DOI:10.1021/jf1022324

JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY
ARTICLE

Improving the Antioxidant Protection of Packaged Food by Incorporating Natural Flavonoids into Ethylene−Vinyl Alcohol Copolymer (EVOH) Films

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Ethylene−vinyl alcohol copolymer (EVOH) films containing catechin or quercetin as antioxidant agents were successfully produced by extrusion. The addition of these bioactive compounds did not modify greatly their water and oxygen permeabilities, T_g, or crystallinity but improved their thermal resistance. Exposure of the films to different food simulants showed that both compounds were released, although the extent and kinetics of release were dependent on the type of food. In aqueous and alcoholic food simulants their release was greater in the case of the catechin-containing samples. Exposure of the films to isooctane and ethanol 95% (fatty food simulants) provided controversial results; no release was observed in isooctane, whereas both bioactive compounds were extracted by ethanol due to their high solubility in alcohol and the plasticizing effect of ethanol on the polymer. Packaging applications of these films can improve food stability and provide a method for adding such bioactive compounds.

KEYWORDS: Flavonoids; active packaging; antioxidant; release; EVOH

INTRODUCTION

Oxidation processes are involved in most deterioration mechanisms present in nature, including both food products and food packages, especially polymeric packages. To protect the polymer during package manufacture and use, most polyolefins contain mixtures of a primary antioxidant, which offers long-term protection to the film, and a secondary antioxidant, which protects the polymer during package manufacture (*1*−*3*). Most of the common antioxidants are phenolic compounds, secondary arylamines, organophosphites, and thioesters of synthetic origin, that are approved by national and international regulations for plastics in contact with foods. Nevertheless, migration of these additives and their degradation products into food during storage may change the sensory properties of the product they contain or even lead to toxicity upon consumption. For these reasons, several research studies have focused on the development of alternative polymer formulas with antioxidants that are considered to be food additives, such as BHT and BHA (*4*−*6*). However, the presence of these synthetic antioxidants in food is questioned, owing to the potential risks, and strict statutory controls are required. The alternative that is being studied widely is the use of natural antioxidants, particularly tocopherol, plant extracts, and essential oils from herbs such as rosemary, oregano, and thyme(*7*−*9*).

Many phenolic compounds are commonly found in plants and have been reported to possess multiple biological effects, including antioxidant activity (*10*). The principal antioxidant activity of these compounds is mainly as radical scavengers. However, many of the constituents of plant essential oils are volatile and difficult to use in conventional packaging manufacturing processes (extrusion, injection). Some initial studies have proved that natural polyphenolic compounds such as catechin or epicatechin can replace synthetic antioxidants in packaging protection (*11*).

To reduce oxidation in sensitive food products, the addition of antioxidants or the design of a suitable packaging technology are the two most common alternatives. Vacuum or modified-atmosphere packaging combined with high-barrier packaging materials can limit the presence of oxygen, although it is not always completely and effectively eliminated because of a residual presence at the time of packing or because it permeates in from the exterior through the package wall. Moreover, some food products such as fresh red meat cannot be packaged without oxygen. Recently, other strategies have been considered including the use of active antioxidant packages (*7*, *12*−*15*).

EU Regulations 1935/2004/EC and 450/2009/EC consider active materials "materials and articles that are intended to extend the shelf-life or to maintain or improve the condition of packaged food" by the purposeful incorporation of components that are released or that absorb substances into or from the packaged food or the environment surrounding the food (*16*−*18*).

Highly reactive species such as free radicals, superoxide, hydroxyl, and singlet oxygen are generated in food or in the surrounding atmosphere by different mechanisms and are involved in oxidation reactions in lipids and other food components, contributing to

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 Published on Web 09/29/2010

their deterioration. Active packaging systems that absorb these reactive species can be a good choice for many products and constitute one of the potential uses of active packaging. Granda-Restrepo et al. developed polyethylene films with α-tocopherol and measured the antioxidant release into milk powder (*14*). Peltzer et al. added carvacrol to polypropylene and measured its migration into water and oil (*15*). To reduce the partial loss of volatile antioxidants during conventional packaging manufacturing processes, Nerín et al. developed a polymeric coating with essential oils to protect meat from oxidation (*12*, *13*). Nevertheless, volatile agents are still released into the atmosphere during storage, reducing the effectiveness of the active materials.

In this work, active antioxidant materials for the packaging of oxygen-sensitive foods, based on an ethylene−vinyl alcohol copolymer (EVOH) and two natural flavonoids, quercetin and catechin, were developed by conventional extrusion. EVOH is a common packaging material that is known for its excellent oxygen barrier properties and its highly hydrophilic nature (*19*−*21*). The main use of this material is to strictly reduce the entrance of oxygen in the package, and in this application the EVOH layer should be sandwiched between polyolefin layers to protect it from the humidity of the environment and of the food. Recently, new data showed the severe effect of humidity on the mass transport of organic compounds, increasing molecular diffusivity several orders of magnitude (*22*). This characteristic is highly profitable because the increment of humidity in the presence of food triggers the agent release and subsequently the antioxidant activity (*22*). The stability of the active materials is guaranteed by dry storage, because the exchange of agents and oxygen is highly restricted.

Quercetin and catechin are phenolic compounds that are commonly found in both edible and nonedible plants. They have been reported to have multiple biological effects, including high antioxidant activity (*23*, *24*). The antioxidant activity of phenolic compounds is due to their ability to scavenge free radicals, donate hydrogen atoms or electrons, or chelate metal cations as a result of their chemical structure (*25*−*28*). Besides their antioxidant character, these two flavonoids were selected because (a) they are nonvolatile, reducing the loss of the agent during packaging manufacturing that occurs with other compounds such as BHT or carvacrol (b) they could protect the polymer during processing, and, furthermore, (c) their release into food increases the product's bioactive compound content instead of resulting in a toxicological risk, as occurs with synthetic antioxidants.

The resulting materials were characterized to analyze the effect of the addition of quercetin and catechin on EVOH functional properties, and their antioxidant activity in food was determined by monitoring the agents' release into different food simulants and by analyzing the scavenging capacity of radical oxidizing compounds.

MATERIALS AND METHODS

Chemicals and Reagents. An ethylene−vinyl alcohol copolymer with a 44% ethylene molar content (EVOH) was obtained from The Nippon Synthetic Chemical Co. (Osaka, Japan). Reagent-grade absolute ethanol, quercetin dihydrate, and 2,2-diphenyl-1-picrylhydrazyl 95% free radical were purchased from Sigma (Madrid, Spain), and (+) catechin hydrate was purchased from Fluka (Barcelona, Spain). Water was obtained from a Milli-Q Plus purification system (Millipore, Molsheim, France).

Film Preparation. EVOH films containing quercetin and catechin at two different concentrations were obtained by flat extrusion. The antioxidants were incorporated at 1 and 5% of quercetin and at 0.5 and 2% of catechin into hydrophilic EVOH, and the antioxidant−EVOH mixture was extruded on a Brabender DSE 20/40 corotating twin-screw extruder (Plastograph, Dusseldorf, Germany) at 200 °C at a screw speed of 100 rpm. The resulting films were approximately 40−50 μm thick, although the thickness of every sample was individually measured before tests using a digital Mitutoyo micrometer (Metrotec, San Sebastian, Spain).

The film samples obtained in this way were vacuum packaged in aluminum/LDPE bags and stored at room temperature until the moment of analysis. Their thermal properties, oxygen and water vapor transport properties, and optical properties were studied.

Flavonoid concentration in the films was determined by extraction in ethanol at 60 °C during 2 h. The concentration was then determined by UV−vis spectroscopy, and the retained antioxidant activity by the DPPH$^{\bullet}$ method.

Thermal Analysis. Thermogravimetric analyses were carried out using a Mettler Toledo TGA/SDTA/851 thermal analyzer (Columbus OH). The samples were heated from room temperature to 900 °C under a nitrogen atmosphere to determine any evaporation of volatile compounds, as well as the degradation temperatures of the flavonoid-containing materials.

The thermal properties of the samples were also determined with a DSC model Q2000 from TA Instruments (New Castle, DE). Thermograms from −50 to 250 °C with 10 °C/min heating and cooling were obtained. The glass transition (T_g) and melting point (T_m) temperatures and the enthalpy (ΔH_m) were calculated. Considering the polymer percentage of each sample, a corrected enthalpy ($\Delta H_{m,cor}$) value was also estimated.

Barrier Properties. *Water Vapor Permeability* (*WVP*). WVP tests were carried out at 50, 75, and 100% relative humidity (RH) and 23 °C using permeability cups (Elcometer, Manchester, U.K.) in accordance with ISO 2528 (*29*). The aluminum cups were filled with 7 g of silica gel and sealed with vacuum silicon grease (Sigma, Barcelona, Spain) and the film to be tested. The film was fixed in place with a flat Viton ring, an aluminum ring, and three press-screws. To ensure the necessary relative humidity, the cups were then stored in desiccators containing salt solutions: magnesium nitrate $Mg(NO_3)_2 \cdot 6H_2O$, sodium chloride (NaCl), and water for 50, 75, and 100% RH, respectively. The cups were weighed daily, and the plot of the weight increment versus time provided the water vapor transmission rate. These values were then divided by the water pressure gradient and multiplied by the sample thickness to obtain the WVP value.

Oxygen Permeability. The oxygen permeation rates of the materials were determined at 50 and 90% RH and 23 °C using a OXTRAN model 2/21 ML Mocon (Lippke, Neuwied, Germany). The film samples were previously conditioned at the RH of the experiment in the desiccators described above. After the samples had been conditioned in the OXTRAN cells for 6 h, the transmission values were determined every 45 min until constant.

Optical Properties. The film color was determined with a Konica Minolta CM-35000d spectrophotometer set to D65 illuminant/10° observer. The film specimens were placed on the surface of a standard white plate, and the CIELAB color space was used to determine the parameters L^*, a^*, and b^*. The color was also expressed using the polar coordinates L^*, C^*, and H^*, where L^* is the same as previously, C^* is the chroma or saturation index, and H^* is the angle. Eight measurements were taken of each sample, and three samples of each film were measured. All of the samples were selected with a thickness of 40 μm to reduce the effect of thickness on color measurements.

Release Studies. A study of the release of the active compounds from the films was carried out by determining the specific migration from the polymer into the different food simulants specified in European law: water was used as an aqueous food simulant, ethanol 10% as an alcoholic food simulant, and ethanol 95% and isooctane as fatty food simulants. Migration studies were conducted at 37 °C, in accordance with EU Regulations (UNE-EN 1186-3) (*30*). Double-sided, total immersion migration tests were performed as follows: a 24 cm^2 piece of each plastic sample and 90 mL of the simulant (area-to-volume ratio around 6 dm^2/L) were placed in a glass vial covered with aluminum foil to protect the content from light. Simulants were deoxygenated by bubbling nitrogen, and a final nitrogen flush was done before closing the cells to reduce the oxygen percentage at the cell headspace. Flavonoid solutions in water and alcohol using this procedure were stable for 1 month. Periodically, three vials were opened, and the concentration of the antioxidant in the simulants was analyzed by UV spectroscopy. Using an absorbance/concentration (g/mL) calibration curve, the results can be expressed as the concentration of quercetin or catechin released into the simulants.

At the same time, the antioxidant activity provided by the films was evaluated through measuring the radical scavenging ability of the food simulants, using the method of Okada and Okada with a slight modification (*31*). The bleaching rate of a stable free radical, 2,2-diphenyl-1-picrylhydrazyl (DPPH•), was monitored at a characteristic wavelength in the presence of the sample. In its radical form DPPH• absorbs at 517 nm, but upon reduction by an antioxidant or a radical compound, its absorption decreases. The percentage inhibition values were calculated using eq 1:

$$I\,(\%) = [(\text{Abs control} - \text{Abs sample})/\text{Abs control}] \times 100 \quad (1)$$

Using a calibrated curve of ascorbic acid concentration versus I (%), the results can be expressed easily as the equivalent ascorbic acid concentration. The antioxidant activity of the two flavonoids (as received) was determined by this method; 0.79 ± 0.03 g of quercetin or 0.89 ± 0.05 of catechin was equivalent to 1 g of ascorbic acid.

Statistical Analysis. One-way analyses of variance were carried out. The SPSS computer program (SPSS Inc., Chicago, IL) was used. Differences in pairs of mean values were evaluated by the Tukey-b test for a confidence interval of 95%. Data are represented as mean ± standard deviation.

RESULTS AND DISCUSSION

In this work, EVOH films containing catechin or quercetin as antioxidant agents were successfully produced by extrusion. The analysis of the ethanol extract of the diverse samples by UV−vis spectroscopy revealed that the final contents of quercetin into Q 1% and Q 5% films were 75.6 ± 1 and 80.1 ± 1.0% with respect to nominal content, respectively. Similar analyses for Cat. 0.5% and Cat. 2% films showed that the final catechin contents were 66.8 ± 1.0 and 67.1 ± 1.0%, respectively. The analysis of the antioxidant activity by the DPPH• method provided similar results: 71.5 ± 2% for Q 1%, 79.5 ± 1% for Q 5%, 69.2 ± 1% for Cat. 0.5%, and 69.8 ± 1% for Cat. 2%, with respect to nominal antioxidant activity. Besides these tests, the extract was analyzed by HPLC with DAD (data not shown). Although some minor peaks were present in the spectra, the content of catechin and quercetin measured was in good coincidence with the values obtained by UV−vis spectroscopy and by the DPPH• test.

Thermal Characterization. The films containing the antioxidants were first characterized by DSC to check for effects on the polymer morphology caused by the addition of the flavonoids.

Figure 1 presents representative first-heating thermograms of the materials developed, and **Table 1** shows the main information obtained from the thermogram analysis. During the first heating, all of the samples presented the same features: glass transition at temperatures of ca. 45 °C, a melting endotherm starting at ∼120 °C and with a minimum value at around 165 °C, and temperatures in agreement with the values reported in the literature for pure EVOH (*21*, *32*). Between the glass transition temperature and the melting temperature, all of the samples presented a small endotherm at temperatures of ca. 88 °C.

During the cooling process (not shown), the polymer showed a crystallization exotherm at 147 °C. During the second heating (not shown), the glass transition and the melting of crystals were observable at temperatures similar to those of the first heating, but there was no sign of the endotherm at 88 °C. In the case of semicrystalline/amorphous thermoplastics, processing results in internal molecular stresses (thermal history effects), which are relieved on first heating (*32*). For all of the samples, the release of these stresses appears as an endothermic relaxation event after the glass transition, approximately at around 88 °C.

As can be seen in **Figure 1** and **Table 1**, the presence of the antioxidants in the polymer did not produce large changes. The glass transition temperatures of the samples were not significantly different ($p < 0.05$), although it would appear that the addition of high concentrations of the flavonoids might result in an increase in polymer rigidity.

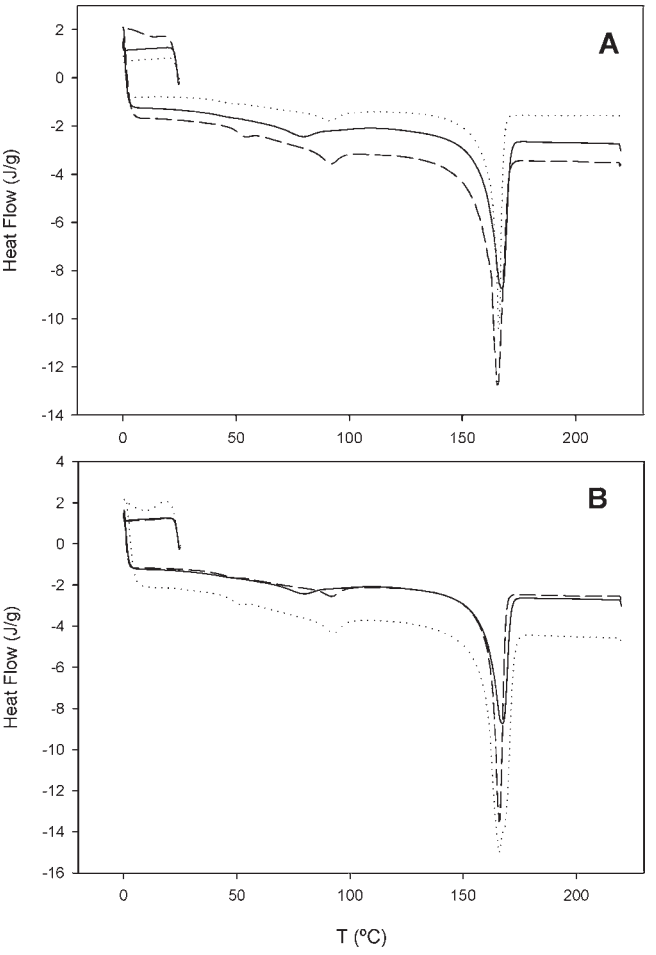

Figure 1. DSC thermograms of EVOH-based materials during the first heating; (**A**) values for quercetin-containing films [(—) blank; (· · ·) Q 1%; and (— — —) Q 5%]; (**B**) values for catechin-containing films [(—) blank; (· · ·) Cat. 0.5%; and (— — —) Cat. 2%].

Table 1. Thermal Parameters from DSC Thermograms of the EVOH-Based Materials during the First Heating[a]

sample	T_g (°C)	T_m (°C)	ΔH_m (J/g)	$\Delta H_{m,cor}$ (J/g)
blank	43.0 ± 1.3 a	167.0 ± 0.3 a	−74.8 ± 1.8	−74.8 ± 1.8 a
Q 1%	42.5 ± 1,3 a	165.7 ± 0.6 b	−75.2 ± 1.8	−75.9 ± 1.8 a
Q 5%	50.3 ± 4.0 b	164.9 ± 0.2 b	−70.4 ± 1.2	−74.1 ± 1.2 a
Cat. 0.5%	42.7 ± 1.3 a	165.4 ± 0.3 b	−74.7 ± 4.2	−75.1 ± 4.2 a
Cat. 2%	44.8 ± 2.5 a	165.6 ± 0.1 b	−73.0 ± 1.8	−74.5 ± 1.8 a

[a] Different letters (a, b) indicate significant differences among the values of the same thermal property.

The melting feature also differed slightly in the materials containing flavonoids. The minimum for the endotherm moved forward significantly in all of the antioxidant-containing samples ($p > 0.05$). Also, the crystallinity (melting enthalpy) of EVOH samples decreased when high concentrations of the antioxidants were added. However, no significant differences were observed when the enthalpy values were corrected for the percentage of polymer in the sample. Also, **Figure 1** shows that the width of the transition increased in the samples with flavonoids. A possible interpretation of these differences is that the antioxidant molecules disrupt the crystal structure, resulting in a more heterogeneous structure.

Because the compounds were melt-blended with the polymer at high temperatures, thermogravimetric analyses were performed

to determine the degradation temperature of the new materials and the thermal stability of the antioxidants.

Figure 2 shows that the stability of the polymer was improved by the addition of the natural antioxidants, because the degradation of the resulting materials occurred at higher temperatures. As can be seen in **Figure 2A**, the degradation temperatures of the materials containing quercetin were higher than those of the blank EVOH sample, which degraded at 414 °C, compared to 430 and 447 °C for 1 and 5% quercetin, respectively. In the case of the catechin-containing samples, shown in **Figure 2B**, the low concentration sample presented a lower degradation temperature, due to earlier degradation of the catechin, but the higher concentration sample possessed the highest stability: the degradation temperature for the sample with 2% catechin was 455 °C.

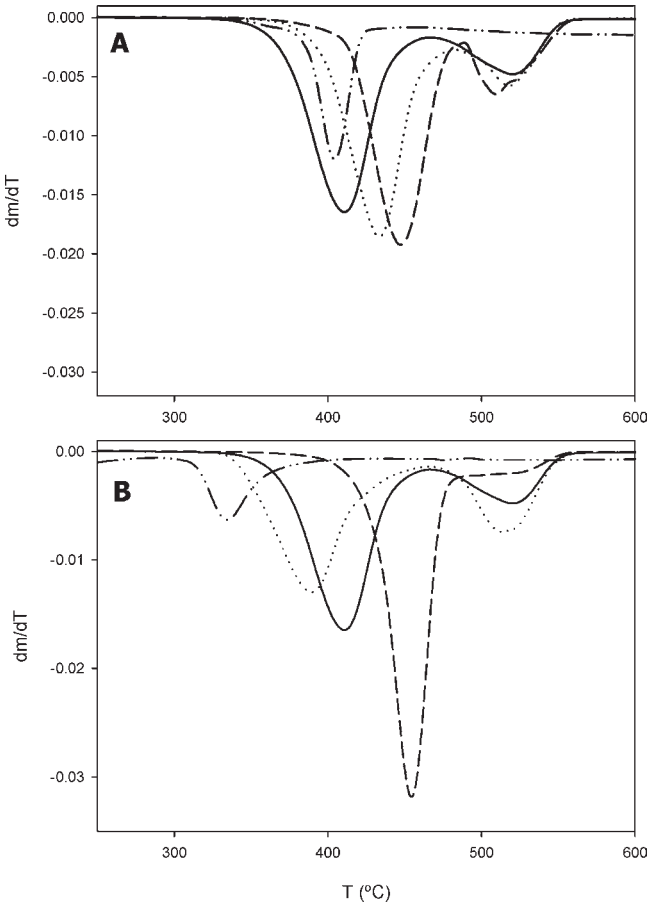

Figure 2. Derivative of the weight loss of natural antioxidants and EVOH materials, measured by TGA: (**A**) values for quercetin-containing films [(—) blank; (· · ·) Q 1%; (— — —) Q 5%; and (— · —) quercetin]; (**B**) values for catechin-containing films [(—) blank; (· · ·) Cat. 0.5%; (— — —) Cat. 2%; and (— · —) catechin].

Barrier Properties. *WVP*. The WVP values were measured for all samples at 50, 75, and 100% RH gradients and 23 °C. As can be seen in **Table 2**, the WVP of all samples increased with the RH gradient, showing the plasticizing effect of water on the polymer matrix at high humidities. In dry conditions, strong interchain interactions among EVOH hydroxyl groups result in high cohesive energy and low chain flexibility. In the presence of humidity, sorbed water molecules interact by hydrogen bonding with the −OH groups of the polymer, reducing the interchain bonding and giving rise to a decrease in the glass transition temperature (T_g) of the polymer and an increase in its flexibility and permeability to gases and vapors (*21*). This effect is in agreement with previous studies in which water permeability was rather constant at low humidity and increased considerably in very humid environments (*33*).

The addition of the antioxidants to EVOH did not produce significant effect on WVP at 50% RH. At 100% RH, the WVPs of all samples with active agents incorporated increased up to 30% with respect to the blank sample. This increment could be a consequence of the more deficient crystallinity of the antioxidant-containing samples. At high humidities, the high plasticization of the polymer may increase the areas suitable for transport, including amorphous areas within small and defective crystal structures, which are forbidden from transport at low humidities. At 75% RH, the WVP values of the developed films fell considerably with respect to the blank sample, by a factor of 4. It is known that mass transport kinetics change drastically when a polymer passes from a vitreous to a rubbery state. The presence of the antioxidants may reduce the plasticizing effect of the sorbed water in such a way that the relative humidity at which the T_g reaches room temperature increases, delaying the exponential growth of permeability toward higher RH values.

Oxygen Permeability. **Table 2** also shows the oxygen permeability values for EVOH samples. As already commented above, the water sorption of the EVOH samples increased with relative humidity, resulting in plastification of the polymer with a sharp fall in its glass transition temperature (T_g) and an increase in its permeability (*33*, *34*).

The incorporation of antioxidants did not modify the barrier properties notably. At 50% RH, the samples with antioxidants presented slightly higher oxygen permeability values ($p > 0.05$). This increment could be caused by the already mentioned reduction of the crystalline fraction and the more irregular crystal structure, as observed in the thermal analysis. At high humidities, the samples with antioxidants presented increased oxygen permeability values, in agreement with those for water permeability. The presence of the antioxidants may increase the amount of sorbed water and, consequently, polymer plasticization. The samples with catechin, the most hydrophilic antioxidant, are those that present the strongest effect.

From the water and oxygen mass transport results, it can be concluded that the addition of the antioxidants did not modify the barrier properties of the EVOH materials. The materials

Table 2. Water Vapor and Oxygen Permeability Values of EVOH-Based Materials[a]

	water vapor permeability [kg·m/(m^2·s·Pa)]			oxygen permeability [kg·m/(m^2·s·Pa)E−03]	
	50% RH	75% RH	100% RH	50% RH	90% RH
blank	2.0 ± 0.1 a	8.9 ± 0.6 c	13.9 ± 1.0 ab	7.2 ± 0.2 a	33.6 ± 0.9 b
Q 1%	1.7 ± 0.1 a	1.9 ± 0.1 a	18.3 ± 1.3 c	6.7 ± 0.4 a	29.4 ± 0.6 a
Q 5%	1.7 ± 0.4 a	1.7 ± 0.1 a	13.2 ± 1.6 a	8.0 ± 0.2 b	36.6 ± 0.3 c
Cat. 0.5%	1.9 ± 0.2 a	1.9 ± 0.2 a	16.5 ± 0.9 bc	7.8 ± 0.1 b	40.2 ± 0.2 d
Cat. 2%	1.8 ± 0.2 a	5.3 ± 0.6 b	17.1 ± 3.0 bc	9.3 ± 0.4 c	36.8 ± 0.4 c

[a] Different letters (a−d) indicate significant differences among the values of permeability at the same RH.

Table 3. Color Parameters of EVOH-Based Materials[a]

	L^*	a^*	b^*	C^*	H (deg)	ΔE
blank	91.1 ± 1.4 a	−0.08 ± 0.02 c	−0.05 ± 0.02 a	0.10 ± 0.03 a	29.3 ± 5.9 b	
Q 1%	92.7 ± 1.3 a	−5.3 ± 0.1 b	11.1 ± 0.2 d	12.3 ± 0.2 d	−64.6 ± 0.3 a	12.4 ± 0.2
Q 5%	92.9 ± 0.8 a	−11.4 ± 0.4 a	28.1 ± 1.33 e	30.3 ± 1.4 e	−67.8 ± 0.3 a	18.2 ± 1.3
Cat. 0.5%	92.2 ± 0.7 a	0.24 ± 0.1 d	2.5 ± 0.2 b	2.5 ± 0.2 b	84.4 ± 0.7 c	10.2 ± 0.1
Cat. 2%	91.9 ± 0.5 a	0.62 ± 0.1 e	4.6 ± 0.6 c	4.7 ± 0.6 c	82.4 ± 0.2 c	12.3 ± 0.4

[a] Different letters (a−d) indicate significant differences among the values of the same color property.

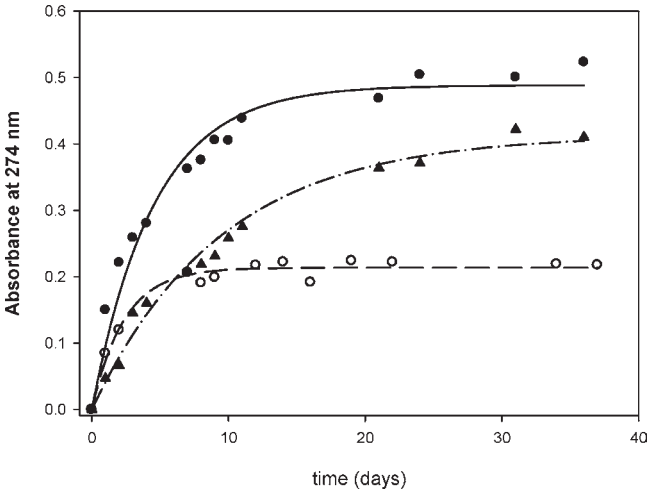

Figure 3. Examples of the release of catechin from EVOH-based materials, showing the effect of concentration and simulant (water and 10% ethanol): (●) Cat. 2% into ethanol 10%; (○) Cat. 0.5% into ethanol 10%; (▲) Cat. 2% into water.

containing these flavonoids provide a medium permeability to water at low relative humidities and a high permeability in humid environments. With respect to oxygen, the materials maintain their status as very high barrier polymers when dry and as high-barrier polymers when humid.

Optical Properties. The color parameters of the extruded EVOH films containing catechin (at 0.5 and 2% w/w) and quercetin (at 1 and 5%) were analyzed, and the results are given in **Table 3**. All of the film samples were highly homogeneous and transparent, as the luminosity values (L) show. The catechin-containing films presented a light brown color, reflected by the rise in the a^* and b^* values and the hue angle values falling in the second portion of the first quadrant. The quercetin-containing EVOH materials developed a yellow color as indicated by the hue angle. These samples presented negative a^* values (green) and large positive b^* values (yellow). As shown in **Table 3**, the chroma and ΔE values increased with the concentration of each antioxidant in the film.

Antioxidant Release. The release of antioxidants from the films into different food simulants was monitored during storage at room temperature. Antioxidant release presented a similar profile with all of the simulants and antioxidants. **Figure 3** shows representative evolutions of catechin release at the two concentrations and using ethanol 10% and water as simulants. The accumulation of antioxidant followed an "exponential growth to a maximum" type of profile, although the extent and kinetics varied markedly between samples.

The first important factor for the release of compounds from the polymer matrix into the simulant was the initial concentration of the antioxidant in the film. In all of the tests, and as expected, the higher the initial antioxidant concentration the higher the amount of antioxidant released.

A second important factor was the food simulant. As can be seen in **Figure 3**, the extent and kinetics of catechin release are higher in the presence of alcohol. The extent of release at equilibrium (after a lengthy exposure time) depends on the compatibility between the migrant and the simulant. The extent of release can be characterized by the partition coefficient (K), defined as the ratio of the concentration of a compound in the polymeric phase to that in the food simulant. The K values are shown in **Figure 4**. Both antioxidants are highly soluble in ethanol and, therefore, the release of these agents into 95% ethanol approached full extraction. The K values for both antioxidants were < 100, without significant differences between samples. In contrast, their solubility in water is limited, especially for quercetin, which had a K value well above 10000. This low compatibility reduces the extent of release considerably. The presence of 10% of alcohol slightly increases the release of the flavonoids. Release tests were also performed with isooctane, although the amount of antioxidant in this simulant, if any, lay below the sensitivity threshold of the technique. The chemical incompatibility between the antioxidants and the isooctane ($K \rightarrow \infty$) and the lack of plasticization of the polymer (very slow diffusion) are responsible for practically preventing their release.

Figure 3 likewise shows that the type of simulant to which the film is exposed can also alter the kinetics of the process. The D coefficient as defined in Fick's laws characterizes the kinetics of transport in polymeric matrices. From the evolution of release during exposure, the D values were calculated according to the method of López-Carballo (*22*). **Figure 3** includes the plots of the theoretical values. It will be seen that they describe the experimental data well, indicating that antioxidant release follows Fick's laws. **Figure 4** compares the diffusion coefficient values obtained for the two agents and the different food simulants.

It is well-known that the presence of high relative humidities results in the plasticization of the polymer, which in turn results in a faster diffusion process. Therefore, one could expect the mass transport to depend on the water gain by the polymer and the release curve not to be described by a model that considers a constant diffusion coefficient. Nevertheless, sorption by the polymer of substances of small molecular size such as water is so fast that the mass transport of the flavonoids can be considered to start once the polymer matrix has been plasticized by water. This consideration has been used successfully before, when describing the effect of humidity on the mass transport of α-pinene in an ethylene−vinyl alcohol copolymer with 32 molar percentage of ethylene, EVOH32 (*22*). In that work, the pinene permeability through EVOH32 increased by a factor of 10000 because of humidity. The effect of humidity on D was also severe. In absolute values, however, the measured diffusivity of pinene in EVOH32 exposed to a humid environment was lower than the diffusion of flavonoids in EVOH44 measured in the present study. This difference is probably caused by the difference in ethylene content: there is a 100 factor difference in oxygen permeability between these two copolymers (*34*). Also, immersion in water could be expected to induce far greater plasticization of the polymer than that caused by humid air.

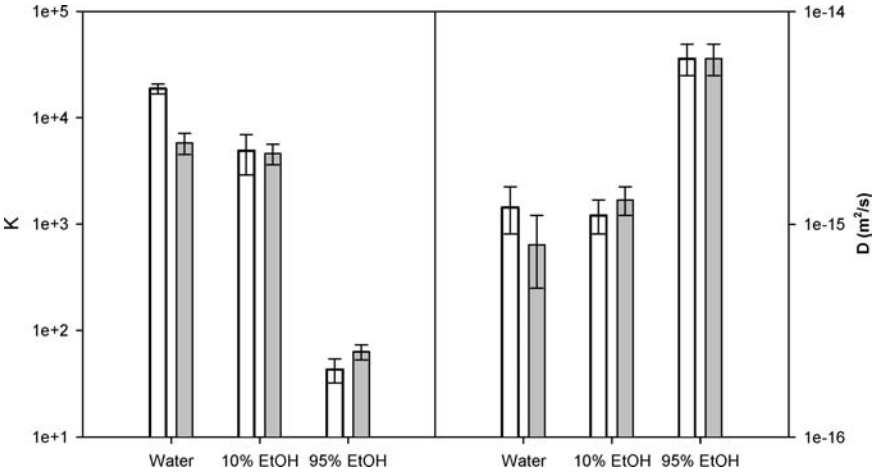

Figure 4. Partition (*K*) and diffusion (*D*) coefficient values for the release of catechin (gray bars) and quercetin (white bars) from EVOH-based materials into the food simulants tested.

Similar results were observed for diffusion in films exposed to a 10% ethanol aqueous solution. However, immersion of the sample into 95% ethanol produced a significant increase in the release rate, as the *D* values reached the 7×10^{-15} m^2/s range. The plasticizing effect of low molecular weight alcohols on EVOH materials is considered to be responsible for this effect (*22*). No differences were observed between the *D* values for the two agents.

The migration of the compounds in isooctane was so low and/or slow that the antioxidant activity, if any, was below the experimental error level. In the films exposed to isooctane, both the low solubility of the antioxidants and the low interaction with the polymer resulted in near-zero release.

To prove that the active films protect the food from oxidation by radicals, the method based on the reduction of DPPH, a stable free radical, was selected to evaluate their antioxidant activity in the food simulants, because the free radical scavenging activity of the phenolic antioxidants incorporated into the films is considered to be due to their hydrogen-donating ability.

The antioxidant activity was observed to be proportional to the antioxidant concentration in the different simulants and showed the same kinetic profile as antioxidant release. Besides the effects of concentration, as already commented, the effect of the food type was clearly noticeable. Films were very active in alcohol-containing simulants and showed less activity in aqueous products. However, their activity in contact with fatty food products is uncertain because the values observed in ethanol 95% and isooctane were clearly divergent. Similar disagreement has been observed in other migration studies (*35*).

Table 4 compares the maximum antioxidant activity (long-term storage) of the food simulants exposed to the antioxidant films. These results refer to the antioxidant activity that a standard 1 L package made with these films will provide for the packaged product. In water and ethanol 10% simulants, films with low and high concentration of catechin provided better results compared to quercetin ones. The reason for this effect is the above-mentioned higher solubility of catechin in these liquid media. In ethanol 95%, the antioxidant protection was proportional to the antioxidant concentration in the films because both quercetin- and catechin-containing films released most of the flavonoid incorporated. Therefore, catechin-containing films presented better characteristics for the production of an all-purpose active package.

These results are indicative of active films having been successfully obtained by adding natural antioxidants to hydrophilic EVOH copolymers through an extrusion process. At the flavonoid concentrations tested, the resulting materials maintained the typical properties of EVOH materials. The films released the active agent as a function of antioxidant concentration and the type of food simulant to which the film was exposed, which thereby acquired antioxidant capacity. The films proved to be active for aqueous and alcoholic food products. However, the activity of the films exposed to fatty foods was ambiguous, because the tests carried out with the two fatty simulants presented opposite outcomes: very high activity in ethanol 95% and nil for isooctane. Further studies are ongoing to measure the activity of these materials with different real oxygen-sensitive products.

Table 4. Maximum Antioxidant Activity, Expressed as Ascorbic Acid Concentration, in All Food Simulants in Contact with EVOH Films Containing Quercetin and Catechin[a]

	antioxidant activity (mg/L ascorbic acid)		
	water	EtOH 10%	EtOH 95%
Q 1%	0.07 ± 0.03 a,x	1.69 ± 0.72 a,y	29.5 ± 3.26 a,z
Q 5%	1.03 ± 0.79 ab,x	4.43 ± 1.56 ab,y	124.71 ± 8.79 b,z
Cat. 0.5%	2.95 ± 1.36 bc,x	4.03 ± 1.67 ab,x	16.48 ± 3.21 c,y
Cat. 2%	4.54 ± 1.8 c,x	6.59 ± 2.57 b,x	84.05 ± 4.68 d,y

[a] Different letters (a−d) indicate significant differences among the diverse films in each simulant. Different letters (x−z) indicate significant differences among the simulants, in each film.

ACKNOWLEDGMENT

Mary Georgina Hardinge provided assistance with the English language text.

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Received for review June 10, 2010. Revised manuscript received August 24, 2010. Accepted September 16, 2010. We acknowledge the financial support of the Spanish Ministry of Science and Innovation, Projects AGL2006-02176, AGL2009-08776, and Fun-C-Food CSD2007-00063, and the C.L.-d.-D. fellowship (FPU program).