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Development of New Antioxidant Active Packaging Films Based on Ethylene Vinyl Alcohol Copolymer (EVOH) and Green Tea Extract

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ABSTRACT: Ethylene vinyl alcohol copolymer (EVOH) films containing green tea extract were successfully produced by extrusion. The films were brown and translucent, and the addition of the extract increased the water and oxygen barrier at low relative humidity but increased the water sensitivity, the glass transition temperature, and the crystallinity of the films and improved their thermal resistance. An analysis by HPLC revealed that the antioxidant components of the extract suffered partial degradation during extrusion, reducing the content of catechin gallates and increasing the concentration of free gallic acid. Exposure of the films to various food simulants showed that the liquid simulants increased their capacity to reduce $DPPH^{\bullet}$ and $ABTS^{\bullet+}$ radicals. The release of green tea extract components into the simulant monitored by HPLC showed that all compounds present in the green tea extract were partially released, although the extent and kinetics of release were dependent on the type of food. In aqueous food simulants, gallic acid was the main antioxidant component released with partition coefficient values ca. 200. In 95% ethanol (fatty food simulant) the *K* value for gallic acid decreased to 8 and there was a substantial contribution of catechins (*K* in the 1000 range) to a greatly increased antioxidant efficiency. Kinetically, gallic acid was released more quickly than catechins, owing to its faster diffusivity in the polymer matrix as a consequence of its smaller molecular size, although the most relevant effect is the plasticization of the matrix by alcohol, increasing the diffusion coefficient >10-fold. Therefore, the materials here developed with the combination of antioxidant substances that constitute the green tea extract could be used in the design of antioxidant active packaging for all type of foods, from aqueous to fatty products, the compounds responsible for the protection being those with the higher compatibility with the packaged product.

KEYWORDS: active packaging, antioxidant, release, EVOH, green tea extract

INTRODUCTION

To reduce oxidation in sensitive food products, the addition of antioxidants and the design of a suitable vacuum or modified atmosphere packaging technology are the two most common alternatives. A novel alternative that is being developed is the design of active antioxidant packaging systems.[1] Various antioxidant agents have been added to polymeric films with the purpose of being delivered to oxygen-sensitive food, improving its chemical stability. Substances such as carvacrol, aromatic plant extract (oregano, barley), or α-tocopherol have been incorporated into polymers for the preparation of active packages.[1−8] Although all of these developments presented significant antioxidant activity, they have some drawbacks, derived from a collateral effect on sensory properties of food owing to the release of highly aromatic substances or the absence of a triggering mechanism. Flavonoids, catechin, and quercetin have also been incorporated into polymers to reduce polymer degradation[9] and/or food oxidation.[10] These substances are well-known antioxidant agents and do not add relevant modification of food flavor; however, although naturally present in many food products, they are not food additives, and therefore these agents should receive the status of food additive before commercialization of the active package.

Green tea extract is a great source of flavonoids with the status of food additive. These compounds have aroused considerable interest recently because of their potential beneficial effects on human health. They have been reported to have antiviral, antiallergic, antiplatelet, anti-inflammatory, antitumor, and antioxidant activities.[11−14] The main compounds responsible for this antioxidant activity are gallic acid and eight major catechins: (+)-catechin (C), (−)-epicatechin (EC), (−)-catechin gallate (CG), (−)-epicatechin gallate (ECG), (−)-gallocatechin (GC), (−)-epigallocatechin (EGC), (−)-gallocatechin gallate (GCG), and (−)-epigallocatechin gallate (EGCG).[15−19]

Tea catechins can act as antioxidants by donation of hydrogen atoms, as acceptors of free radicals, as interrupters of chain oxidation reactions, or by chelating metals.[20] It has also been shown that green tea extract has greater antioxidant activity than a reproduced mixture of catechins, a well-known synergistic effect.[21,22] Possible interactions must be considered and not only the content of phenolic components.

Green tea extract (GTE) has been already incorporated in food to extend its shelf life, resulting in food protection and no visible color or perceivable odor change in treated samples when the extract was incorporated at optimum concentrations.[23−25]

Green tea extract has also been investigated as a potential source of antioxidants to be used as additives in plastic to protect them during polymer processing/manufacturing. The performance of

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polypropylene samples stabilized with GTE at 0.05% (w/w) was evaluated by Dopico-Garcia et al.[9] Results showed GTE can be an interesting source of antioxidants for plastics because the stability provided by the addition of GTE was comparable to the stability provided by synthetic additives.[9]

The purpose of this work was to develop an antioxidant active packaging material to improve food protection by the incorporation of GTE in a hydrophilic plastic layer, ethylene vinyl alcohol copolymer (EVOH), by flat extrusion. The material developed was characterized by the analysis of (i) its optical, thermal, and barrier properties, (ii) its antioxidant efficiency, and (iii) the release kinetics of the antioxidant agents in several food simulants.

MATERIALS AND METHODS

Chemicals and Reagents. An ethylene vinyl alcohol copolymer with a 44% ethylene molar content (EVOH) was kindly supplied by The Nippon Synthetic Chemical Co. (Osaka, Japan). Green tea extract was supplied by Plantextrakt (The Nature Network), Baceiredo S.L. (Vitoria, Spain). Reagent grade absolute ethanol, methanol, formic acid, 2,2-diphenyl-1-picrylhydrazyl 95% free radical, 2,2′-azinobis(3-ethylbenzothiazoline-6-sulfonic acid), and acetic acid were purchased from Sigma (Madrid, Spain). Gallic acid, caffeine, catechin hydrate, and epigallocatechin gallate (EGCG) were purchased from Fluka Biochemika (Barcelona, Spain). Water was obtained from a Milli-Q Plus purification system (Millipore, Molsheim, France).

Film Preparation. EVOH films containing GTE were obtained by flat extrusion. The extract was incorporated at 5% (w/w) concentration through a side port for solids, and the antioxidant−EVOH mixture was extruded on a Brabender DSE 20/40 corotating twin-screw extruder (Plastograph, Düsseldorf, Germany) at 200 °C at a screw speed of 100 rpm. The resulting films presented an average thickness of 35 ± 3 μm, although the thickness of every sample was individually measured before tests using a digital Mitutoyo micrometer (Metrotec, San Sebastian, Spain). The film samples obtained were vacuum packaged in aluminum/LDPE bags and stored at room temperature (23 ± 2 °C) until the moment of analysis.

Antioxidant Activity of GTE and Film of EVOH with GTE Incorporated. Antioxidant activities of GTE and films containing GTE were measured by two different antioxidant assays, the DPPH$^{\bullet}$ method and the ABTS$^{\bullet+}$ assay, that measure antioxidant effectiveness by monitoring the inhibition of oxidation of a suitable substrate. The DPPH$^{\bullet}$ method is based on the bleaching rate of a stable free radical, 2,2-diphenyl-1-picrylhydrazyl (DPPH$^{\bullet}$), monitored at a characteristic wavelength in the presence of the sample. In its radical form DPPH$^{\bullet}$ absorbs at 517 nm, but upon reduction by an antioxidant or a radical compound its absorption decreases.[26] The ABTS$^{\bullet+}$ assay is based on the inhibition by antioxidants of the radical cation 2,2′-azinobis(3-ethylbenzothiazoline-6-sulfonate), ABTS$^{\bullet+}$, which has a characteristic long-wavelength absorption spectrum showing a main absorption maxima at 415 nm.[27] These two indicator radicals are neutralized either by direct reduction via electron transfers or by radical quenching via H atom transfer.[28] In both assays, the percentage inhibition values were calculated using this equation:

$$I\,(\%) = (A_{control} - A_{sample})/A_{control} \times 100$$

To standardize the results, scavenging activities of the DPPH$^{\bullet}$ and ABTS$^{\bullet+}$ radicals were expressed as ascorbic acid concentration, using a calibrated curve of ascorbic acid concentration versus I (%).

The antioxidant activities of the extract and the film were determined by these methods to study the degree of immobilization of the antioxidant in the film and its final active concentration in the film after undergoing the process of flat extrusion. Film extraction was performed by two consecutive extractions in ethanol at 65 °C for 3 h. The antioxidant activity was determined by the DPPH$^{\bullet}$ method. Similarly, extraction was done under water, and the antioxidant activity was measured by the ABTS$^{\bullet+}$ assay. In both cases, no antioxidant activity was observed in the second extraction, indicating that the agent was fully extracted in the first process.

Chromatographic Study of GTE and Film of EVOH with GTE Incorporated. Green tea extract and the ethanolic extraction of the film (described in the previous section) were analyzed by UPLC-q-TOF to identify their major compounds using an Acquity BEH C18 (100 mm × 2.1) column with 1.7 μm particle size. The mobile phase consisted of (A) Milli-Q water and (B) methanol with 0.1% formic acid. Solvent flow was 0.3 mL/min with a linear gradient from 5 to 95% of B. The detection system was a q-TOF equipped with an electrospray source in positive mode, with the mass range m/z between 50 and 5000, a capillary voltage of 1 kV, and source and desolvation temperatures of 100 and 400 °C, respectively. The cone and desolvation flows were 20 and 600 L/h of nitrogen, respectively.

Quantitative analysis was performed on a 1200 series HPLC equipped with an SPD-M10Avp diode array detector, a DGU-14A degasser, an SCL-10Avp system controller, and a Class-VP v.6.14 chromatography data system (Agilent Technologies España, Las Rozas, Spain). A stainless steel column, Gemini C6-Phenyl 110A, 4.6 × 150 mm, 5 μm particle size, protected by an RP_18 precolumn, both from Phenomenex (Torrance, CA), was used. The flow rate was 1 mL/min, and the injection volume was 20 μL. The mobile phases were 0.3% formic acid (solvent A) and methanol−0.3% formic acid (solvent B). The gradient started with 12% solvent B, changing linearly to 40% solvent B over a period of 25 min, then changing linearly to 60% until min 60 was reached, and then changing to 100% over a period of 1 min. This was maintained for 5 min, followed by a change to 12% solvent B for 10 min. Peaks were detected by measurement of absorbance at 280 nm. A tentative identification was performed by comparison of the chromatograms with those reported in the literature and the results of the UPLC-q-TOF chromatography. Catechin, caffeine, gallic acid, and EGCG were identified by injection of standards.

Optical Properties. The film color was determined with a Konica Minolta CM-35000d spectrophotometer set to D65 illuminant/10° observer. The film specimen was 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 35 μm to reduce the effect of thickness on color measurements.

Thermal Analysis. Thermogravimetric analyses were carried out using a Mettler Toledo TGA/SDTA/851 thermal analyzer (Columbus, OH). The samples (ca. 10 mg) were heated in 100 μL ceramic pans from room temperature to 900 °C under a nitrogen atmosphere at 10 °C/min to determine any evaporation of volatile compounds, as well as the degradation temperatures of the antioxidant-containing materials.

The thermal properties of the samples were also determined with a model Q2000 DSC from TA Instruments (New Castle, DE). Previously dried 7 mg samples were inserted in 40 μL hermetic Tzero aluminum pans. Thermograms were obtained from 25 to 220 °C with 10 °C/min heating, cooling to 25 °C, and a second heating process to 220 °C. The glass transition (T_g) and melting (T_m) temperatures and the enthalpy (ΔH_m) were calculated from the first heating process. 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 90% relative humidity (RH) and 23 °C using permeability cups (Elcometer, Manchester, U.K.) in accordance

with ISO 2528. 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, leaving a permeable surface of 10 cm^2. To ensure the necessary relative humidity, the cups were then stored in desiccators containing salt solutions: magnesium nitrate, $Mg(NO_3)_2$; 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 film area 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 an OXTRAN model 2/21 ML Mocon (Lippke, Neuwied, Germany). After the samples had been conditioned in the OXTRAN cells for 6 h, the transmission values were determined every 45 min until constant. After the permeation tests were completed, continuous permeation experiments were carried out on each sample to determine the diffusion coefficient (D). From the transmission rate values measured during the transient state, the value of D was assessed from the solution to Fick's second law for the boundary conditions of an isostatic permeation experiment.[29]

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 various food simulants specified in European law: water was used as an aqueous food simulant, 3% acetic acid as an acidic food simulant, 10% ethanol as an alcoholic food simulant, and 95% ethanol as fatty food simulant. Migration studies were conducted at 40 ± 1 °C, in accordance with EU regulations.[30] Double-sided, total immersion migration tests were performed as follows: 80 cm^2 of each plastic sample and 100 mL of the simulant (area-to-volume ratio around 6 dm^2/L) were placed in tubes (12.5 cm high × 4.3 cm diameter) covered with aluminum foil to protect the contents from light. Simulants were deoxygenated by bubbling nitrogen, and a final nitrogen flush was done before the cells were closed to reduce the oxygen percentage in the cell headspace. Periodically, three vials were opened, and the antioxidant activity provided by the films was evaluated by measuring the radical scavenging ability of the food simulants, using the $ABTS^{\bullet+}$ assay in the case of aqueous simulants and the $DPPH^{\bullet}$ method in the case of the 95% ethanol simulant. Both assays are described under Antioxidant Activity of GTE and Film of EVOH with GTE Incorporated.

At the same time, the release of the various green tea components was analyzed by HPLC. Simulants were previously concentrated by evaporation using a water bath at 60 °C and flushing with nitrogen gas. The resulting dry residues were redissolved on mobile phase solvents, filtered, and analyzed by HPLC. The conditions are described under Chromatographic Study of GTE and Film of EVOH with GTE Incorporated.

Statistical Analysis. One- and two-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 test for a confidence interval of 95%. Data are presented as the mean ± standard deviation.

RESULTS AND DISCUSSION

Antioxidant Activity and Composition. First, the antioxidant activity (AA) of green tea extract was determined by the $DPPH^{\bullet}$ and $ABTS^{\bullet+}$ methods. One g of GTE dissolved in ethanol presented an AA versus the $DPPH^{\bullet}$ radical equivalent to 7.43 g of ascorbic acid. The AA versus $ABTS^{\bullet+}$ of 1 g of the extract dissolved in water was equivalent to 3.80 g of ascorbic acid. These results confirm the high antioxidant activity of the extract, especially in ethanol. Differences in the measurement of the AA with these two radicals have often been reported.[28,31,32] Besides the use of different solvent media in the two methods, reactivity patterns and mechanisms are difficult to interpret without detailed information about the composition and structures of the antioxidants tested. Both assays are usually classified as single electron transfer (SET) reactions, but these two indicator radicals may in fact be neutralized either by direct reduction via electron transfers or by radical quenching via H atom transfer.[28,31,32]

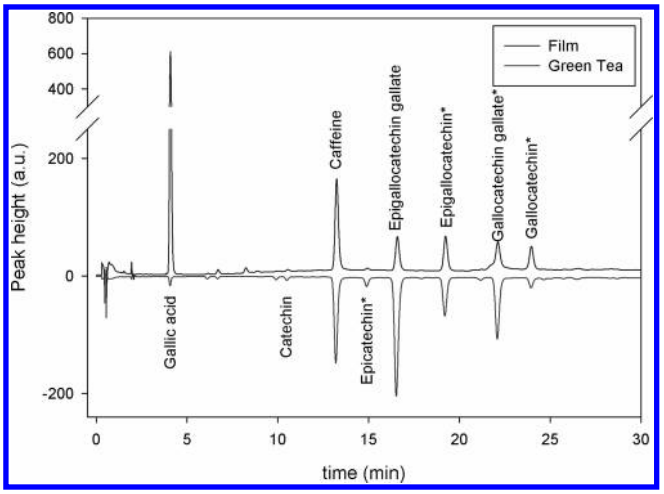

Figure 1. Chromatogram of green tea extract (GTE) and the resulting ethanolic extraction from EVOH_GTE5% (film). *, tentative identification.

To determine the real concentration of GTE in the polymeric film, extractions with water and ethanol were conducted at 65 °C. To check for any degradation effect of the extraction process, an aqueous solution of GTE underwent the extraction process. The extract suffered a large degradation, with the AA decreasing to 20.4% of the initial value. When the film was submitted to the extraction process, the aqueous extract presented an equivalent activity of 4.33×10^{-3} g of ascorbic acid per gram of film versus the $ABTS^{\bullet+}$ radical (nominal value was 0.0795). According to this result, only 5.5% of the nominal extract maintained AA after processing and extraction.

The same procedure was followed with ethanol as solvent. An ethanolic solution of the extract was maintained for 3 h at 65 °C and then analyzed by the $DPPH^{\bullet}$ method. The GTE maintained 88.3% of the nominal activity. Next, the ethanol extraction of the film presented an equivalent activity of 0.264 g of ascorbic acid per gram of film versus the $DPPH^{\bullet}$ radical (nominal value was 0.371 g), 71% of the nominal activity.

According to these results, the extrusion process resulted in a degradation of the extract of about 20%, as the evaluation of the ethanolic extract suggests, possibly due to the potential polymerization of phenols and the degradation of some catechins. The AA of green tea is greatly deteriorated when heated in water, this extraction procedure being unsuitable.

Green tea extract and the ethanolic extraction of the film were also studied by HPLC to analyze their major compounds. UPLC-qTOF chromatography allowed the identification in the tea extract of gallic acid (GA), caffeine (CF), catechin (C), gallocatechin (GC), and gallocatechin gallate (GCG) through the molecular ions, although isomers could not be distinguished. Figure 1 shows the chromatograms obtained by HPLC-UV for both the GTE and the film. The original GTE consisted of several compounds, eight of them identified as GA, epigallocatechin (EGC), C, CF,

Table 1. Color Parameters of EVOH-Based Materials: Control Film (Blank) and Film Containing 5% of Green Tea Extract (EVOH_GTE5%)[a]

	L^*	a^*	b^*	C^*	H^*
blank	91.1 ± 1.4 b	−0.08 ± 0.02 a	−0.05 ± 0.02 a	0.10 ± 0.03 a	29.3 ± 5.9 a
EVOH_GTE5%	81.1 ± 1.3 a	6.30 ± 0.30 b	12.80 ± 0.60 b	14.20 ± 0.70 b	63.9 ± 0.3 b

[a] "a" and "b" indicate significant differences among the values of the same color property.

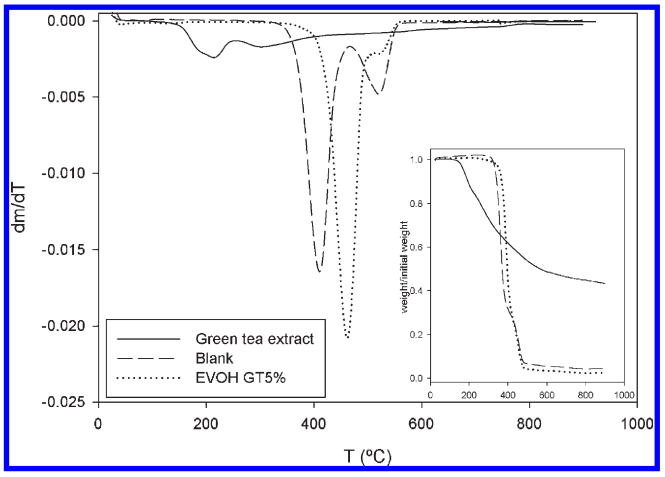

Figure 2. Weight loss and derivative of the weight loss of green tea extract and EVOH materials.

epigallocatechin gallate (EGCG), GCG, epicatechin gallate (ECG), and catechin gallate (CG) by comparison with other papers in the literature.[17−19,33−35] The resulting ethanolic extractions of the film were also analyzed by HPLC with UV detection. Chromatograms revealed that the extract incorporated was affected by thermal and mechanical stresses during the extrusion process, implying changes in the concentration of some compounds. Several tea catechin concentrations decreased considerably, especially the four catechin gallates. Apparently, the ester bond of the gallate breaks during film manufacture, resulting in a severe increase of gallic acid concentration in the film.

Optical Properties. The films containing the extract were transparent and brown and clearly different from the blank samples. Color parameters of the extruded EVOH films were analyzed, and the results are given in Table 1. The incorporation of the GTE slightly reduced the luminosity of the film, as the L values show. The brownish color of the films developed is reflected by the rise in the a^* and b^* values and by falling hue angle values in the second portion of the first quadrant. The incorporation of dehydrate catechin in EVOH by extrusion did not produce such relevant color change.[10,36] This film color change may be a consequence of sugar caramelization during the extrusion process, because the extract contains sugars, free or as glycosylated catechins, that can undergo caramelization reactions. Partial polymerization of phenols during heating may also be involved in the development of the brown color.

Thermal Characterization. Thermal analyses of the films were carried out to study the influence of the addition of the GTE on the thermal stability and morphology of the polymer. As Figure 2 shows, in the thermogram of the GTE there is a broad degradation band that starts at 150 °C, with a maximum at approximately 200 °C. The catechins from the extract are partially glycosylated, and a "caramelization" of these sugars takes place, probably due to heating. A partial degradation of the catechins, leading to the formation of gallic acid, and the polymerization of phenols might be related to this transition, too. The degradation continues over a wide temperature range, with a final ash content of ca. 50% of the initial weight.

Table 2. Thermal Properties Obtained from DSC Thermograms during the First Heating for the EVOH-Based Materials: Control Film (Blank) and Film Containing 5% of Green Tea Extract (EVOH_GTE5%)[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 b	−74.8 ± 1.8 a	−74.8 ± 1.8 a
EVOH_GTE5%	49.4 ± 1.7 b	163.4 ± 2.6 a	−84.3 ± 5.4 b	−88.5 ± 5.4 b

[a] "a" and "b" indicate significant differences among the values of the same thermal property.

TGA of the two polymeric samples showed that the degradation occurred in a main step at a temperature above 400 °C. The EVOH blank sample presented its maximum loss rate at 414 °C. The film containing the GTE had improved thermal stability, the center of the transition being at 463 °C maximum. The presence of the antioxidant mixture in the matrix is probably responsible for this stability, and this is in agreement with the results observed in EVOH materials containing catechin or quercetin.[10] The addition of antioxidants (mainly synthetic ones) to prevent thermal degradation of polymers is a common procedure.

Materials were also analyzed by DSC, to study the effect of the extract addition on the polymer morphology. Table 2 summarizes the main information obtained from the DSC analysis. As can be seen, the glass transition temperature increased significantly in the EVOH_GTE sample, indicating increased rigidity with respect to the blank sample. This effect can be caused by interactions of the OH substituents of the polymers with the agents, resulting in increased interchain forces and cohesive energy. Also, the presence of GTE modified the crystallinity of the composite material. The thermogram showed a decrease in the melting point (minimum of the endotherm) and an increase in the melting enthalpy. These two observations can be interpreted as if the additive produces a nucleating effect on the polymer, increasing the crystalline percentage of the polymer, although the crystal structure is more deficient. Similar properties were observed in EVOH films containing catechin and quercetin, although the effects of these two flavonoids on the thermal behavior of EVOH were not as significant.[10]

Barrier Properties. *Water Vapor Permeability.* As Table 3 shows, WVP was measured at 50, 75, and 100% RH gradients at 23 °C. Both samples presented the same profile: permeability increased significantly with the RH gradient (statistics not shown), showing the plasticizing effect of water on the polymer matrix at high humidities. In dry conditions, EVOH is well-known for its good barrier properties, owing to strong interchain interactions among EVOH hydroxyl groups that result in high cohesive energy and low chain flexibility. However, as the humidity increases, water

Table 3. Water Vapor and Oxygen Permeability Values for the EVOH-Based Materials: Control Film (Blank) and Film Containing 5% of Green Tea Extract (EVOH_GTE5%)[a]

	water vapor permeability 10^{-16} (kg·m/[m^2·s·Pa])			oxygen permeability 10^{-21} (m^3·m/[m^2·s·Pa])	
sample	RH 50	RH 75	RH 100	RH50	RH90
blank	2.0 ± 0.1 b	8.9 ± 0.6 b	13.9 ± 1.0 a	7.2 ± 0.2 b	33.6 ± 0.9 a
EVOH_GTE5%	1.2 ± 0.2 a	2.5 ± 0.1 a	17.3 ± 1.0 b	3.5 ± 0.3 a	43.8 ± 0.5 b

[a] "a" and "b" indicate significant differences among the values of permeability at the same RH.

molecules are sorbed and interact with the polymer chains, reducing intermolecular interactions and increasing flexibility, and as a consequence the molecular diffusion is accelerated. This effect is in agreement with several studies in which water permeability increased considerably in very humid conditions.[37,38]

The addition of the GTE to EVOH improved barrier properties at low and medium relative humidities, decreasing up to 50% with respect to the blank sample, in agreement with thermal analysis that showed a more crystalline morphology for antioxidant-incorporated samples. Nevertheless, the films appeared to be more water sensitive than the pure polymer because at 100% RH the water permeability values of the material developed with the active agent were higher than those of the blank sample, probably owing to the higher water affinity of the extract components. This effect was also shown in a previous work.[10] The high plasticization of the polymer may increase the areas suitable for transport, including amorphous areas within small and defective crystal structures, where transport is impeded at low humidities.

Oxygen Permeability. Oxygen transmission through EVOH is limited by the semicrystalline structure and the high intermolecular and intramolecular cohesive energies. Accordingly, EVOH films have a very low oxygen permeability in the 10^{-21} m^3·m/(m^2·s·Pa) range.[39,40] However, EVOH is hygroscopic and absorbs water at high relative humidity and then loses much of its oxygen barrier performance. Water molecules interact with the hydroxyl groups in the polymer matrix, weakening the existing hydrogen interchain bonds, increasing polymer segment mobility, and facilitating the diffusion of oxygen. As Table 3 shows, the permeability to oxygen presented a profile similar to that of permeability to water vapor. Oxygen permeability values increased significantly with RH for both samples, in agreement with previous papers.[38,39] The incorporation of the extract improved the oxygen barrier at low humidity, an effect that can be attributed to the previously commented increase in polymer crystallinity. At high humidity, the active film presented higher values than the blank polymer as a result of increased water sensitivity, in agreement with the effect observed in the water permeability.

The diffusion coefficient was also calculated by applying the analytical solution to Fick's law for the boundary conditions of an isostatic permeation process.[29] The diffusion coefficient was measured at 90% RH, and the values were 1.125×10^{-13} and 1.63×10^{-13} m^2/s for the EVOH blank and EVOH_GTE5% films, respectively. The values obtained were very similar to the value obtained from the literature for an EVOH copolymer with 44% ethylene content, 1×10^{-13} m^2/s.[38] The higher value obtained for the sample containing the extract revealed a faster diffusivity of oxygen in this film as a consequence of a more plasticized polymer structure.

Green Tea Extract Release. Release studies were carried out by exposure of the films developed to the food simulants included in EU regulations for migration studies.[30] The antioxidant activity of the GTE released on food simulants was evaluated periodically by measuring the radical scavenging ability, using the ABTS$^{\bullet+}$ assay in the case of aqueous simulants and the DPPH$^{\bullet}$ method in the case of 95% ethanol.

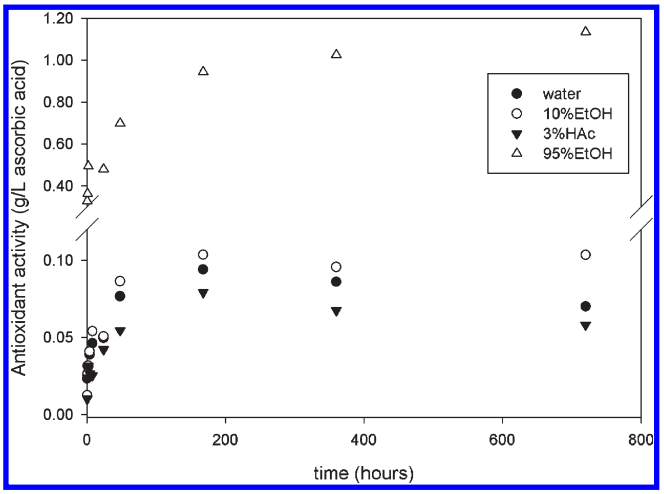

Figure 3. Antioxidant activity of green tea extract found in the food simulants tested during exposure to the EVOH-based materials determined by the DPPH$^{\bullet}$ method (95% ethanol) and by the ABTS$^{\bullet+}$ method for the aqueous simulants.

Figure 3 presents the antioxidant activity of food simulants exposed to GTE-containing films, expressed as equivalent ascorbic acid concentration. The kinetics presented similar profiles with all of the simulants, following an "exponential growth to a maximum" type profile. The effect of the type of food was clearly noticeable, presenting around 10 times higher antioxidant activity on 95% ethanol, probably owing to the better solubility of the active agents in this liquid. Catechins, the most powerful antioxidants present in GTE, are slightly soluble in water and highly soluble in ethanol. The nominal antioxidant activity toward the DPPH$^{\bullet}$ radical that could be provided by the film to the 95% ethanol simulant (considering 5% of GTE, no degradation, and full release) was 1.25 g/L ascorbic acid equivalent. According to the results of the release (Figure 3), the extraction of the green tea components advances toward an almost complete release after 2 weeks of exposure.

For the aqueous simulants, the nominal antioxidant activity against ABTS$^{\bullet+}$ that could be provided by the film to the water, 3% acetic acid, and 10% ethanol simulants (considering 5% of GTE, no degradation, and full release) was 0.267 g/L ascorbic acid equivalent. According to the release results (Figure 3), the extraction of the green tea components advances toward an equilibrium value that is ca. 35% of the nominal value. Besides a partial degradation of the GTE during extrusion, the low compatibility between the catechins and water reduced the extent of

Table 4. Concentration of Four Identified Green Tea Components (A) Present in the EVOH_GTE5% Material Developed and (B) Released into the Different Food Simulants

	gallic acid	caffeine	catechin	EGCG
(A) EVOH_GTE5%, mg/g film	4.84	5.01	2.71	2.48
(B) simulant, mg/g film (% released from film)				
water	2.86 (59%)	4.85 (97%)	<1%	<1%
acetic acid 3%	2.79 (57%)	4.63 (97%)	<1%	<1%
ethanol 10%	3.03 (63%)	4.80 (93%)	<1%	<1%
ethanol 95%	4.73 (98%)	4.86 (96%)	0.81 (10%)	0.32 (6%)

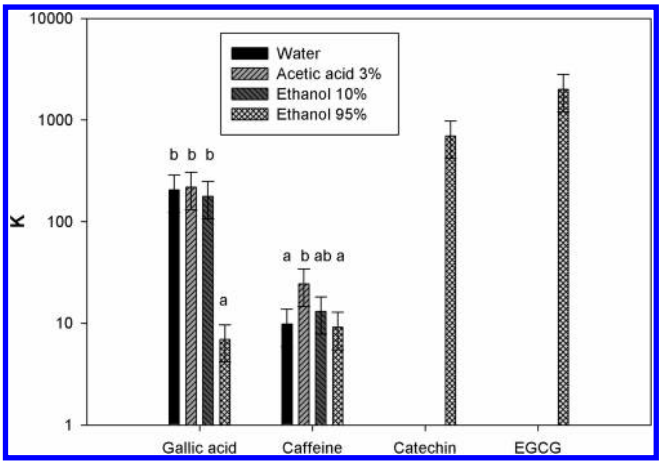

Figure 4. Partition coefficients of gallic acid, caffeine, catechin, and EGCG between EVOH and food simulants. "a" and "b" indicate significant differences among *K* values.

their release and subsequently considerably reduced the antioxidant activity on aqueous food simulants. According to the experimental values (Figure 3), equilibrium is achieved after 10 days for the three simulants. The highest extent was obtained for 10% ethanol, in the 0.10 g/L range. Neutral and acidic aqueous simulants presented a maximum extent at 0.09 and 0.07 g/mL, respectively, reaching this value after 200 h. A reduction in this value was observed at longer exposure times, probably due to partial degradation of the active compounds in these solvents.

To determine the components of the GTE that were responsible for the antioxidant activity measured, the simulants were sampled and analyzed by HPLC. All compounds identified in the extracts (Figure 1) were observed in the four simulants, although the concentrations of the compounds were dependent on the type of simulant. The two main compounds that were released in all simulants were gallic acid and caffeine. Also, the five catechins were released from the polymeric films, although this release is more evident in 95% ethanol owing to the higher solubility of catechins in this solvent.

The identification of four of the compounds was confirmed by injection of pure standards: gallic acid, caffeine, catechin, and epigallocatechin gallate. The response of the HPLC was calibrated for these four compounds, and their individual release was monitored during storage.

Table 4 summarizes the maximum release reached at equilibrium of each identified compound (expressed as mg of compound/g film) and as a percentage of the nominal content of each compound in the film. The release of gallic acid was very similar for all aqueous simulants, and caffeine was practically all extracted from the material developed. The release of all identified components was higher in the 95% ethanol simulant, especially for catechin and epigallocatechin gallate, the releases of which on aqueous simulants were very small and within the limit of sensitivity of the experimental procedure (quantification was not attempted).

The extent of release at equilibrium (after a long exposure time) depends on the compatibility of the migrant with both the food simulant and the polymeric film. The higher the solubility in the simulant, the higher the release that should be expected. Caffeine with a water solubility of 23 g/L is the most soluble compound in water and presented the largest extent.

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 values of K are presented in Figure 4 and show that caffeine was the compound with the highest release in all simulants, migration being slightly lower in the acidic food simulant. Similar K values were obtained for gallic acid in the three aqueous simulants. The partition value favored the release into 95% ethanol to a larger extent ($p < 0.05$). For the two catechins, the values obtained for the equilibrium constant were higher than for the other two compounds, indicating a limited release. Because both substances are very soluble in ethanol, a larger release close to full extraction could be expected as well as K values approaching 0. Possibly, these compounds are chemisorbed in the EVOH matrix, immobilized, and not available for mass transport process and can be extracted only in strong conditions. No values were calculated for the release of catechins into aqueous simulants because the concentrations present large errors, although they could be expected to be well above 30000.

The kinetics of the release of the various compounds was also monitored. Figure 5 shows the release of gallic acid and caffeine in the four simulants. As can be seen, the release kinetics of the compounds followed the same profile as antioxidant activity. Similar curves were obtained for the other components of the extract. The diffusion coefficient (D) as defined in Fick's laws is commonly used to characterize the kinetics of transport in polymeric matrices. Kinetically, the release process depends on the diffusion of the migrant and the extent of the process. Considering that the release agents are rapidly dispersed in the food simulant, so that their concentration is homogeneous at all times, the release of a given compound from the polymer phase into the liquid phase at time t ($m(t)$) can be expressed as a function of the release at equilibrium (m_{eq}) and the values of the D and K

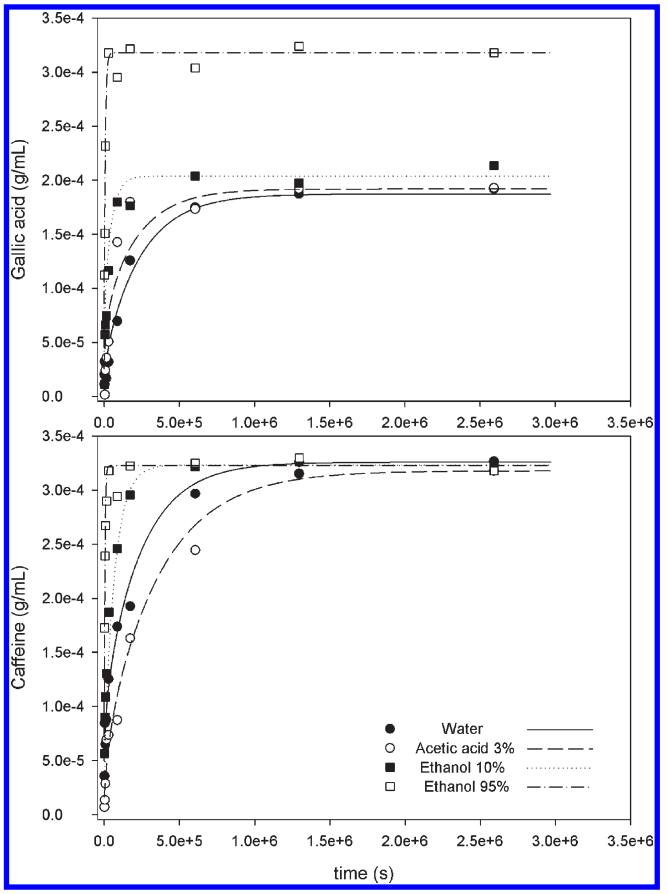

Figure 5. Release of gallic acid and caffeine from EVOH-based material into food simulants: symbols represent experimental data, and lines are values predicted using eq 1 with K and D values included in Figures 4 and 6.

coefficients:[41]

$$\frac{m(t)}{m_s^f} = 1 \sum_{n=1}^{\infty} \frac{2\alpha(1+\alpha)}{1+\alpha+\alpha^2 q_n^2} exp(-4Dq_n^2 t/l^2) \qquad (1)$$

where α represents the ratio between the mass of compound in the liquid and that in the polymer at equilibrium ($\alpha = V_S/(KV_P)$, V_S and V_P are the volumes of simulant and polymer) and q_n are the positive solutions of the equation

$$tg(q_n) = -\alpha q_n$$

Figure 5 shows the curves obtained from eq 1 using the K values included in Figure 4 and the D values that yielded the best fit to the experimental values (shown in Figure 6). As can be seen, there was good agreement between the theoretical values and the experimental release data.

The D values for all compounds and simulants are plotted in Figure 6. As can be seen, gallic acid and caffeine had similar D values in all simulants. With respect to the effect of simulant on the diffusivity of the antioxidants in the polymer matrix, the release from films contacting 95% ethanol is faster than that from films exposed to aqueous simulants. The large interactions reported between low molecular weight alcohols and EVOH could result in a strong plasticization of the matrix, accelerating the agent release.[10,42]

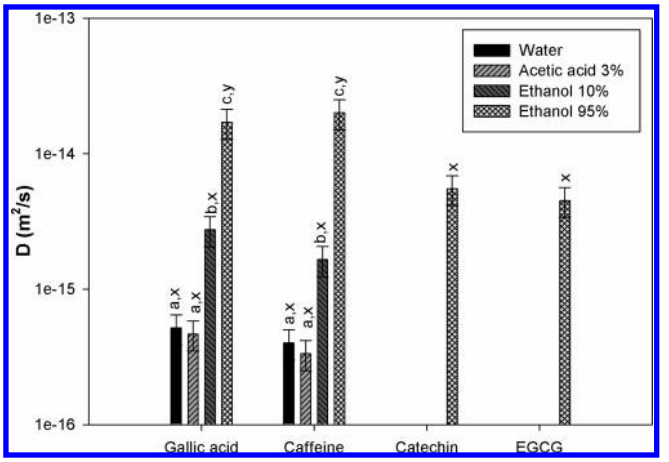

Figure 6. Diffusion coefficients of gallic acid, caffeine, catechin, and EGCG in films exposed to the tested food simulants obtained by fitting the experimental release using eq 1 for gallic acid and caffeine and using eq 2 for catechin and EGCG. "a"−"c" indicate significant differences among D values of a substance between food simulants. "x" and "y" indicate significant differences among D values of diverse substances when exposed to the same simulant.

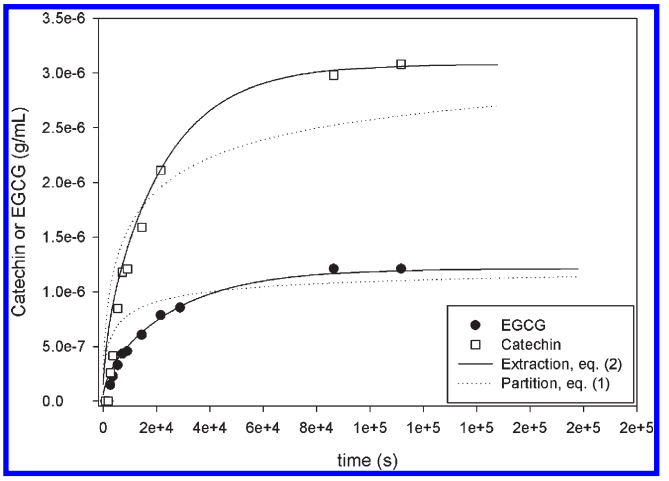

Figure 7. Release of catechin and EGCG from EVOH-based material into ethanol: dots are experimental data, and lines are the theoretical description obtained using eqs 1 and 2.

The release of the two catechins is shown in Figure 7. As can be seen, the curves obtained with eq 1 did not describe the experimental results. To cast some light on the reason for this deviation, the model applicable to the release of compounds in an extraction process was attempted. Equation 2 is the Fick's solution to this case, in which diffusion is the only variable controlling the process:

$$\frac{m(t)}{m_s^f} = \left[1 - \frac{8}{\pi^2} \sum_{n=0}^{\infty} \frac{1}{(2n+1)^2} exp\left(\frac{-D(2n+1)^2 \pi^2 t}{l^2}\right)\right] \qquad (2)$$

This figure also shows the theoretical results obtained using eq 2. It is obvious that the extraction model describes the release of these two compounds better than the partition model. These results indicated that both catechin and EGCG were fully extracted

during the exposure test; therefore, the remains of these two compounds in the polymer were not available for transport. That is, they were immobilized in the polymeric matrix. Only during the extraction process in hot ethanol were they released from the EVOH.

The *D* values that best fit the experimental results for the two catechins have been included in Figure 6. As can be seen, they are much lower than those calculated for gallic acid and caffeine. This difference was expected, because catechin and EGCG have a much larger molecular size than the other two compounds, and a larger size results in more difficult transport and a lower *D* value.

From these results, it can be stated that GTE can be successfully added into EVOH by extrusion, preserving its antioxidant activity. The presence in the extract of antioxidant agents with different molecular structures and polarities results in a general purpose antioxidant solution, releasing antioxidant agents to any type of food from aqueous to fatty products. Therefore, the materials here developed with the cocktail of antioxidant substances that constitute the green tea extract could be used in the design of antioxidant active packaging for all type of foods, from aqueous to fatty products, the compound responsible for the protection being that with the higher compatibility with the packaged product.

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