released into the contained product. Migration is the result of diffusion, dissolution, and equilibrium processes.[8−10] The apparent diffusion coefficient (D) measures the rate at which the diffusion process occurs, and it is described by Fick's second law.[9]

Catechin and epicatechin are natural antioxidants that belong to the flavonoid group. These compounds are present in green tea or in fruits like grapes.[11,12] Catechin and epicatechin are two flavan-3-ols stereoisomers with similar radical scavenging and antioxidant activity due to their structure. Both have the capacity to scavenge hydroxyl, peroxyl, and 2,2-diphenyl-1-picrylhydrazyl (DPPH) radicals and chelate the iron ion.[13] Their antioxidant activity is 2.4 mM Trolox equivalents, and they can retard lipid oxidation.[14] The thermal stability of epicatechin (265 °C) is higher than that of catechin (227 °C) in the presence of oxygen.[15] Catechin is commercially available as catechin hydrate, which shows a considerable loss of weight from 50 to 110 °C due to water evaporation.[16]

In recent years, research related to the development of active packaging containing natural antioxidants has increased. Ethylene vinyl alcohol (EVOH) films added with catechin were produced by extrusion.[17] Catechin diffused to water and water−ethanol food simulants following a Fickian release behavior. Also, PLLA films added with resveratrol were obtained by the blow-extrusion fabrication process.[18] Resveratrol is a stilbene with a similar structure to catechin and epicatechin. It proved to diffuse from PLLA to ethanol at different temperatures showing a Fickian release behavior with

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D values between 10^{-13} and 10^{-10} cm^2/s and energy of activation of 176 kJ/mol.

The aim of the present study was to develop films of PLLA containing catechin and epicatechin to be used as antioxidant membranes for food, pharmaceutical drugs, or cosmetics. The kinetics of the diffusion of catechin and epicatechin from the PLLA matrix into several food simulants was determined as well as the activation energy for the diffusion. Additionally, the antioxidant activity of the PLLA antioxidant films was indirectly determined.

MATERIALS AND METHODS

Chemical and Reagents. Poly(94% L-lactic acid) resin (PLLA 4042D) was obtained from NatureWorks LLC (Blair, NE). Catechin hydrate, epicatechin (90% purity), DPPH, Tween 65, and tetrahydrofuran (THF) were acquired from Sigma (St. Louis, MO). Butylated hydroxytoluene (BHT) was supplied by TCI (Portland, OR). Methanol and ethanol (ACS grade) were bought from Fermont (Monterrey, NL, México), and formic acid (98−100% purity) was from Merck (Darmstadt, Germany). Water and methanol [high-performance liquid chromatography (HPLC) grade] were supplied by J. T. Baker (Toluca, Edomex, México). Deodorized soybean oil was provided by Industrializadora Oleofinos S.A. de C.V. (Zapopan, Jal, México).

Production of PLLA-Catechin and PLLA-Epicatechin Films. Pellets and films were manufactured at the Centro de Investigación en Alimentación y Desarrollo, A.C. campus Hermosillo, Sonora, México. A PLLA-catechin formulation was obtained by blending PLLA with 20.51 mg/g of catechin hydrate. Because of the catechin hydrate contained 14.2% of water; the effective concentration of catechin added was 17.60 mg/g. A PLLA-epicatechin formulation was obtained by blending PLLA with 21.38 mg/g of epicatechin. One more formulation without antioxidant was used as a control. Before blending, PLLA was ground (Thomas-Wiley, Laboratory Mill, model 4, Philadelphia, PA) to reduce the particle size and to obtain a homogeneous distribution of the antioxidants. The three formulations were further extruded in a pilot plant-scale extrusion machine (Beutelespacher, México D.F., México) equipped with a filament die to obtain pellets. Extrusion temperatures for zones 1−4 of the extruder were 135, 140, 140, and 140 °C, respectively. To ensure a better homogenization of the antioxidants, each formulation was subjected to a second pelletization process, including the control pellets. The three formulations of pellets were processed by the flat extrusion process in the same extrusion machine at temperatures for zones 1−4 of 140, 145, 160, and 160 °C, respectively. Finally, three films were obtained as follows: PLLA-catechin, PLLA-epicatechin, and PLLA-control. To avoid contamination of each film type with the other flavonoid, the extruder was purged with enough pure PLLA between PLLA-catechin and PLLA-epicatechin processing. The film thickness was determined with a micrometer model DTT, E.J. Cady & Co. (Wheeling, IL).

Extraction and Quantification of Catechin and Epicatechin in the Pellets and Films. Pellets (0.1 g) and PLLA films cut into 0.25 cm^2 pieces (0.1 g) were stirred in 20 mL of methanol at 40 °C for 24 h in the dark to extract the antioxidants. To protect the antioxidants from degradation during the extraction period, 100 μg/mL of BHT was added to the solutions. The extractions were performed several times on the same material to ensure complete extraction of the antioxidants. Three replicates were carried out for each case. Quantification was achieved with a HPLC (Varian 9012, México D.F., México) equipped with a fluorescence detector (Varian 9075) at 280 nm of excitation wavelength and 310 nm of emission wavelength. A 5 μL loop and a Microsorb C_{18} column 100 mm × 4.6 mm (Varian) protected with a C_{18} guard column were employed, and an isocratic elution of 10:90 methanol:water (5% formic acid) at a flow rate of 1 mL/min and a temperature of 25 °C was applied to elute the catechin or epicatechin from the column. Standard solutions of catechin and epicatechin in methanol at concentrations from 0.5 to 32.0 μg/mL were used to elaborate calibration curves for the quantification of the analytes. The retention times for catechin and epicatechin were 8.6 and 22.9 min, respectively. The limit of quantification (LOQ) was 0.5 μg/mL. Analytical recoveries were determined by spiking solutions containing pieces of the control films with known concentrations of catechin and epicatechin. The solutions were extracted according to the aforementioned method, and the results indicated that recoveries of 88.30 ± 2.56 and 95.28 ± 3.03% were obtained for catechin and epicatechin, respectively. All analytical data were corrected for recoveries. The concentration of catechin and epicatechin was expressed as mg/g of pellets or film.

Diffusion of Catechin and Epicatechin from PLLA Films into Food Simulants. The amount of catechin and epicatechin that diffused from PLLA-catechin and PLLA-epicatechin films into 95% ethanol, 50% ethanol, soybean oil, soybean oil:water emulsion, and soybean oil:water mixture was quantified. A test cell of 40 mL glass vial with a screw cup and a sealing PTFE/silicon septum as recommended by ASTM D4754-98[19] was used. The vials were covered with aluminum foil to protect the content from light, and 100 μg/mL of BHT was added to prevent degradation of the antioxidants. Circle films of 2.0 cm in diameter were cut from the PLLA-catechin and PLLA-epicatechin films. The circled films were inserted in stainless steel wires, separated by glass beads, and introduced in each cell containing the food simulant conditioned at the experimental temperature. The cells were kept under constant agitation. Three cell replicates of PLLA-antioxidant films were used per temperature.

95% Ethanol. According to the U.S. Food and Drug Administration (U.S. FDA), 95% ethanol can be considered as a fatty food simulant for polyolefins.[20] There is no food simulant specifically established for PLLA. Four sets of triplicate cells were filled with 38 mL of 95% ethanol and conditioned at 20, 30, 40, and 50 °C. Eight circled films were introduced in each cell (volume/area ratio = 1.51 mL/cm^2) complying with ASTM D4754-98,[19] which establishes a ratio between 155 and 0.31 mL/cm^2. The vials were stored at 20, 30, 40, and 50 °C, and samples were periodically collected over 240, 168, 20, and 14 h of storage, respectively. Samples were injected directly onto the HPLC, and catechin/epicatechin quantification was performed as described in the previous section. To establish when the system reached the equilibrium of diffusion, the concentration (μg/mL) versus time of each sample were plotted until the slope of the curve reached zero.

50% Ethanol. According to the U.S. FDA[20] and European Union Directives,[21] 50% ethanol can be considered as a simulant for alcoholic beverages and oil in water emulsions. The experimental conditions were equal to those for 95% ethanol, but this was carried out only at 40 °C.

Soybean Oil. This experiment was carried out with refined and deodorized soybean oil. Circled films (32) were introduced in each cell containing 38 mL of soybean oil (volume/area ratio = 0.378 mL/cm^2) complying with ASTM D4754-98.[19] The cells were stored under constant agitation at 40 °C. Samples of soybean oil (triplicates) were taken frequently to quantify catechin and epicatechin using HPLC with the method previously described. Standard solutions of catechin and epicatechin in the oil at the proper concentrations were used to elaborate calibration curves for the quantification of the analytes.

Water. According to the European Union Directives, water is an aqueous food simulant.[21] Circled films (24) were introduced in each cell and filled with 38 mL of water HPLC grade (volume/area ratio = 0.504 mL/cm^2) and stored under constant agitation at 40 °C. Samples of water were taken frequently, and the concentration of each antioxidant was quantified by HPLC with the method previously described. Standard solutions of catechin and epicatechin in water at the proper concentrations were used to elaborate calibration curves for the quantification of the analytes.

Emulsion. An emulsion of water−oil (50:50) was prepared using water HPLC grade, soybean oil, and Tween 65 (5%) (polyoxyethylenesorbitan tristearate). Circled films (30) were introduced in each cell, filled with 38 mL of the emulsion (volume/area ratio of 0.398 mL/cm^2) and stored at 40 °C under constant agitation. Samples were taken frequently to quantify the concentration of each antioxidant using HPLC.

Oil–Water. A mixture of oil–water (50:50) was prepared using water HPLC grade and soybean oil. The experiment was performed as previously described for the emulsion.

Catechin and Epicatechin Release Models. The migration process is described by the diffusion kinetics of the migrant in the film, and it is expressed by D. To determine D into 95 and 50% ethanol, analytical solutions of Fick's second law equation were used. When the diffusion is in one dimension and there is a limited volume of film in a finite volume of solution,[9,22] the analytical solution is

$$\frac{M_t}{M_\infty} = 1 - \sum_{n=1}^{\infty} \frac{2\alpha(1+\alpha)}{1+\alpha+\alpha^2 q_n^2} \exp\left(-\frac{Dq_n^2 t}{l^2}\right) \quad (1)$$

where M_t/M_∞ is the concentration of the antioxidant diffused at time t divided by the concentration of the antioxidant diffused at equilibrium: l is the thickness of the film and the q_n values are the nonzero positive roots of $\tan q_n = \alpha q_n$ and α is

$$\alpha = \frac{V_S}{K_{P,S} V_P} \quad (2)$$

where V_S and V_P are the molar volume of the simulant and the polymer and $K_{P,S}$ is the partition coefficient of the antioxidant between the PLLA and the simulant, which at a lower concentration can be assumed constant and calculated from the ratio of the concentration of the antioxidant in the PLLA film ($C_{P,\infty}$) and the simulant ($C_{S,\infty}$) at equilibrium:

$$K_{P,S} = \frac{C_{P,\infty}}{C_{S,\infty}} \quad (3)$$

If the amount of simulant can be considered infinite (i.e., $\alpha \gg 1$ since $V_S \gg V_P$ and/or $K_{P,S} < 1$), the analytical solution of Fick's second law equation[10] is

$$\frac{M_t}{M_\infty} = 1 - \frac{8}{\pi^2} \sum_{m=0}^{\infty} \frac{1}{(2m+1)^2} \exp\left(-\frac{D(2m+1)^2 \pi^2 t}{l^2}\right) \quad (4)$$

To fit the data to either eq 1 or eq 4, M_t/M_∞ was plotted versus time t. D (cm^2/s) was calculated for each antioxidant at the different experimental temperatures. To determine the fit of the experimental data to the eqs 1 and/or 4, the nonlinear regression (nlin-fit) function in MATLAB R2010b (MathWorks, Natick, MA) was applied to the data.[10]

Activation Energy for the Diffusion (E_a) of Catechin and Epicatechin into 95% Ethanol. To determine the effect of temperature on the diffusion of catechin and epicatechin from PLLA films into 95% ethanol, the E_a was calculated using the Arrhenius equation for diffusion:

$$D = D_O e^{[-E_a/RT]} \quad (5)$$

where D is the diffusion coefficient, D_O is the pre-exponential factor of diffusion, E_a is the activation energy of diffusion, R is the ideal gas constant (8.3145 J/Kmol), and T is the temperature in K. E_a was obtained from the slope of a plot of the reciprocal of temperature ($1/T$) vs the logarithm of D ($E_a = -\text{slope} \times 2.303R$).[23]

Effect of the Manufacturing and Diffusion Conditions on the Weight Average Molecular Weight of PLLA. The weight average molecular weights (M_W) of PLLA resin, pellets, and films were analyzed to determine if the processing conditions caused hydrolysis of the polymer. Also, the M_W of the circled films after the diffusion experiments was analyzed to determine if the contact with the simulants, temperature, and time of contact caused hydrolysis of the PLLA. PLLA samples were dried under vacuum, and 20–30 mg of PLLA was dissolved in 10 mL of THF stabilized by BHT. The M_W of the samples was determined with a gel permeation chromatograph (GPC) (Waters 1515, Waters, Milford, MA) equipped with a refractive index detector (Waters 2414). A serial of columns (each 7.8 mm × 300 mm, Waters Styragel) were placed between the injector and the detector. Column HR4 was located closest to the injector, followed by HR3 and HR2. An isocratic elution of THF at a flow rate of 1 mL/min and a temperature of 25 °C was applied, and the temperature of the detector was set to 35 °C. A M_W calibration curve was constructed from a standard polystyrene kit (STD KIT SM 105, Shodex standard, Waters), which contained a M_W range of 1310–3640000 Da. The experiments were conducted in triplicate. The Mark–Howink constants for correction were $K = 0.0174$ mL/g and $a = 0.736$ for PLLA solutions in THF at 30 °C.

Effect of Manufacturing and Diffusion on the Antioxidant Activity. The effect of the manufacture process on the antioxidant activity of PLLA-catechin and PLLA-epicatechin films was indirectly determined by extracting the antioxidants from the films. An additional extract was obtained from the PLLA-control film. The extractions were performed with methanol at 40 °C for 24 h in darkness, and quantification of catechin and epicatechin was carried out by HPLC as previously described. After quantification, equal fresh solutions of the pure antioxidants in methanol were prepared as controls. Finally, the antioxidants activities were determined.

The effect of the diffusion process on the antioxidant activity of PLLA-catechin and PLLA-epicatechin films was determined by taking samples of the simulant when the system reached the equilibrium. No BHT was added to the systems.

Antioxidant activities of the extracts or solutions containing catechin or epicatechin were measured by DPPH method proposed by Brand-Williams et al.[24] with some modifications. This assay is based on the measurement of the scavenging ability of antioxidants toward the stable radical DPPH. A 3.9 mL amount of a 0.0634 mmol/L of DPPH radical solution in methanol (2.5 mg of DPPH in 100 mL of methanol with an OD = 0.7) was added to 0.1 mL of standard solutions of catechin (46.42 μg/mL), epicatechin (57.52 μg/mL), and film extracts that resulted with the same concentration of antioxidants, respectively. The mixture was shaken in a vortex and kept 30 min in the dark. The absorbance was then read using an UV–vis spectrophotometer (Cary 50 Bio, Varian, Victoria, Australia), at a wavelength of 515 nm against a blank (3.9 mL of the DPPH radical solution reacted with 0.1 mL of 80% methanol). The antioxidant activity was expressed as DPPH radical scavenging (%) according to the following equation. Three replicates were carried out for each sample.

$$\% = \left(\frac{A_{blank} - A_{sample}}{A_{blank}}\right) 100 \quad (6)$$

These experiments were based on a completely randomized design with equal replications. Analysis of variance for the treatments was performed using NCSS statistical software. Mean comparisons of the studied parameters among treatments were done using the Tukey–Kramer test ($p < 0.05$).

RESULTS AND DISCUSSION

Quantification of Catechin and Epicatechin in the Pellets and Films. The final concentrations of catechin and epicatechin in the pellets were 11.05 ± 2.21 and 16.74 ± 0.70 mg/g, respectively (Table 1). Catechin and epicatechin remained in the pellets at 62.8 ± 12.6 and 78.3 ± 3.3%, respectively. The losses were due to degradation of the

Table 1. Concentration of Catechin and Epicatechin in the PLLA Films after Pelletization and Film Extrusion

	flavonoid (mg/g)			
films	added before pelletization	quantified in the pellets[a]	quantified in the films	flavonoid remained in the films (%)
PLLA-catechin	17.60	11.05 ± 2.21	12.76 ± 0.45	72.5
PLLA-epicatechin	21.38	16.74 ± 0.70	15.03 ± 0.48	70.3

[a]Two pelletization processes were applied.

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antioxidants during the two pelletization process, and also, they may be influenced by adherence of the antioxidants to the screw and barrel walls of the extruder. Regarding the higher losses of catechin as compared to epicatechin, they were due to the PLLA-catechin pellets residing in the extruder for 7 min as compared to 5 min for PLLA-epicatechin pellets. The addition of catechin hydrate containing 14.2% of water added a 0.29% of moisture to the PLLA, affecting the viscosity of the molten polymer, decreasing the flow rate, and increasing the residence time. After the films were processed, the concentration of catechin and epicatechin was 12.76 ± 0.45 (1.28%) and 15.03 ± 0.48 mg/g (1.50%), respectively. Therefore, no practical loss of catechin was obtained after processing the pellets to the film. However, in the case of epicatechin, 10.2% was lost. The difference between the process of extruding a filament for pelletization and extruding the film was that the film was immediately cooled after coming out from the die. Meanwhile, for the filament, it took 10−15 min to cool down at room temperature. During the pelletization process, a water bath is normally used to cool down the filaments. Because PLLA is water sensitive, it was not used to avoid absorption of water by the formulations of PLLA with the flavonoids. A similar behavior was reported for the production of PLLA added with α-tocopherol.[10] Total losses of catechin and epicatechin after the two pelletizations and the film extrusion processes were 27.5 and 29.7%, respectively. López-de-Dicastillo et al.[17] reported that 66.8−67.1% of the catechin added to EVOH films remained after processing at 200 °C. The catechin in our PLLA film remained at 72.5% after processing at a temperature up to 160 °C. However, the material and the antioxidant were exposed three times to melting temperatures. Therefore, temperature and residence time, among other factors, influenced the remaining of the compounds in the final product.

Diffusion to 95% Ethanol. Figure 1 shows the diffusion behavior of catechin and epicatechin from the PLLA films to 95% ethanol at 20, 30, 40, and 50 °C. The equilibriums for both systems were obtained at 7.04 (169 h), 5.0 (120 h), 0.54 (13 h), and 0.42 days (10 h), respectively. The release of catechin was 19.0, 75.5, 99.0 and 99.3%, and the release of epicatechin was 17.2, 69.0, 98.3, and 99.1% at 20, 30, 40, and 50 °C, respectively. The equilibrium time and the release of each antioxidant were indirect and directly proportional to the temperature, respectively, due to the dependence of the diffusion on this variable.

Catechin and epicatechin diffusions followed Fick's second law at 40 and 50 °C. At 20 and 30 °C, an apparent equilibrium for both antioxidants at 1.0 (24 h) and 1.5 days (36 h), respectively, was observed. However, the release of the antioxidants after that time started to increase again to reach the final equilibrium of the systems. Manzanarez-López et al.[10] found the same diffusion behavior of α-tocopherol to ethanol from PLLA films at 33 °C. Soto-Valdez et al.[18] also reported a similar diffusion of resveratrol from PLLA to ethanol at 33 °C. This behavior could be due to the combination of temperature and a period of time in which the PLLA discs were penetrated by ethanol causing separation of the polymer chains and increasing the release. Mascheroni et al.[22] mentioned the sorption of ethanol by PLLA matrix and a consequent creation of voids spaces favoring the migration of phenolic compounds.

The $K_{P,S}$ value expresses the relative solubility of the migrant between the plastic and the simulant at equilibrium. $K_{P,S} > 1$ indicates a higher concentration of migrant in the polymer as compared to that in the simulant. Table 2 shows high $K_{P,S}$ values at 20 °C for catechin and epicatechin that coincide with the low migration from the PLLA films. This means that after 7.04 days (169 h), the antioxidants concentration was much higher in the films than in 95% ethanol. At 30 °C, the antioxidants concentration was still high in the films at 5.0 days (120 h). At 40 and 50 °C, $K_{P,S}$ values significantly decreased ($p < 0.05$), which coincided with the release of more than 98% of catechin and epicatechin to ethanol.

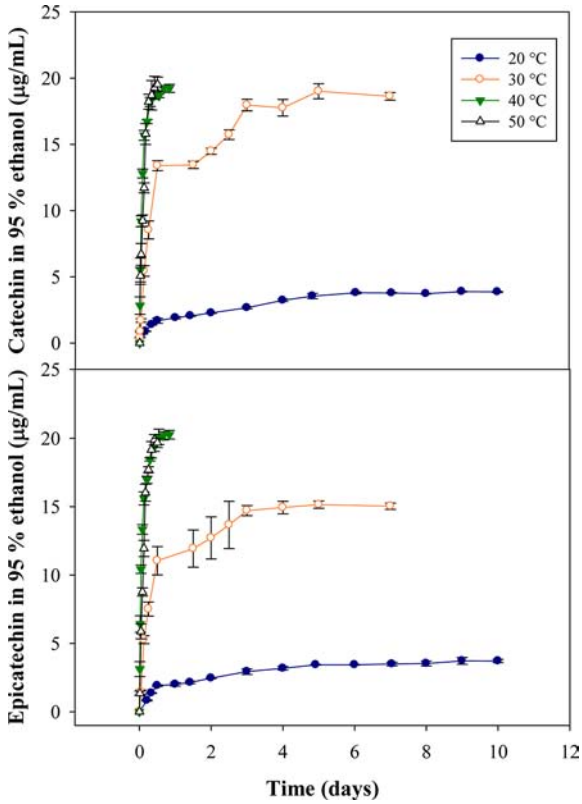

Figure 1. Diffusion of catechin and epicatechin from PLLA films into 95% ethanol at 20, 30, 40, and 50 °C. The graphs show the concentration of catechin and epicatechin in 95% ethanol (μg/mL) vs time (days).

Figure 2 shows the diffusion graphs according to Fick's second law at 20, 30, 40, and 50 °C in 95% ethanol. D values at 20 and 30 °C were calculated according to eq 1 due to the high $K_{P,S}$ values obtained at these temperatures (Table 3) showing a finite system. D values at 40 and 50 °C were calculated using eq 4 due to low $K_{P,S}$ values. In these cases, the systems were considered with an infinite simulant volume because the release of the antioxidants was almost complete (~99%). Also, eq 4 provided a better fit to obtain D than eq 1.

The diffusion of both antioxidants showed a similar behavior regarding the temperature due to the chemical structure similarities of catechin and epicatechin. At 20 °C, D values for catechin and epicatechin were 0.49×10^{-11} and 0.88×10^{-11} cm^2/s, respectively. At 30 °C, the D values increased by 2 orders of magnitude to 13.1×10^{-11} and 13.7×10^{-11} cm^2/s for catechin and epicatechin, respectively. This was the temperature range with the highest increase on diffusion. At 40 °C, the D values were kept at the same order of magnitude than at 30 °C but significantly higher ($p < 0.05$), being 47.9×10^{-11} and 51.2×10^{-11} cm^2/s for catechin and epicatechin, respectively. At 50 °C, the D values unexpectedly decreased to 31.5×10^{-11}

Table 2. Partition Coefficients ($K_{P,S}$) and α Values of Catechin and Epicatechin for the Diffusion from PLLA Films into 95% Ethanol at 20, 30, 40, and 50 °C[a]

	$K_{P,S}$		α	
temperature (°C)	catechin	epicatechin	catechin	epicatechin
20	1453.79 ± 14.95 c	1569.79 ± 9.69 c	0.36 ± 0.00 a	0.32 ± 0.00 a
30	74.43 ± 8.06 b	115.80 ± 10.74 b	5.33 ± 0.59 a	3.68 ± 0.34 a
40	3.06 ± 0.11 a	4.88 ± 0.70 a	158.09 ± 5.98 b	94.08 ± 12.71 b
50	2.02 ± 0.03 a	2.75 ± 0.18 a	220.96 ± 3.38 c	179.06 ± 12.27 b

[a]Thickness of the films was 57−77 μm. Values are means ± standard deviations of three replicates. Different letters within the same columns indicate statistically significant different values ($p < 0.05$).

and 34.9×10^{-11} cm^2/s for catechin and epicatechin, respectively. Probably, partial degradation of the antioxidants once dissolved in the simulant caused an underestimation when the antioxidants were quantified, decreasing the apparent diffusion rate.

Ortiz-Vazquez et al.[25] reported D values for BHT from PLLA to 95% ethanol as 2.95×10^{-11}, 8.95×10^{-11}, and 190.4×10^{-11} cm^2/s at 23, 31, and 43 °C, respectively. These values show a trend in which diffusion of BHT is faster than catechin and epicatechin in PLLA. The molecule of BHT has only one hydroxyl group; meanwhile, catechin and epicatechin have five hydroxyl groups. Therefore, these flavonoids could have more interactions with the molecules of PLLA. Also, Soto-Valdez et al.[18] reported D values for resveratrol from PLLA to ethanol as 3.06×10^{-11}, 41.70×10^{-11}, and 82.60×10^{-11} cm^2/s at 23, 33, and 43 °C, respectively. These values are also higher than those for catechin and epicatechin. The molecule of resveratrol has three hydroxyl groups, which provide less interaction with PLLA chains than catechin and epicatechin. López-de-Dicastillo et al.[17,26] reported the incorporation of pure catechin and catechin from green tea in other polymers like EVOH with D values of 7×10^{-11} cm^2/s to 95% ethanol at 37 and 40 °C. This value is lower than our value for catechin in PLLA, indicating that this antioxidant makes strong interactions with the EVOH molecule.

Figure 3 shows the dependence of D on temperature by means of the Arrhenius equation. The behavior of the log D versus $1/T$ gave a linear plot and E_a of 110.43 and 98.91 kJ/mol for catechin and epicatechin, respectively. The E_a can be defined as the energy required for a migrant to move among the chains forming the polymer matrix. It is assumed that the migrant, when given sufficient energy, can "jump" into an adjacent space if that space is large enough to accommodate the migrant. A net diffusion flux results if another migrant molecule makes a "jump" onto the space that was previously occupied by the first molecule.[23] The energy is provided by temperature that has an effect on the migrant itself, on the polymer matrix, and on the medium in which it is in contact. In this work, the E_a for both antioxidants in PLLA films are comparable with values like 96.2, 164.7, and 176.0 kJ/mol reported for α-tocopherol,[10] BHT,[25] and resveratrol[18] incorporated in PLLA films, respectively.

Diffusion to 50% Ethanol. Figure 4 shows catechin and epicatechin diffusion graphs following Fick's second law for the experiments carried out in 50% ethanol at 40 °C. The systems reached the equilibrium at 2.50 days (60 h), being 1.96 days (47 h) longer than in 95% ethanol at the same temperature. The release of the antioxidants from the PLLA films was of 73.9 and 61.9%, and the $K_{P,S}$ values were 120.22 and 221.46 for catechin and epicatechin, respectively (Table 4). The $K_{P,S}$ values indicate that after the equilibrium was reached, epicatechin remained in the PLLA films at a higher concentration than catechin ($p < 0.05$), similar to the behavior in the 95% ethanol system. D values were calculated according to eq 1 due to the high $K_{P,S}$ values and low α values obtained, which belong to a finite system. The catechin diffusion rate between PLLA chains was higher than epicatechin ($p < 0.05$). The diffusion of the antioxidants resulted in a slower rate than in 95% ethanol due to the influence of the simulant composition. The penetration capacity of ethanol in the PLLA matrix and the good dissolution of the antioxidants in this solvent caused that the greater the proportion of ethanol as food simulant the higher the diffusion rate of the antioxidants. The U.S. FDA[20] and European Union Directives[21] recommend this food simulant for alcoholic beverages and oil in water emulsions. According to our results, this system could simulate the interaction between PLLA and alcoholic beverages but hardly could simulate the interaction between this polymer and oil in water emulsion food types, as shown in the following section.

Diffusion to Soybean Oil, Water, Emulsion, and Oil−Water Mixture. No release of catechin and epicatechin from the PLLA films in soybean oil, water, emulsion, and oil−water mixture was detected higher than the LOQ (0.5 μg/mL) of the analytical procedure at 40 °C. As it was explained in the previous section, catechin and epicatechin molecules have five hydroxyl groups through which intermolecular association by hydrogen bonding with the carbonyl groups of the PLLA could happen.[27] This fact and the limited solubility of the antioxidants in oil or water contributed to keep the phenolic compounds trapped between the long molecules of the polyester.[27] Even at temperatures as high as 40 °C, there was not enough energy to break the interactions and promote the diffusion. In the case of ethanolic simulants, the molecule of ethanol penetrated among the PLLA chains, weakening the interactions and increasing the possibilities of the flavonoids to diffuse to a medium in which they were rapidly dissolved. However, in the simulant with a low proportion of ethanol (50%), the diffusion rate decreased as compared to that in 95% ethanol. This fact strengthens the theory of the influence of the ethanol penetration between the PLLA chains on the diffusion of phenolic compounds. However, this also suggests that ethanol solutions cannot be used as food simulants for packaging made of PLLA since the food-packaging interaction is not properly simulated.

Effect of the Manufacturing and Diffusion Conditions on the M_W of PLLA. The effect of the processing conditions on the M_W of PLLA is shown in Table 5. The M_W of PLLA resin was 109.6 kDa; however, after the pelletization of PLLA with catechin, epicatechin, and control, the M_W was 106.8, 110.1, and 110.0 kDa, respectively. Only the M_W of PLLA-catechin pellets was significantly lower ($p < 0.05$) than that of the PLLA resin. Moisture present in PLLA can cause a

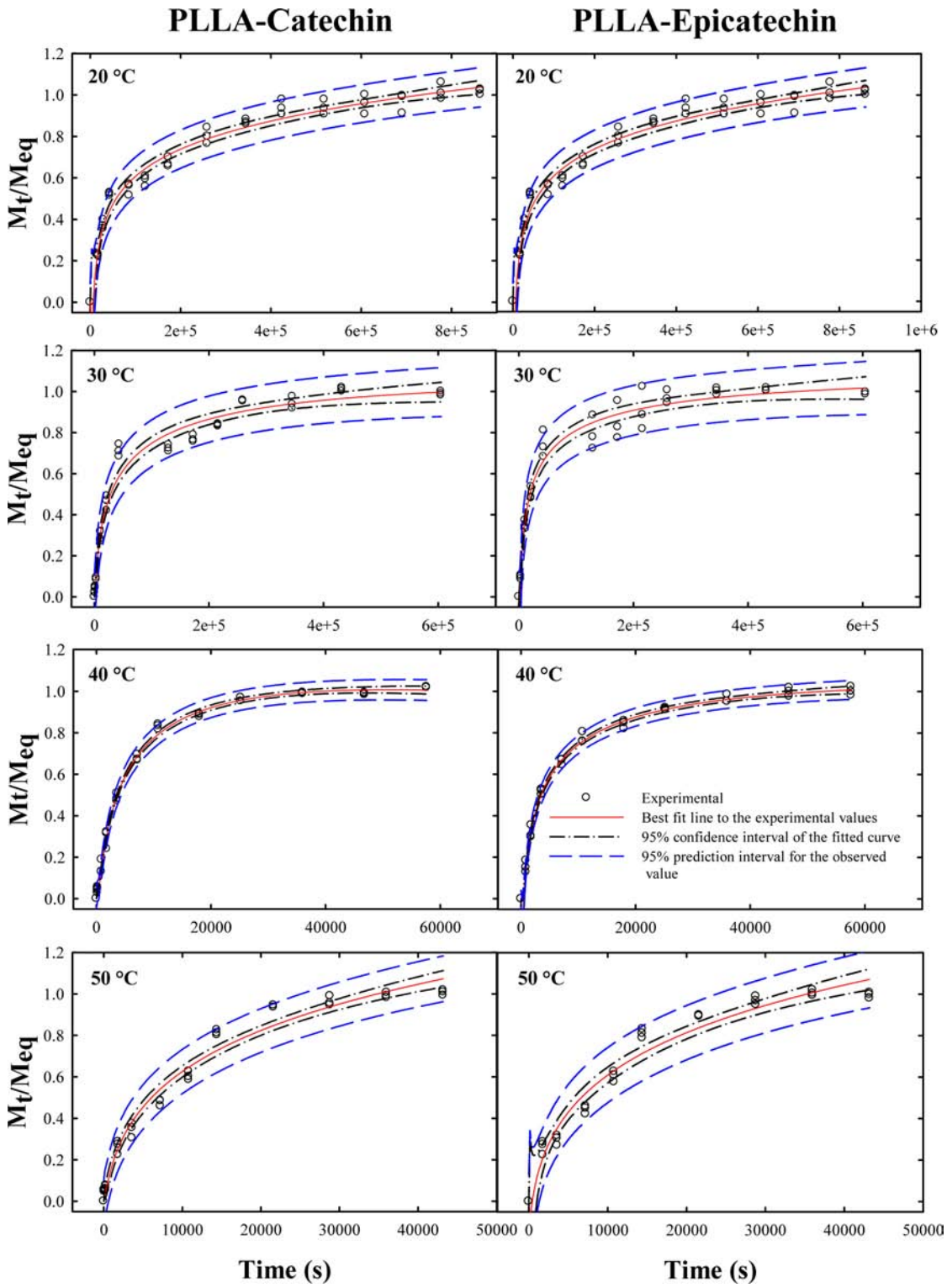

Figure 2. Diffusion of catechin and epicatechin from PLLA films into 95% ethanol at 20, 30, 40, and 50 °C according to the Fick's second law. The *y*-axis shows the mass of catechin and epicatechin diffused at time *t*, divided by the mass of catechin or epicatechin diffused at equilibrium (M_t/M_{eq}). The *x*-axis shows *t* in s. The thickness of the films was 57−77 μm.

molecular weight drop during melt extrusion due to hydrolytic degradation.[28] In the present work, 0.29% of moisture was introduced to PLLA in the catechin hydrate additive. Taubner and Shishoo[29] processed PLLA with 0.30% of moisture and reported a decrease on the M_W when processing at 210 °C; however, at higher temperatures (240 °C), there was no effect due to water evaporation, although there was effect of the high temperature on the M_W. Our pellets were processed at temperatures up to 140 °C; therefore, there are high probabilities that the moisture remained during the two pelletization processes applied. Taubner and Shishoo[29] also reported an effect of the residence time on the M_W of the

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Table 3. Diffusion Coefficients (D) of Catechin and Epicatechin from PLLA Films into 95% Ethanol at 20, 30, 40, and 50 °C[a]

temperature (°C)	$D \times 10^{-11}$ (cm²/s)	
	catechin	epicatechin
20	0.49 ± 0.1 a	0.88 ± 0.2 a
30	13.1 ± 4.3 b	13.7 ± 3.5 b
40	47.9 ± 1.8 d	51.2 ± 1.9 d
50	31.5 ± 1.4 c	34.9 ± 2.0 c

[a]The thickness of the films was 57−77 μm. Values are means ± standard deviations of three replicates. Different letters within the same columns indicate statistically significant different values ($p < 0.05$).

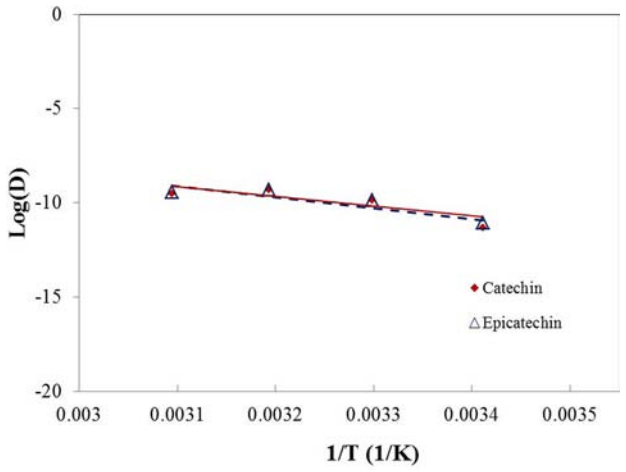

Figure 3. Activation energy for the diffusion of catechin ($y = -5767.3x + 8.7366$; R^2, 0.7597) and epicatechin ($y = -5165.6x + 6.8683$; R^2, 0.7815) from PLLA films into 95% ethanol. The slope of each line was equal to $-E_a/2.303R$ from which the E_a of catechin and epicatechin was 110.43 and 98.92 kJ/mol, respectively.

PLLA. In the present work, the moisture affected the viscosity of the molten PLLA added with catechin; consequently, the PLLA-catechin pellets resided in the extruder for 2 min more as compared to the PLLA-epicatechin and control pellets. This also contributed to the hydrolytic degradation of PLLA added with catechin. After the films were manufactured, the M_W of PLLA-catechin, PLLA-epicatechin, and PLLA control was 96.9, 105.8, and 103.4 kDa, respectively. Temperatures up to 160 °C were used in this stage of the process, which contributed to decrease the M_W of the three materials. Therefore, moisture, residence time, melting, or higher temperatures induce hydrolysis of PLLA even at short times and may affect the properties of the finished article.

The effect of the diffusion conditions on the M_W of PLLA is shown in Table 6. The M_W of PLLA-catechin and PLLA-epicatechin films in contact with 95% ethanol did not change ($p > 0.05$) except in PLLA-epicatechin at 30 °C. At 40 and 50 °C, the films were in contact with 95% ethanol for 24 and 14 h, a very short time to induce hydrolysis. At 30 °C, the contact time increased to 168 h with the highest decrease in M_W for both films; however, only PLLA-epicatechin films showed a significant decrease ($p < 0.05$). At 20 °C, the contact time increased to 240 h, but no hydrolysis was induced due to the relatively low temperature. A similar behavior was reported for PLLA films added with resveratrol and stored in contact with ethanol at 23 °C for 40 days.[18] Regarding the M_W of the films

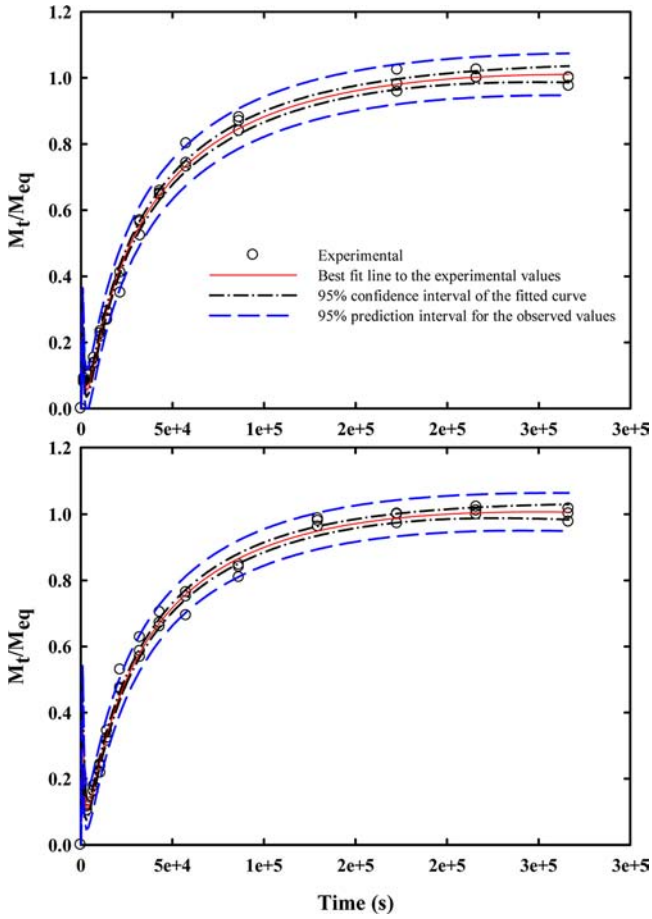

Figure 4. Diffusion of catechin (top) and epicatechin (bottom) from PLLA films into 50% ethanol at 40 °C according to the Fick's second law. The y-axis shows the mass of catechin and epicatechin diffused at time t, divided by the mass of catechin or epicatechin diffused at equilibrium (M_t/M_{eq}). The x-axis shows t in s. The thickness of the films was 57−77 μm.

Table 4. Partition ($K_{P,S}$) and Diffusion (D) Coefficients of Catechin and Epicatechin from PLLA Films into 50% Ethanol at 40 °C[a]

antioxidant	$K_{P,S}$	α	$D \times 10^{-11}$ (cm²/s)
catechin	120.22 ± 10.44 a	3.97 ± 0.33 b	11.18 ± 0.03 b
epicatechin	221.46 ± 10.43 b	2.23 ± 0.10 a	9.41 ± 0.03 a

[a]The thickness of the films was 57−77 μm. Values are means ± standard deviations of three replicates. Different letters within the same columns indicate statistically significant different values ($p < 0.05$).

Table 5. Effect of Processing Conditions on the M_W of PLLA Pellets and Films Added with Catechin and Epicatechin[a]

material	M_W (kDa)		
	PLLA-control	PLLA-catechin	PLLA-epicatechin
pellets	110.0 ± 1.12 c	106.8 ± 3.77 bc	110.1 ± 0.83 c
films	103.4 ± 0.83 b	96.9 ± 0.72 a	105.8 ± 0.58 bc

[a]The M_W of the PLLA resin was 109.6 kDa. Values are means ± standard deviations of three replicates. Different letters within the same rows or columns indicate statistically significant different values ($p < 0.05$).

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Table 6. Effect of Diffusion Conditions on the M_W of PLLA Films Added with Catechin and Epicatechin[a]

simulant	T (°C)	time (h)	M_W (kDa) PLLA-catechin	M_W (kDa) PLLA-epicatechin
film			96.9 ± 0.72 c	105.8 ± 0.58 d
95% ethanol	20	240	96.4 ± 0.12 c	102.9 ± 0.77 cd
95% ethanol	30	168	94.8 ± 0.96 c	101.9 ± 0.83 c
95% ethanol	40	24	96.7 ± 0.92 c	102.1 ± 0.38 cd
95% ethanol	50	14	97.9 ± 3.20 c	103 ± 0.80 cd
50% ethanol	40	98	88.5 ± 2.84 b	93.8 ± 0.62 b
100% water	40	480	71.3 ± 2.69 a	79.7 ± 2.14 a

[a]Values are means ± standard deviations of three replicates. Different letters within the same column indicate statistically significant different values ($p < 0.05$).

in contact with 50% ethanol, both PLLA-catechin (88.5 kDa) and PLLA-epicatechin (93.8 kDa) presented hydrolysis after 98 h at 40 °C. Hydrolysis of PLLA is accelerated by the presence of moisture,[3] as it was observed in the M_W of PLLA-catechin and PLLA-epicatechin films in contact with water at 40 °C, which presented 71.3 and 79.7 kDa, respectively. However, diffusion of the antioxidants was not induced by the chain scission of the PLLA, confirming the strong interactions between the five hydroxyl groups of the flavonoids and the carbonyl groups of PLLA and the low affinity with the simulant. Films in contact with soybean oil were not analyzed for M_W since it was known from previous experiments that no hydrolysis was present in PLLA films added with BHT in contact with coconut oil for 100 days at temperatures as high as 43 °C.[25]

Effect of Manufacturing and Diffusion on the Antioxidant Activity. DPPH radical scavenging activity (%) of catechin and epicatechin extracted from the PLLA films and equivalent solutions freshly prepared with the standards are shown in Figure 5. The extract of the PLLA-catechin film contained 46.42 μg/mL of the antioxidant, and a freshly prepared solution at the same concentration showed 32.90 ± 0.85% of DPPH radical scavenging activity. Otherwise, the extract of PLLA-epicatechin film contained 57.52 μg/mL of epicatechin, and a freshly prepared solution at the same concentration showed 36.68 ± 1.14% of DPPH radical scavenging activity. The extracts obtained from the PLLA-flavonoids films showed a higher percentage of scavenging the DPPH radical than the freshly prepared solutions, although there were only significant differences for the PLLA-epicatechin extract ($p < 0.05$). Therefore, PLLA could contain other antioxidants that contributed to the antioxidant activity of the extracts. To confirm this, the PLLA control film was extracted under the same conditions than the antioxidant films, obtaining a 5.60 ± 0.59% of DPPH radical scavenging activity.

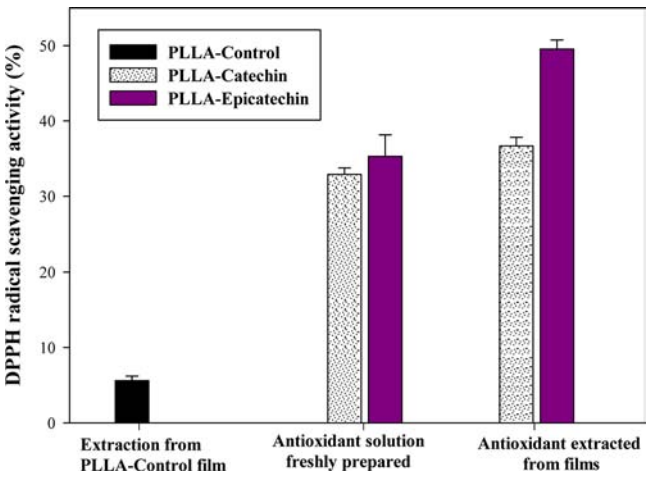

Figure 5. DPPH radical scavenging activity (%) of catechin (46.42 μg/mL) and epicatechin (57.52 μg/mL) in extracts from the PLLA films and in freshly prepared solutions. Results are means of three replicates. Bars indicate standard deviation.

The antioxidant activity for catechin and epicatechin diffused from the PLLA films into 95% ethanol (at equilibrium) is shown in Figure 6. The DPPH radical scavenging activity (%) of the food simulants was proportional to the diffusion temperature from 20 to 40 °C ($p < 0.05$). However, at 50 °C, the percent of DPPH radical scavenging did not increase ($p > 0.05$). This confirms that the diffusion at 50 °C was lower than at 40 °C, probably due to decomposition on the antioxidants to other species with no antioxidant activity. In spite of that, positive correlations between the antioxidant concentration and the percent of DPPH radical scavenging for catechin ($r^2 = 0.9918$) and epicatechin ($r^2 = 0.9056$) were obtained. This behavior has been reported for migration of catechin, epicatechin, quercetin, gallic acid, caffeine, etc., to simulants in which the antioxidant activity was proportional to the antioxidant concentration in the simulant.[17,26] Also, in extracts from different varieties of grapes, the antioxidant activity increases with the concentration of the phenolic compounds.[30,31]

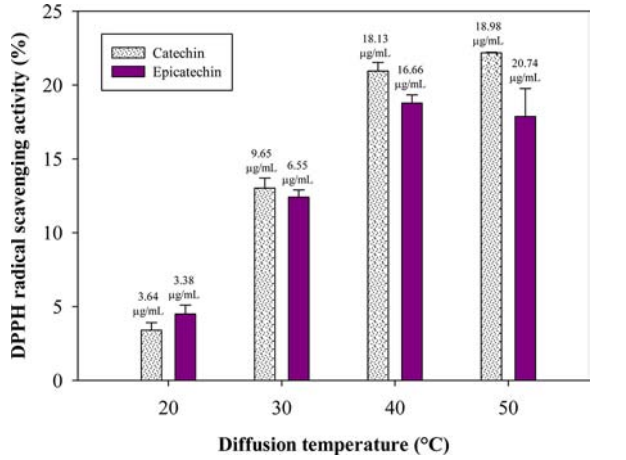

Figure 6. DPPH radical scavenging activity (%) of catechin and epicatechin after diffusion into 95% ethanol at 20, 30, 40, and 50 °C. Concentrations above the columns indicate the antioxidant concentration in the simulant when the system reached the equilibrium. Results are means of three replicates. Bars indicate standard deviation.

In conclusion, the development of biodegradable antioxidant active films based on catechin and epicatechin was obtained through PLLA extrusion. The extrusion process affected the presence of the added antioxidants; however, the remained content presented antioxidant activity. The release of the phenolic compounds was obtained in ethanolic systems adding antioxidant activity to the media. The films produced in this study could be an attractive alternative to control oxidations problems in the pharmaceutical, medical, food, or cosmetic areas.

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Notes

The authors declare no competing financial interest.

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