

Fabrication of Poly(lactic acid) Films with Resveratrol and the Diffusion of Resveratrol into Ethanol

Herlinda Soto-Valdez,¹ Rafael Auras,² Elizabeth Peralta¹

¹Centro de Investigación en Alimentación y Desarrollo, A.C., CTAOV, Apdo. Postal 1735 Hermosillo, Sonora 83304, México

²School of Packaging, Michigan State University, East Lansing, Michigan 48824-1223

Received 26 July 2010; accepted 31 October 2010

DOI 10.1002/app.33687

Published online 25 February 2011 in Wiley Online Library (wileyonlinelibrary.com).

ABSTRACT: Poly(94% L-lactic acid) (PLA) films containing 0, 1, and 3 wt % resveratrol (PR0, PR1, and PR3) were extruded in a pilot-plant scale blown-extrusion machine. Yellow-colored PLA films with reduced crystallinity were obtained, and the films absorbed UV-visible light at 350–200 nm. The diffusion kinetics of resveratrol into ethanol at 9, 23, 33, and 43°C displayed Fick's behavior, and diffusion coefficients between 10^{-13} and 10^{-10} cm²/s were obtained. According to the Arrhenius equation, the energy of activation for the diffusion of resveratrol from

PR1 and PR3 films was 175 and 177 kJ/mol, respectively. The temperature of diffusion had a stronger effect on the reduction of the weight average molecular weight of PLA than the processing conditions and the contact time with ethanol. However, diffusion of resveratrol was not affected by the degradation of PLA. © 2011 Wiley Periodicals, Inc. *J Appl Polym Sci* 121: 970–978, 2011

Key words: activation energy; diffusion; biopolymers

INTRODUCTION

Poly(lactic acid) (PLA) is a polyester produced by the polymerization of lactic acid (2-hydroxy propionic acid) or lactide (the cyclic diester of lactic acid). PLA is biodegradable, compostable, biocompatible, and suitable for delivery and release applications.¹ Currently, PLA can be processed as a textile to produce surgical sutures, molded to manufacture implants, extruded to produce films, coextruded to form multi-layer films, thermoformed to produce trays, and injection-stretch blow-molded to produce bottles. In 2009, the world capacity of PLA was 140,000 metric tons,² and the production of PLA is expected to increase due to the recent expansion in the demand for sustainable and compostable plastics.

Because PLA is a relatively new polymer, new studies on natural PLA additives continue to emerge. Additives not only maintain or improve the properties of polymers, but also are used to apply the material as a delivery device for antioxidants,

antimicrobial agents, drugs, and so forth. Among natural antioxidants, resveratrol ((E)-5-(4-hydroxy-styryl)-benzene-1,3-diol) has significant potential as a PLA additive. Resveratrol is a stilbene that is produced in plants as a response to fungal infection and is found in grapes, grape juice, wine, peanuts, and a number of other plant species.³ Pure resveratrol is a white powder with a yellow cast that melts at 253–257°C. It is soluble in ethanol (50 mg/mL), dimethyl sulfoxide (DMSO) (16 mg/mL), and water (<3 mg/100mL).⁴ Resveratrol contains two aromatic rings and three hydroxyl groups (Fig. 1), and has a higher radical-scavenging capacity than propyl gallate, ascorbic acid, and α -tocopherol.^{5,6} Resveratrol has been shown to display cardioprotective, chemopreventive, and antitumor activity, and has been used for the prevention of Alzheimer's disease.^{5,7–9} Gülçin¹⁰ demonstrated that 30 μ g/mL of resveratrol inhibited 89.1% of the lipid peroxidation of a linoleic acid emulsion. In contrast, butylated hydroxyanisole (BHA), butylated hydroxytoluene (BHT), α -tocopherol, and trolox inhibited only 83.3, 82.1, 68.1, and 81.3% of lipid peroxidation, respectively. Thus, resveratrol has the potential for use as an antioxidant additive for polymers, and PLA-resveratrol could be used as an antioxidant release membrane for pharmaceutical, medical, and food applications.

The release of species from plastic materials is often described as migration, which is the mass transfer of low molecular weight substances from the parent material to the medium. Migration is the result of diffusion, dissolution, and equilibrium

Correspondence to: H. Soto-Valdez (hsoto@ciad.mx).

Contract grant sponsor: CONACYT, México; contract grant numbers: 2007-CB-81697, 2008-CB-106224.

Contract grant sponsors: Center for Food and Pharmaceutical Packaging Research (CFPPR) of the School of Packaging, MSU, Fulbright-García Robles Program.

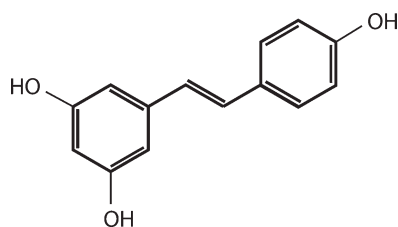


Figure 1 Resveratrol.

processes.^{11,12} The diffusion coefficient (D) represents the rate at which diffusion occurs and can be described by the Fick's second law.¹¹

The aim of this study was to develop PLA-resveratrol films by a blow-extrusion fabrication process. The effect of resveratrol on PLA films was studied, and the kinetics of the diffusion of resveratrol from the PLA matrix into ethanol were determined.

EXPERIMENTAL

Materials

Poly(94% L-lactic acid) resin (PLA 4042D) was obtained from NatureWorks LLC (Blair, NE), and *trans*-resveratrol (99.4%) was obtained from Chroma-Dex (Santa Ana, CA). Butylated hydroxytoluene (BHT, 99%) was used as an antioxidant (SAFC Supply Solutions, St. Louis, MO), and absolute ethanol (Aaper Alcohol and Chemicals, Shelbyville, KY) was used as the releasing media. All solvents used for the quantification of resveratrol were HPLC grade (Sigma-Aldrich, St. Louis, MO).

The production of PLA-resveratrol films

Pure PLA films and PLA films with 1 and 3% wt/wt resveratrol (PR0, PR1, and PR3, respectively) were extruded in a pilot-plant scale blow-extrusion machine (Beutelespacher, México). Extrusion temperatures for zones 1 through 4 of the extruder were 165, 170, 170, and 170°C, respectively.¹³ To obtain a homogeneous distribution of the antioxidant in the films, pelletization was performed with the same extrusion machine. During the production of pellets and films, the heads and tails of each batch were discarded to avoid sections with variable concentrations of resveratrol. All of the films were 2 mils (50.8 μm) thick.

Quantification of resveratrol

PLA films were cut into 0.25 cm^2 pieces and stirred in methanol or ethanol at 40°C for 24 h in the dark to extract resveratrol from the films. To protect the antioxidant from degradation during the extraction

period, 100 $\mu\text{g}/\text{mL}$ of BHT was added to the solution. The extraction was performed twice to ensure complete extraction of the antioxidant, and quantification was achieved with a high performance liquid chromatograph (HPLC) (Waters model 2695, Milford, MA) equipped with a Waters dual λ absorbance detector (model 2487, 295 nm). A 10- μL loop and a Novapack C_{18} column (150 \times 3.9 mm) (Waters) protected with a C_{18} guard column were employed, and an isocratic elution of 98 : 02 methanol:water at a flow rate of 1 mL/min and a temperature of 25°C was applied to elute the analyte from the column.¹³ Standard solutions of resveratrol in methanol at concentrations from 2 to 40 $\mu\text{g}/\text{mL}$ were used to produce a calibration curve for the quantification of the analyte.

The retention time of resveratrol was 1.2 min, and the limit of quantification (LOQ) was 0.09 $\mu\text{g}/\text{mL}$. Analytical recoveries were determined by spiking a solution containing pieces of the PR0 film with a known concentration of resveratrol. The solutions were extracted according to the aforementioned method, and the results indicated that recoveries of $99.5 \pm 1.5\%$ were obtained.

Effect of resveratrol on the optical properties of PLA films

The transmission of UV-visible light through the films was measured with a Perkin-Elmer Lambda 25, UV-visible spectrophotometer (Perkin-Elmer Instruments, Beaconsfield, UK) between 200 and 800 nm at a rate of 480 nm/min, a slit width of 1.0 nm and an interval of 1.0 nm.¹³ An integrating reflectance spectroscopy accessory (model RSA-E-20, Labsphere®, North Sulton, NH) was also employed.

Effect of resveratrol on the thermal properties of PLA films

A differential scanning calorimeter (DSC 2920, TA Instrument, New Castle, DE) was used to determine the effect of the antioxidant on the glass transition temperature (T_g), melting temperature (T_m), and degree of crystallinity (χ_c) of the materials. Samples of the films were subjected to a heat-cool cycle, and the temperature was varied from 10 to 190°C at a heating rate of 10°C/min. The results were analyzed with Universal Analysis Software (Version 3.9A), and χ_c was determined according to ASTM D3417-99¹⁴ and eq. (1):

$$\chi_c(\%) = 100 \times \frac{\Delta H_c + \Delta H_m}{\Delta H_m^c}, \quad (1)$$

where ΔH_c is the enthalpy of cold crystallization, ΔH_m is the enthalpy of fusion, ΔH_m^c is the heat of melting crystalline PLA.¹⁵

Diffusion of resveratrol from PLA films

The amount of resveratrol that diffused from PR1 and PR3 into ethanol was determined using a migration cell recommended by ASTM D4754-98.¹⁶ Eight round discs (2 cm diameter) cut from the films were inserted in a stainless steel wire and placed in a 40 mL vial containing 38.5 mL of ethanol. Each disc was separated by means of a glass bead to expose both sides to liquid. The liquid volume/area ratio was 0.77 mL/cm² complying with ASTM D4754-98 which establishes a ratio between 155 and 0.31 mL/cm².¹⁶ Experiments were performed at 9, 23, 33, and 43°C, and ethanol samples were collected periodically over 278, 10, 4, and 2 days of storage, respectively. All of the storage conditions were controlled to within ±0.5°C. Samples were injected directly onto the HPLC, and resveratrol quantification was performed as described above. Four replicates were conducted, and the calibration curve for resveratrol in ethanol was prepared from standard solutions containing 0.09–120 µg/mL of resveratrol. The concentration of resveratrol was expressed as µg/mL, and the LOQ of the method was 0.09 µg/mL.

An analytical solution representing one-dimensional diffusion in limited volume of film and infinite volume of solution¹¹ was used to determine the diffusion coefficient of the Fick's second law (D)

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

To fit the data to eq. (2), the ratio of the resveratrol concentration in solution at time t to the resveratrol concentration in solution at equilibrium (M_t/M_∞) was plotted against the square root of time (s), and the diffusion coefficients (D , cm²/s) were calculated for each concentration and temperature. In eq. (2), l (cm) is the thickness of the film. The value of D was determined by minimizing the sum of the squares errors (SSE) of the measured and estimated values. To determine the fit of the experimental data to eq. (2), the nonlinear regression (nlinfit) function in MATLAB R2008a (MathWorks, Natick, MA) was applied to the data.¹⁷

Determination of partition coefficients ($K_{p,f}$)

The chemical equilibrium in a diffusion system is expressed by $K_{p,f}$ [eq. (3)]:

$$K_{p,f} = \frac{C_{p,\infty}}{C_{f,\infty}} \quad (3)$$

where $C_{p,\infty}$ (µg/cm³) and $C_{f,\infty}$ (µg/cm³) are the equilibrium concentration of the resveratrol in

the polymer and the food (ethanol in this case), respectively.

Activation energy for the diffusion (E_a) of resveratrol into ethanol

To determine the effect of temperature on the diffusion of resveratrol from the PLA films into ethanol, the E_a was calculated using the Arrhenius equation for diffusion:

$$D = D_0 e^{[-E_a/RT]}, \quad (4)$$

where D is the diffusion coefficient, D_0 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 ($1/T$) versus the logarithm of D ($E_a = -\text{slope} \times 2.303R$).¹⁸

Effect of the diffusion conditions on the weight average molecular weight of PLA

The weight average molecular weights (M_w) of PLA resin, pellets, and films were analyzed before and after the diffusion experiments to determine if PLA was hydrolyzed under the experimental conditions. PLA samples were dried under vacuum, and 20–30 mg of PLA were dissolved in 10 mL of tetrahydrofuran (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 × 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, Milford, MA), which contained a M_w -range of 1310–3,640,000 Da. The experiments were conducted in duplicate.

RESULTS AND DISCUSSION

Figure 2 shows the appearance of pure PLA films (PR0) and PLA films with 1 and 3 wt % resveratrol (PR1 and PR3).

Quantification of resveratrol after film processing

After processing the films, 83.3 and 74.5% of the resveratrol remained in the PR1 and PR3 films,



Figure 2 The appearance of PR0 (left), PR1 (middle), and PR3 (right).

respectively, (Table I). During the pelletization and film extrusion process, the material was poured into the feeder at 165–170°C. The material resided in the extruder for 5–10 min, and friction was applied between the barrel and screw. This stage of the pelletization and film production process normally causes antioxidants in the parent material to degrade.^{19,20} The loss of resveratrol from PR1 and PR3 was 16.7 and 25.5%, indicating that the amount of resveratrol lost during the extrusion process increased with an increase in resveratrol concentration. Similarly, coextruded polyamide 6/66-LDPE films with 0.8 and 1.4% of BHT lost 1.5 and 24.3% of BHT during the extrusion process.¹⁹ Moreover, extrusion of LDPE films containing 2 and 4% α -tocopherol resulted in the loss of 4.65 and 24.55% of the antioxidant, respectively.²⁰ Therefore, films with resveratrol concentrations greater than 1% may require additional considerations to avoid antioxidant losses.

Effect of resveratrol on the optical properties of PLA films

PLA-resveratrol films displayed a yellowish color with varying intensities (Fig. 2). Using PR0 as a reference, Soto-Valdez et al.¹³ reported the color of the films in CIE $L^*a^*b^*$ nomenclature. The addition of the antioxidant did not affect the lightness (L^*) of the films, and L^* remained constant at 89.5. Minor differences in the green (a^*) and yellow (b^*) color of the films were observed, and films containing 1 and 3% wt resveratrol displayed a total difference in color ($\Delta E = ((\Delta a^*)^2 + (\Delta b^*)^2 + (\Delta L^*)^2)^{0.5}$) of 0.32 and 0.67, respectively. Differences in single film layers were not perceptible to the naked eye, but the yellowish color of the individual layers could be observed in film rolls (Fig. 2). Figure 3 shows the light transmission spectra of PLA films with and without resveratrol.¹³ At a film thickness of 2 mils (50.8 μm), the transmission of light from 350 nm (5%

of transmission) to 200 nm through PR1 and PR3 was less than that of PR0. According to the certificate of analysis of resveratrol published by Chroma-Dex,²¹ resveratrol has two maximum wavelengths of absorption at 308 and 225 nm, which agrees with the results shown in Figure 3.

Effect of resveratrol on the thermal properties of PLA films

Figure 4 shows the T_m and T_g of PR0, PR1, and PR3 films during the first DSC run. PR0 displayed a lower degree of crystallinity (5.7%) than a similar PLA film manufactured via bio-orientation (25%).¹ During the blow extrusion process, the molten plastic is forced through a die with a circular opening. An air outlet is located in the center of the die, and compressed air is forced into the center of the extruded circular profile, creating a bubble, which causes the extruded circular cross section to expand to different proportions. In this study, the PLA bubble was immediately cooled, and expansion did not occur. Therefore, the PLA did not crystallize, and only slight orientation was obtained. Moreover, the crystallinity of the films decreased with an increase in the percent resveratrol due to the plastization effect of resveratrol on the PLA structure. The T_m of PLA decreased by almost 1°C due to the addition of 1 and 3% of resveratrol. There was also an effect of the antioxidant on the T_g of PLA. However, the T_g of PR1 and PR3 were similar.

Diffusion of resveratrol from PLA films into ethanol

Bottles made of PLA could be used to contain wine, beer or any alcoholic beverages susceptible to oxidation. So, it was important to test the films with resveratrol to know the potential of this application. Figure 5 shows the diffusion of resveratrol from PLA films into ethanol at four different temperatures. The results indicated that the equilibration time decreased with an increase in temperature due to the dependence of diffusion on this variable. At 43 and 33°C, equilibrium was obtained after 12 and

TABLE I
The Concentration of Resveratrol in the PLA Films After Extrusion

Films	Resveratrol (mg/g)		Resveratrol remained in the films (%)
	Before extrusion	After extrusion ^a	
PR1	10.2	8.5 \pm 0.1	83.3
PR3	30.2	22.5 \pm 0.6	74.5

^a The reported data are the mean of three replicates \pm standard deviation.

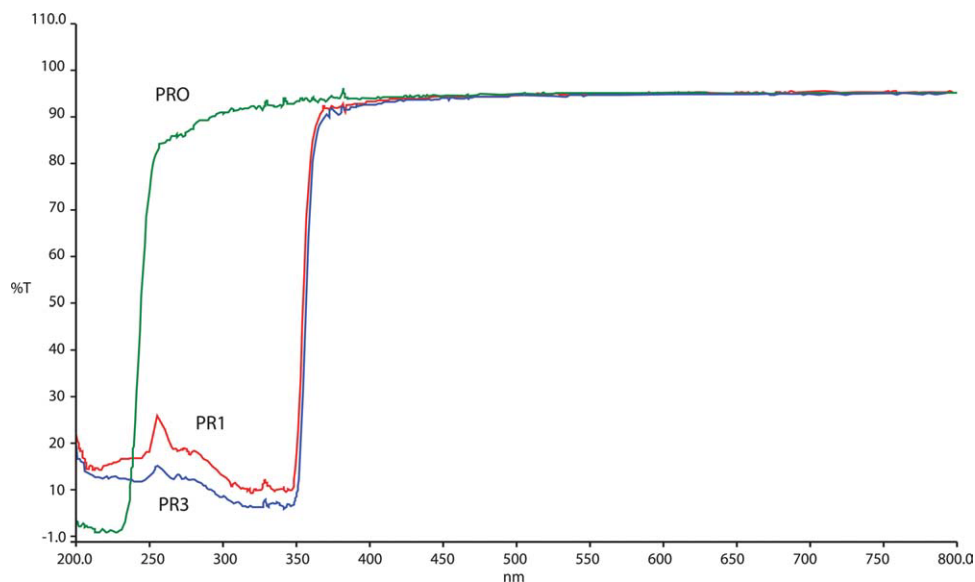


Figure 3 UV-visible light transmission spectra of PR0, PR1, and PR3.¹³ All of the films were 2 mils (50.8 μm) thick. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

27 h, respectively, and 95–99% of the resveratrol in PR1 and PR3 was released into solution. $K_{p,f}$ at 43°C for these systems was determined to be 2.9 and 2.8 for the PR1 and PR3 films, respectively. Meanwhile, $K_{p,f}$ at 33°C was 15.3 and 8.5 for PR1 and PR3 films, respectively, (Table II). Due to most of resveratrol was released at these temperatures the system was

considered of infinite volume for which eq. (2) is applicable. At 23°C, equilibrium was achieved after 130 h, and 85.9 and 78.8% of the resveratrol in PR1 and PR3 was released, respectively. $K_{p,f}$ at this temperature was 52.3 and 71.8 for the PR1 and PR3 films, respectively, (Table II). More than 10% of the resveratrol was not released at 23°C due to its lower

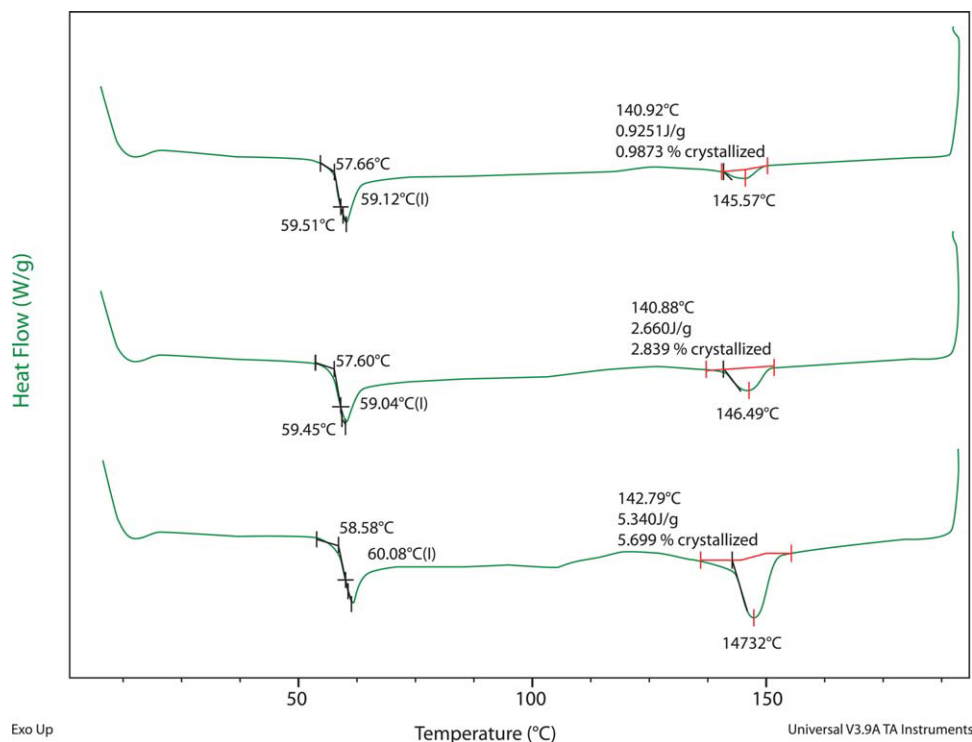


Figure 4 DSC thermograms of PR0 (bottom), PR1 (middle), and PR3 (top). Samples were subjected to a heat-cool cycle, and the temperature was varied from 10 to 190°C at a rate of 10°C/min. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

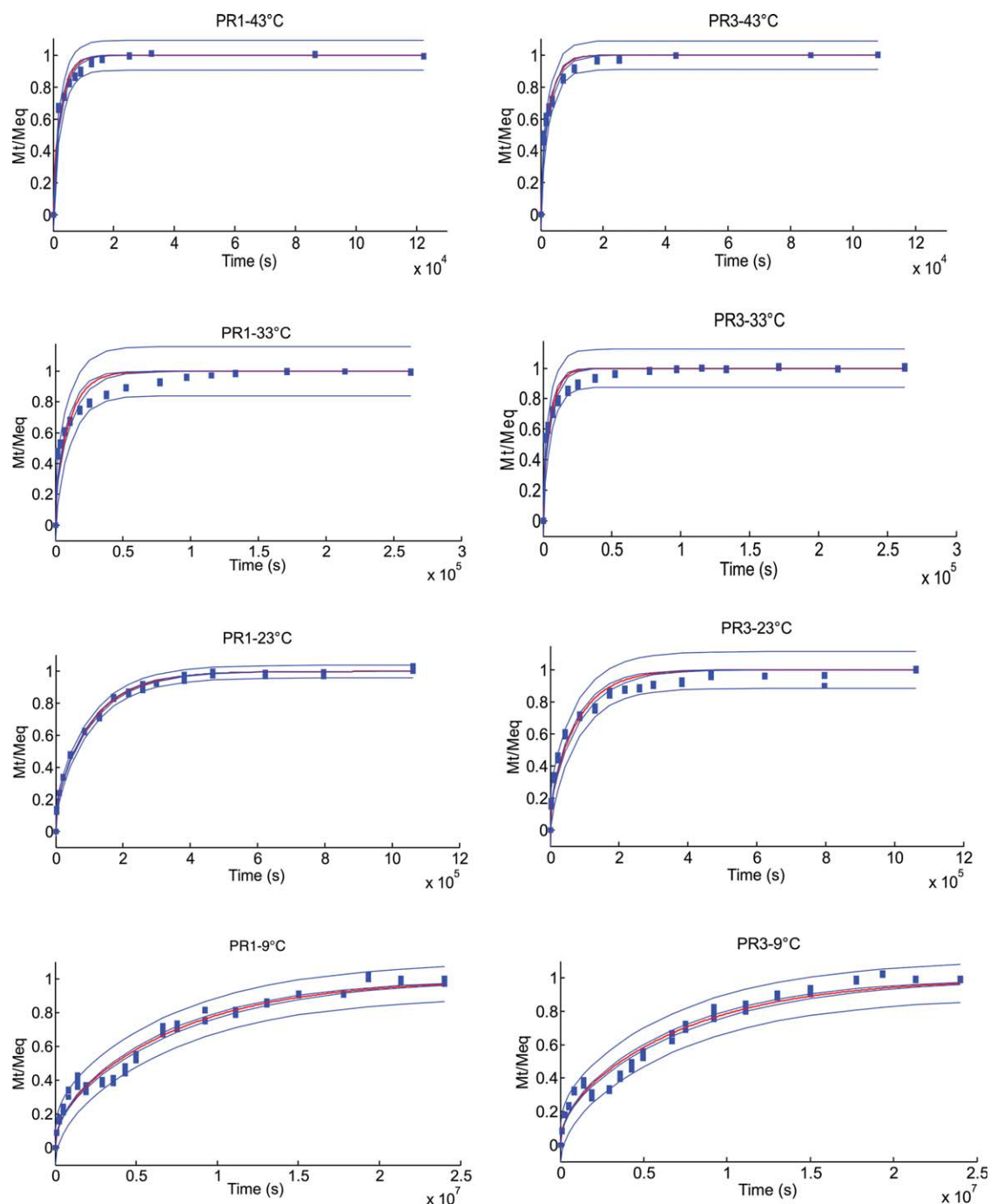


Figure 5 Diffusion of resveratrol from PR1 and PR3 into ethanol at 43, 33, 23, and 9°C, according to Fick's second law [eq. (2)]. The y -axis is the ratio of the concentration of resveratrol in solution at time t to the concentration of resveratrol in solution at equilibrium (M_t/M_{eq}), and the x -axes are time (t) in s. The central line shows the best fit to the experimental data, and the outer lines are the predicted intervals for the experimental values. The inner lines around the best fit curve are the confidence intervals. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

solubility in ethanol and the low diffusion rate of small molecules in glassy polymers. This behavior was more evident at 9°C, in which the equilibrium was apparently attained after 5372 h, and only 22.5 and 36.8% of the resveratrol in PR1 and PR3 was released, respectively. The experiment was stopped at 6667 h because a change in the resveratrol concen-

tration in solution was not observed and according to the graph (Fig. 5, PR1-9°C and PR3-9°C) it could take months before it could happen. Consequently, $K_{p,f}$ values as high as 1143.1 and 506.1 for the PR1 and PR3 films were obtained at 9°C (Table II). From the graphs shown in Figure 5, the value of D for PR1 and PR3 was estimated according to eq. (2), and

TABLE II
Partition Coefficients ($K_{p,f}$) of Resveratrol Between PR1 and PR3, and Ethanol at 9, 23, 33, and 43°C

Temperature (°C)	$K_{p,f}$ ^a	
	PR1	PR3
9	1143.1 ± 62.7	506.1 ± 39.6
23	52.4 ± 4.0	71.8 ± 2.1
33	8.5 ± 3.5	15.3 ± 3.3
43	2.9 ± 0.3	2.8 ± 0.4

^a The data are averages of four samples ± the standard deviation.

the results are shown in Table III. The D values obtained at 43 and 33°C were $\sim 10^{-10}$ cm²/s, and the D values decreased to 10^{-11} cm²/s at 23°C. Alternatively, at 9°C, the D values were $\sim 10^{-13}$ cm²/s. In spite of the high $K_{p,f}$ values at 23 and 9°C, which show that some resveratrol remained in the films at equilibrium, D values were also determined by eq. (2). PLA-resveratrol films are a new release system for antioxidants; thus, literature data was not available for comparison. However, the D values obtained in this study were similar to those reported by Heirlings et al.,²² who studied the diffusion of α -tocopherol from conventional polymers such as LDPE and EVA (ethylene vinyl acetate) into ethanol at 7°C. D values of 2.64×10^{-11} and 4.23×10^{-11} cm²/s were obtained, respectively, and 95% of the α -tocopherol in the films diffused into solution. Although the D values obtained from the aforementioned study are on the same order of magnitude as the D values of resveratrol at 23°C, significantly lower temperatures were employed, indicating that diffusion of resveratrol from PLA is slower than that of commercial antioxidants from polyolefins. This was expected due to the fact that between 7 and 23°C polyolefins are above their T_g , in a rubbery state. Meanwhile, at this range of temperatures PLA is below its T_g , in a glassy state.

Activation energy of the diffusion (E_a) of resveratrol into ethanol

In the absence of anomalies such as melting or glass transitions, the diffusion of an additive through a polymer matrix is adequately described by the Arrhenius equation, where E_a is the energy required to produce an opening between the polymer chains that is large enough for an additive molecule to move through.¹⁸ Figure 6 shows the Arrhenius plots of the diffusion of resveratrol from PR1 and PR3 at 9, 23, 33, and 43°C. For PR1 and PR3, plots of D versus $1/T$ produced a straight line with an E_a of 175 and 177 kJ/mol, respectively.

TABLE III
Diffusion Coefficients (D) of Resveratrol from PR1 and PR3 into Ethanol at 9, 23, 33, and 43°C

Temperature (°C)	D ^a (cm ² /s)	
	PR1	PR3
9	$(3.47 \pm 0.10) \times 10^{-13}$	$(3.49 \pm 0.10) \times 10^{-13}$
23	$(2.30 \pm 0.03) \times 10^{-11}$	$(3.06 \pm 0.14) \times 10^{-11}$
33	$(2.26 \pm 0.15) \times 10^{-10}$	$(4.17 \pm 0.25) \times 10^{-10}$
43	$(8.51 \pm 0.38) \times 10^{-10}$	$(8.26 \pm 0.32) \times 10^{-10}$

All of the films were 2 mils (50.8 μ m) thick.

^a The data are expressed as the best fit of four samples ± the standard deviation.

Reynier et al.²³ reported that the E_a of the diffusion of a series of additives (Irganox 1076, Irganox 1330, Irgafos 168, octadecanol, tripalmitin, among others) from polypropylene ranged between 100 and 250 kJ/mol. Thus, the activation energies obtained in this study are similar to those of other polymers with hydrocarbon chains (polyolefins).

Effect of the diffusion conditions on the M_w of PLA

The effect of the processing conditions and media on the M_w of PLA is shown in Figure 7. The M_w of PLA resin was 168 kDa; however, after the pelletization of PLA with 1 and 3% resveratrol, the M_w decreased to 161 and 163 kDa, respectively. Moreover, after the films were manufactured, the M_w of PR1 and PR3 was 162 and 159 kDa, respectively. Therefore, the pelletization process had a stronger effect on the degradation of the polymer than the manufacturing procedure. During pelletization, the

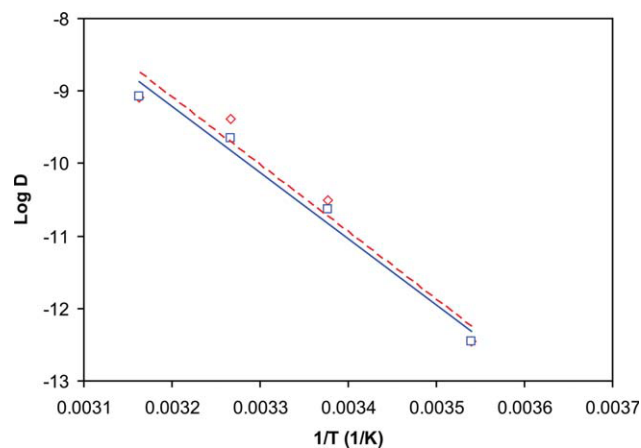


Figure 6 The activation energy of the diffusion of resveratrol from PR1 (\square , —, $Y = -9127.2X + 19.999$, $R^2: 0.982$) and PR3 (\diamond , - -, $Y = -9265.4X + 20.555$, $R^2: 0.956$) into ethanol. The slope of each line was equal to $-E_a/2.303R$, and the E_a of diffusion from PR1 and PR3 was 175 and 177 kJ/mol, respectively. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

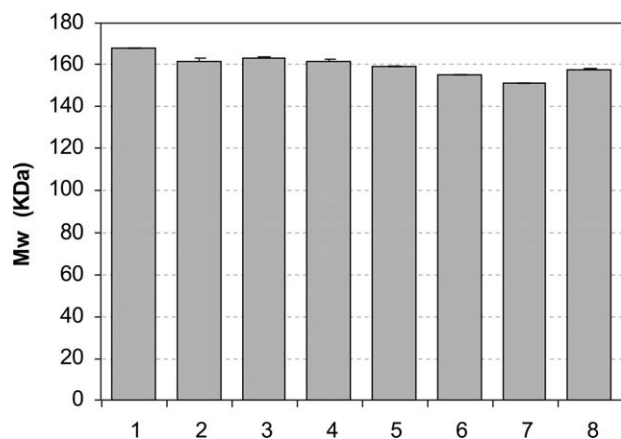


Figure 7 Effect of processing and diffusion conditions on the M_W of PLA with resveratrol. Bars indicate the average of two replicates. (1) PLA resin, (2) PR1 pellets, (3) PR3 pellets, (4) PR1 film, (5) PR3 film, (6) PR3 film after contact with ethanol at 23°C for 40 days, (7) PR3 film after contact with ethanol at 43°C for 2 days, and (8) PR0 film after contact with ethanol at 43°C for 2 days.

cooling time of the filaments was greater than 15 min. Alternatively, during film production, the films were cooled immediately after bubble formation.

After 2 days of contact with ethanol at 43°C, the M_W of PR3 was 151 kDa. Thus, the M_W of PR3 decreased by $\sim 5\%$ due to contact with ethanol. Alternatively, the M_W of PR0 was 158 kDa under the same conditions. The PR0 film was not subjected to pelletization in the present work so that reducing the thermal history of the PLA. Thus, the degradation of the polymer during contact with ethanol was lower than that of PR3. The M_W of PR3 after 40 days of contact with ethanol at 23°C was 155 kDa, which represents a $<3\%$ decrease in M_W . Despite the longer contact time, less polymer degradation was observed at 23°C than at 43°C. Therefore, the temperature of the solution had a stronger effect than time on the loss of M_W due to contact with ethanol. The hydrolytic degradation mechanism, rate, and behavior of PLA are affected by a number of parameters such as polymer morphology and structure and surrounding environment parameters such as pH, temperature, catalytic species, etc. With respect to the environment, hydrolytic degradation of PLA in acidic solution proceeds via a chain-end scission mechanism in the lactyl monomer unit, forming lactic acid. In the present case where the experiments were run in neutral to slightly acidic ethanol solution between 9 and 43°C, we could expect that hydrolysis can happen at some degree at 43°C via the described mechanism (chain-end scission mechanism in the lactyl monomer unit, forming lactic acid).²⁴

The effect of PLA degradation on the release of resveratrol could have been observed at 43°C, but diffusion reached equilibrium during the first 12 h of contact. By that time, $>95\%$ of resveratrol was

released into solution, and further loss of resveratrol from the film was not observed after 48 h. M_W of the films was measured after 48 h of contact. At 23°C, equilibrium was attained at 130 h of contact, and 78.8% of resveratrol was released into solution. The films were placed in ethanol for up to 960 h to determine if the antioxidant remaining in the film diffused into solution. The results indicated that a further increase in the release of resveratrol between 130 and 960 h was not detected, and the M_W of the films was measured after 960 h of contact. Therefore, PLA degradation did not have an effect on the diffusion of resveratrol under the studied conditions.

CONCLUSION

PLA films containing 1 and 3 wt % resveratrol were manufactured, and 16–25 wt % of resveratrol degraded during the production process. PLA films with 1 and 3% resveratrol were transparent and yellow in color, and the films adsorbed UV-visible radiation at 350–200 nm. The results revealed that the addition of resveratrol to PLA decreases the crystallinity of the films. Moreover, the diffusion of resveratrol from PLA films into ethanol showed Fick's behavior, and D values between $(3.47 \pm 0.10) \times 10^{-13}$ and $(8.51 \pm 0.38) \times 10^{-10}$ cm²/s were obtained. The temperature dependence of the D values was in accordance with the Arrhenius equation, and the activation energy of the diffusion of resveratrol in ethanol was 176 kJ/mol. The results indicated that the temperature of the release experiments had a stronger effect on the weight average molecular weight of the PLA films than the processing conditions and the contact time with ethanol. However, the diffusion of resveratrol was not affected by the degradation of PLA. Thus, the films produced in this study could be used as antioxidant release membranes for a variety of pharmaceutical, medical, and food applications.

We gratefully acknowledge Gaurav Dhoot, Sukeewan Detyothin, and Thitisilp Kijchavengkul for their assistance with MATLAB and the determination of weight average molecular weights.

References

1. Auras, R.; Harte, B.; Selke, S. *Macromol Biosci* 2004, 4, 835.
2. NatureWorks. Available at <http://www.natureworksllc.com/news-and-events/press-releases/2010/03-15-10-lactide.aspx>. Natureworks Press Releases, published online 15 March 2010, retrieved 10 May 2010.
3. King, R. E.; Bomser, J. A.; Min, D. B. *Comp Rev Food Sci F* 2006, 5, 65.
4. Sigma. Product Information: Resveratrol (R5010); Sigma-Aldrich: Saint Louis, MS, 1997.
5. Soares, D. G.; Andrezza, C.; Salvador, M. *J Agric Food Chem* 2002, 51, 1077.

6. Bader, Y.; Quint, R. M.; Getoff, N. *Rad Phys Chem* 2008, 77, 708.
7. Pirola, L.; Fröjdö S. *IUBMB Life* 2008, 60, 323.
8. Karuppagounder, S. S.; Pinto, J. T.; Xu, H.; Chen, H. L.; Beal, M.F.; Gibson, G. E. *Neurochem Int* 2009, 54, 111.
9. Penumathsa, S. V.; Maulik, N. *Can J Phys Pharm* 2009, 87, 275.
10. Gülçin, I. *IFSET* 2010, 11, 210.
11. Crank, J. *The Mathematics of Diffusion*; Oxford Science Publications: Oxford, UK, 1975.
12. Garde, J. A.; Catalá, R.; Gavara, R.; Hernández, R. J. *Food Addit Contam* 2001, 18, 750.
13. Soto-Valdez, H.; Peralta, E.; Auras, R. In 16th IAPRI Conference, Bangkok, Thailand, June 2008.
14. ASTM D 3417-99. Standard Test Method for Enthalpies of Fusion and Crystallization of Polymers by Differential Scanning Calorimetry (DSC); Annual Book of ASTM Standards: Conshohocken, PA, 1999; Vol. 08-02.
15. Auras, R. A.; Singh, S. P.; Singh, J. J. *Pack Technol Sci* 2005, 18, 207.
16. ASTM D 4754-98. Standard Test Method for Two-Sided Liquid Extraction of Plastic Materials Using FDA Migration Cell; Annual Book of ASTM Standards: Conshohocken, PA, 2006; Vol. 08-02.
17. Dhoot, G.; Auras, R.; Rubino, M.; Soto-Valdez, H.; Dolan K. *Polymer* 2009, 50, 1470.
18. Limm, W.; Hollifield, C. *Food Addit Contam* 1996, 13, 949.
19. Soto-Cantú, C.; Graciano-Verdugo, A.; Peralta, E.; Islas-Rubio, A.; González-León, A.; González-Córdova, A.; Soto-Valdez, H. *J Dairy Sci* 2008, 91, 11.
20. Graciano-Verdugo, A. Z.; Soto-Valdez, H.; Peralta, E.; Cruz-Zárate, P.; Islas-Rubio, A. R.; Sánchez-Valdes, S.; Sánchez-Escalante, A.; González-Méndez, N.; González-Ríos, H. *Food Res Int* 2010, 43, 1073.
21. Resveratrol. Certificate of Analysis; ChromaDex Inc.: Santa Ana, CA, 2007.
22. Heirlings, L.; Siró, I.; Devlieghere, F.; Bavel, E.; Cool, P.; De Meulenaer, B.; Vansant, E. F.; Debevere, J. *Food Addit Contam* 2004, 21, 1125.
23. Reynier, A.; Dole, P.; Feigenbaum, A. *Food Addit Contam* 2002, 19 (Suppl), 42.
24. Tsuji, H. In "Hydrolysis" in Poly(lactic acid) Synthesis, Structures, Properties, Processing, and Applications; Auras, R., Lim, L. T., Selke, S., Tsuji, H., Eds.; Wiley: Hoboken, 2010; Chapter 21.