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Improving the Capacity of Polypropylene To Be Used in Antioxidant Active Films: Incorporation of Plasticizer and Natural Antioxidants

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ABSTRACT: Two types of active antioxidant food packages with improved release properties, based on polypropylene (PP) as one of the most common polymers used in food-packaging applications, were developed. Incorporation of catechin and green tea as antioxidant provided PP with 6 times higher stabilization against thermal oxidation. Release of natural antioxidants (catechins, gallic acid, caffeine, and quercetin) into various food simulants from that nonpolar matrix were improved by blending poly(propylene glycol)-*block*-poly(ethylene glycol)-*block*-poly(propylene glycol) (PPG-PEG-PPG) as plasticizer into the polymer formulation. Increasing release levels between 10- and 40-fold into simulant A and between 6 and 20-fold into simulant D1 resulted from the incorporation of catechin and green tea as antioxidants and PPG-PEG-PPG as plasticizer into the film formulation. The efficiency of the antioxidants in the food simulants after the release process was also corroborated through antioxidant activity tests. Therefore, the developed PPG-PEG-PPG-modified polypropylene resulted in a potential system to be used in active packaging.

KEYWORDS: *active packaging, antioxidant, PP, PPG-PEG-PPG, green tea, flavonoids*

INTRODUCTION

Oxidative processes and microbial spoilage are primary causes for the deterioration of food quality. Traditionally, besides containment, convenience, and communication,[1] packaging also provides protection against possible contamination caused by external agents such as water, light, or odorants. However, increasing safety and quality demands have led to the development of new alternatives in the food-packaging industry. Active packaging with controlled release of active compounds to foodstuffs has emerged as a promising technology.[1−3] Among them, antioxidant active packaging, in which an antioxidant is incorporated into the polymer to be released into the packaged foodstuff, provides a continuous antioxidant effect to prevent lipid oxidation and avoids its rapid depletion compared with its direct addition to food,[1−4] extending the packaging's shelf life.[5,6]

Synthetic antioxidants such as butylated hydroxytoluene (BHT) or butylated hydroxyanisole (BHA)[7−9] traditionally used in packing have been replaced by natural preservaties due to safety concerns.[7,10,11] α-Tocopherol,[5,12−18] carvacrol, and aromatic plant extracts such as oregano or barley, among others,[13,19−21] have been used. Nowadays, polyphenols such as catechins have also aroused high interest as natural antioxidants, being present in several species of the plant kingdom, especially tea.[22−24] Flavonols such as quercetin (Quer) and other compounds such as caffeine (Caff) are, as well, important constituents of tea, also providing it with antioxidant and mood-cognitive-enhancing properties, respectively.[22]

According to their molecular weight and their nonvolatile character, those compounds should be likely to be able to diffuse between the packaging material and the food product and/or partition at the interface when they are used in active food packaging. Recently, some research related to the incorporation of catechins, quercetin, or caffeine as active agents to active polymer packaging has been developed on the basis of polyethylene terephthalate (PET),[25] ethylene vinyl alcohol (EVOH),[26−28] or biodegradable materials such as polylactic acid (PLA).[29−31] Nevertheless, those reported active packagings with hydrophilic and/or biodegradable polymers are intended only for short shelf life products, besides not being as widely used as low-density polyethylene (LDPE) and polypropylene (PP) in food-packaging applications.[16] No important developments of active packaging with those latter polymers have been reported, though, which could be attributed to the few release capacities of catechins or quercetin reported from those polymers despite their highly polar nature. This capacity was then more limited toward the release of lower molecular weight compounds such as caffeine or gallic acid or the release in contact with food simulants of very highly ethanolic content (95%).[25−32]

Some additives such as plastizicers can be used to modify polymer properties, especially workability, flexibility, and extensibility of the polymer. Plasticizers have been blended into polymer matrices to modify polymeric physical characteristics, which lead to enhanced physicochemical polymer properties such as stability, degradability, or permeability. For example, PPG, PEG, or their copolymers have been reported as potential plasticizers into film formulations providing polymers, specially polyesters, with higher biocompatibility and degradation rates, and, thus, modifying their properties.[33,34] Based on

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Table 1. Composition of the Prepared Film Samples and OIT Values for Stabilized and Nonstabilized PP at 200 °C[a]

				natural antioxidants (%)		
sample code	matrix (PP)	commercial antioxidants I168 (%)	plasticizer PPG-PEG-PPG (%)	catechin	green tea	OIT value (min^{-1})
M0-A	X	0.2				4.5 ± 1.03a
M0-B	X	0.2	2			5.82 ± 2.75a;A
M1	X	0.2		2		46.4 ± 5.65b
M2	X	0.2	2	2		65.0 ± 7.43c,e;B
M3	X	0.2	5	2		68.5 ± 1.15c
M4	X	0.2		5		34.8 ± 4.10d
M5	X	0.2	2	5		58.0 ± 3.35e;B,C
M6	X	0.2	5	5		56.4 ± 2.35e
M7	X	0.2			2	45.0 ± 5.19f,g
M8	X	0.2	2		2	38.1 ± 7.10f;C
M9	X	0.2	5		2	50.3 ± 6.30f,g
M10	X	0.2			5	46.0 ± 4.81f,g
M11	X	0.2	2		5	53.4 ± 6.50g;B,C
M12	X	0.2	5		5	55.4 ± 5.90g

[a]OIT data expressed as mean value ± standard error of mean (n = 3). Different lowercase letters (a−g) within a column indicate significant differences between data according to Tukey's test (p = 0.1). Different capital letters (A−C) within groups indicate significant differences between data according to Tukey's test (p = 0.1). Groups: M0, M1−M3 vs M4−M5 vs M7−M8 vs M10−M12.

their role in drug release[35] and following our preliminary study on modified films,[36] those plasticizers may also be an alternative to modify polymer properties and, therefore, mass transport of active agents.

Therefore, the aim of this work was to develop a new antioxidant PP active material to improve food protection. Individual catechin and green tea extract, as well as poly(propylene glycol)-*block*-poly(ethylene glycol)-*block*-poly(propylene glycol) (PPG-PEG-PPG) were incorporated by extrusion. The new materials were characterized and compared in terms of release capacity of the catechins, gallic acid, quercetin and caffeine. The influence of the type and amount of antioxidant, amount of plasticizer, type of food simulant and the contact time were also studied. Finally, the antioxidant efficiency of the antioxidants in the food simulants after the release process was tested too.

MATERIAL AND METHODS

Chemicals and Reagents. Polypropylene ISPLEN^R PP 070 G2M was provided by Repsol YPF (Madrid, Spain). Irgafos 168 (tris(2,4-di-*tert*-butylphenyl)phosphate; I168), (−)-epicatechin (EC), (+)-catechin hydrate (C), (−)-epigallocatechin (EGC), (−)-epigallocatechin gallate (EGCG), (−)-epicatechin gallate (ECG), (−)-gallocatechin gallate (GCG), (−)-catechin gallate (CG), quercetin, gallic acid monohydrate (GA), caffeine, 2,2′-azinobis(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS), and poly(propylene glycol)-*block*-poly(ethylene glycol)-*block*-poly(propylene glycol) (average $M_n \sim 2000$) were supplied by Sigma-Aldrich (Steinheim, Germany). Green tea extract was kindly donated by the group of Packaging Lab, Instituto de Agroquímica y Tecnología de Alimentos (CSIC, Valencia, Spain). Methanol and ethanol (EtOH) HPLC gradient for instrumental analysis were supplied by Merck (Darmstadt, Germany). Formic acid 98−100% puriss p.a. was from Sigma-Aldrich. Water was purified using a Milli-Q Ultrapure wate purification system (Millipore, Bedford, MA, USA).

Film Preparation. Monolayer polypropylene compounding films containing PPG-PEG-PPG (0, 2, or 5%) as plasticizer and individual catechin (2 or 5%) or green tea extract (2 or 5%) as antioxidants were obtained by extrusion. Commercial antioxidant I168 (0.2%) was also added to protect the polymer during the extrusion process. Specific composition of each sample is shown in Table 1. Both catechin as individual compound and the green tea extract were incorporated as solids into the compounding mixture before extrusion. Films without plasticizer were also prepared as reference materials.

Extrusion was carried out using a miniextruder equipped with twin conical corotating screws and a capacity of 7 cm^3 (Minilab Haake Rheomex CTW5 (Thermo Scientific)). Screw rotation rate of 40 rpm, temperature of 180 °C, and 1 min of residence time were used. The resulting films presented an average thickness of 1.5 ± 0.14 mm, although the thickness of every sample was individually measured before tests using an electronic digital micrometer (Comecta S.A., Barcelona, Spain).

Standard and Sample Preparation. Individual stock standard solutions (1000 mg L^{-1}) were prepared into simulants A and D_1 for catechins, caffeine, gallic acid, and quercetin. Work standard solution containing all compounds was prepared from individual stock standard solutions in both stimulants with concentration ranging from 0.1 to 40 mg L^{-1} for all compounds except quercetin (0.004−2 mg L^{-1}). Ethanolic extracts at 10 and 50% of ethanolic content of green tea sample were prepared at 2 and 5% (p/v) of green tea in food simulant. The final samples were filtered through AcrodiscR PTFE CR 13 mm, 0.2 μm filters (Waters, Milford, MA, USA) and transferred into HPLC vials.

Chromatographic Study. HPLC coupled to mass detection was used to identify and quantify the natural antioxidants used, catechins, quercetin, and caffeine, and the plasticizer, PPG-PEG-PPG.

An Agilent 1200 series Rapid Resolution LC system (Agilent Technologies, Waldbronn, Germany) equipped with an online degasser, a binary pump delivery system, a high-performance SL autosampler, and a thermostated column department and online coupled to a mass spectrometer detector (MS) was used for analysis. Samples were filtered through a 0.2 μm Acrodisc PTFE CR and injected in a Zorbax SB-C18 (50 × 2.1 mm, 1.8 μm) column (Agilent Technologies). Two mobile phase systems consisting of mixtures of water/0.1% formic acid (A) and methanol (B) under the following gradient systems were used: mobile phase initially set at 25% B was linearly increased to 100% B in 4 min, maintained for 1 min, and brought back to initial conditions, for analysis of catechins, gallic acid, caffeine, and quercetin. Thirty percent B linearly increased to 100% B in 3 min and was maintained for 13 min, for the determination of the

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plasticizer. The mass spectrometer was an Agilent 6410 triple-quadrupole LC-MS (Agilent Technologies). The column effluent was directly introduced into the triple-quadrupole mass detector operated in a positive ionization mode. Ions were formed using electrospray ionization (ESI). The following ESI source parameters were used: Temperature of the drying gas (N_2) was set to 350 °C and flowed at 10 mL min^{-1}. Nebulizing pressure (N_2) was maintained at 35 psi. Capillary voltage was set at 4 kV. Integration and data elaboration were performed using Agilent MassHunter Workstation software, version B03.00 (Agilent Technology, Santa Clara, A, USA). The full mass scan range m/z 100−1000 (1 s/scan) and the target ions generated by catechins, gallic acid, caffeine, quercetin, and PPG-PEG-PPG corresponded to $[M + H]^+$. Selective ion monitoring (SIM) was used to quantify the target ions. Mass spectral data and retention time were used for peak identification. Quantification of plasticizer was based on an external standard calibration method.

Thermal Stability. *Film Thermal Stability.* Film samples (Table 1) were taken out for oxidation induction time (OIT) measurements to obtain information on polymer stability and antioxidant effectiveness. OIT was measured on a Perkin-Elmer series 7 differential scanning calorimeter (DSC) isothermally at 200 °C under inert atmosphere, which was subsequently switched to oxygen atmosphere. Analyses were carried out according to EN 728:1997.[37] The OIT was measured as the onset point at which the DSC thermogram suffers a sudden drop with respect to the instrument baseline. The obtained results are the mean of three measurements.

Antioxidant Thermal Stability. Epimerization, that is, the conversion of catechins to their corresponding isomers, can occur under hot conditions at the C-2 position.[38] Measurements of the stability of the antioxidants were made in the two selected simulants under the set exposure conditions by storing a solution of the additive in the simulant in parallel with the release tests. Analyses were carried out using the same procedure as for the samples by means of HPLC-QqQ.

Release Studies. Release tests were performed by total immersion of rectangular strip film pieces (80 ± 0.099 × 3.4 ± 0.26 × 1.5 ± 0.14 mm) in 10 mL of food simulant contained in glass-stoppered tubes with polytetrafluoroethylene (PTFE) closures. Milli-Q water was deoxygenated by bubbling nitrogen. The migration test parameters were based on European Commission Regulation 10/2011.[39] Two food simulants were selected to mimic some foods usually and/or able to be packed in plastic films: A (10% ethanol), representing one of the assigned foods that has a hydrophilic character (such as sugar and its products, nuts, vegetables, fish, meat, cheese, sauces, sandwiches); and D_1 (50% ethanol) for foods with lipophilic character and an alcoholic content above 20% (preserved fruits, preserved vegetables, milk, yogurt, cream and soup cream, processed cheese, among others).[39] Release studies were conducted at 40 °C over 5 and 10 days of storage. Test materials were also run simultaneously to check for interferences.

After the contact period, an aliquot of food simulant was filtered through Acrodisc PTFE CR 13 mm, 0.2 μm, filters and analyzed by means of HPLC-QqQ. Release data were corrected with the information obtained from stabilization of the antioxidant under the exposure conditions and expressed as milligrams of compound released per kilogram of film.

The release process is normally described by the kinetics of the diffusion of the antioxidant in the film and is expressed by the diffusion coefficient (D). D is usually estimated using the Fickian diffusion model.[40] When release of antioxidant reaches equilibrium, eq 1 is used as the rigorous model for describing the migration controlled by Fickian diffusion in a packaging film:

$$\frac{M_t}{M_{F,\infty}} = 1 - \sum_{n=0}^{\infty} \frac{8}{(2n+1)^2\pi^2} \exp\left[\frac{-D(2n+1)^2\pi^2 t}{L_P^2}\right] \quad (1)$$

M_t is the mass of the migrant in the food at a particular time t (s); $M_{F,\infty}$ is the mass of migrant in the food at equilibrium; L_P (cm) is the film thickness; D ($cm^2\ s^{-1}$) is the diffusion coefficient; and t is time (s). Nevertheless, when release is slow and equilibrium is not reached at the end of the experiment, eq 2 can be used when M_t/M_P is <0.6:

$$\frac{M_t}{M_P} = \frac{4}{L_P}\left(\frac{Dt}{\pi}\right)^{0.5} \quad (2)$$

M_P is the initial loading of antioxidants in the film; D is estimated from the slope of the plot of M_t/M_P versus $t^{0.5}$.

Diffusion coefficients for samples doped with green tea were calculated as the sum of all studied compounds.

Antioxidant Activity. 2,2′-Azinobis(3-ethylbenzothiazoline-6-sulfonate) (ABTS) was selected to study the radical scavenging behavior of the developed materials after contact period with simulants. The assay is based on the inhibition by antioxidants of the absorbance of the radical cation $ABTS^{\bullet+}$, which has a characteristic wavelength absorption spectrum with a main absorption maxima at 417 nm and secondary absorption maxima at 660, 734, and 820 nm. Radicals $ABTS^{\bullet+}$ are neutralized either by direct reduction via electron transfers or by radical quenching via H atom transfer.[41,42]

Approximately 30 mg of each material was immersed in 10 mL of $ABTS^{\bullet+}$ radical solutions, and their absorbance was kinetically monitored. ABTS radical cations were produced by reacting 7 mM ABTS in water with 2.45 mM potassium persulfate ($K_2S_2O_8$) and then stored in the dark at room temperature for 16 h. The ABTS radical solution was diluted to give an absorbance value of 1 at 734 nm. All experiments were performed in triplicate.

When these radicals are neutralized, the absorbance decreases. The percentage inhibition values were calculated using eq 1:

$$I\ (\%) = [(\text{Abs control} - \text{Abs sample})/\text{Abs control}] \times 100 \quad (3)$$

Using a calibrated curve of gallic acid concentration versus I (%), the results can easily be expressed as the equivalent gallic acid concentration.[26]

A sample of each migrated simulant, after the contact period, was mixed with a solution of radicals $ABTS^{\bullet+}$ of known concentration at a rate of 9:1 (sample/$ABTS^{\bullet+}$ solution). After 15 min of timeout, absorbance was measured.

Statistical Analysis. Data were analyzed by a one-way analysis of variance (ANOVA) test using SPSS statistics software (SPSS Inc., Chicago, IL, USA). Significant differences among the different samples were evaluated by using Tukey's test at a confidence interval of 95%. Data were expressed as the mean ± standard deviation. Box plot representations were also used to display differences between groups of data.

RESULTS AND DISCUSSION

In this work, PP films containing catechin or green tea extract as antioxidant agents and PPG-PEG-PPG as plasticizer were successfully produced by means of extrusion. Release from those extruded materials (Table 1) has been evaluated in this study. Release of seven catechins, gallic acid, quercetin, and caffeine was tested. Not only have the active substances added to the film been evaluated but also the plasticizer PPG-PEG-PPG, which could migrate to foodstuffs.

Antioxidant Content in Green Tea. Table 2 compiles the quantification data for extracts of green tea in 10 and 50% ethanol−water by means of HPLC-PDA-QqQ. The content of green tea catechins together with gallic acid, quercetin, and caffeine was found to be approximately 71% of its weight. A very similar content was obtained in both 10 and 50% ethanolic extracts.

Seven catechins were determined, EGCG, ECG, and GCG being the most abundant in green tea sample, constituting up to 80% of the content of green tea in catechins (without considering the percentage of gallic acid and caffeine). Therefore, the largest percentage of catechins present in green tea exists as gallate forms, which are also the more polar catechins. Caffeine represents 13% of the total content of green tea in catechins, gallic acid, and caffeine, whereas gallic acid was

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Table 2. Antioxidant Content of Green Tea Extract

	$mg_{compound}\ g^{-1}_{green\ ta}$	
	10% ethanolic extract	50% ethanolic extract
GA	13.67 ± 0.24	ND
EGC	49.64 ± 0.29	10.24 ± 0.93
CATE	17.41 ± 1.18	17.40 ± 1.08
EGCG	303.36 ± 3.10	235.57 ± 26.60
EPI	38.73 ± 1.78	40.01 ± 0.91
GCG	73.00 ± 5.36	32.02 ± 7.89
ECG	106.76 ± 0.52	103.51 ± 2.08
CG	14.99 ± 0.54	12.77 ± 0.26
Caff	94.48 ± 1.94	93.54 ± 0.71
Quer	ND	ND

Table 3. Stability of the Studied Antioxidants under Time and Temperature Conditions[a]

	simulant A		simulant D_1	
	5 days	10 days	5 days	10 days
GA	ND	ND	ND	ND
EGC	14.59	7.52	3.71	5.88
CATE	2.91	5.02	7.83	8.06
EGCG	65.55	67.06	ND	ND
EPI	5.61	4.05	19.52	12.63
GCG	19.00	27.64	1.06	13.35
Caff	9.48	48.96	0.09	15.12
ECG	8.49	8.64	1.77	1.19
CG	15.53	6.00	7.51	ND
Quer	ND	ND	ND	ND

[a]Data expressed as relative standard deviation (RSD).

found to be in only <2% of the total content, which is in consonance with those studies that claim that high levels of gallic acid in tea samples should be more related with a degradation process of the tea sample.[26]

Therefore, those seven catechins, gallic acid, caffeine, and quercetin were selected to study the release of green tea components from active film formulation.

Thermal Analysis. *Stability of the Film.* Table 1 shows the OIT values for the studied films. Longer OIT value show that material is more stable against oxidation degradation at that temperature. The results of the OIT measurements revealed that addition of plasticizer did not influence polypropylene stability if antioxidant is not added (no significant differences were observed between those values: M0-A and M0-B, Table 1). Nevertheless, the longest OIT obtained for polypropylene doped with catechin or green tea (OIT > 30 min) confirmed that these compounds provided polypropylene with stabilization against thermal oxidation. These results are also confirmed by statistical analysis. It is worth remarking that catechin and green tea provided polypropylene with similar stability, especially when the highest amount of both antioxidants is used. However, on the other hand, as the concentration of catechin in films increases from 2 to 5%, OIT decreases. This could be related to the possible loss of effectiveness when the amount of additive employed exceeds the ideal percentage and, thus, the effective rate and reaches the so-called waste percentage.[43]

When antioxidant and plasticizer are simultaneously added to film formulations, significant differences were observed with reference to blank samples. Moreover, higher OIT values are observed at higher plasticizer amounts, which could be an indicator of a possible effect of the plasticizer on the fixing of the antioxidant onto the matrix or a possible protective effect against the oxidant reaction.

Antioxidant Stability. Catechin and green tea extract stability through time and with temperature tested through HPLC measurements revealed that extracts were affected by thermal and temporal conditions. Changes in the concentration of some compounds were observed (Table 3). Epimerization of catechin to epicatechin was observed at 40 °C over prolonged contact time. Several catechin concentrations decreased considerably, especially the gallate species. Ethanol is also important because catechin stability increases with increasing percentage of ethanol.

Stability of green tea antioxidants through the extrusion process was also considered. According to López de Dicastillo et al.,[26] thermogravimetric analysis of the green tea sample revealed a broad degradation band that starts at 150 °C, with a maximum at approximately 200 °C. Nevertheless, the specific amount of antioxidant lost during that process was not considered in this work for the following data, because the aim of the present work was to study how the use of plasticizers improved the release of antioxidants and how it could be used for future packaging applications, where losses through the different processes should be assumed.

Release of Catechins. Different migration profiles were observed when catechin or green tea extracts were incorporated into film formulations with increasing amounts of PPG-PEG-PPG as plasticizer (Figures 1 and 2).

Differences were observed in the release from films extruded with commercial catechin and with green tea, which can be clearly attributed to the different compositions of green tea extracts reported beforehand.

The use of a plasticizer in film formulation improved the release capacity of the modified films. In films prepared with commercial catechin (M1−M6, Table 1), the use of PPG-PEG-PPG showed a significant effect with regard to the release of catechin (Figure 1). Levels of catechin released from films with 2% of catechin and 2 and 5% of PPG-PEG-PPG (M2 and M3, respectively) between 30- and 40-fold higher than the corresponding films without plasticizer (M1) into simulant A were observed. Increases between 6- and 20-fold were observed in simulant D_1. When samples with 5% of catechin (M5, M6) were considered, increasing release values between 2- and 5-fold were observed, though. Adding 5% of catechin into film formulation also meant increasing the migration level when compared with those films doped with 2% of catechin (between 3- and 7-fold higher).

In films prepared with green tea (M7−M12), the use of PPG-PEG-PPG as plasticizer also showed a significant effect in the release of catechins, as well as gallic acid, caffeine, and quercetin (Figure 2).

Statistical comparison through box plots (Figures 1a and 2a) also shows differences between release data from films with different percentages of plasticizer.

Individual release data of each catechin, gallic acid, caffeine, and quercetin from PP/PPG-PEG-PPG/green tea films (M7−M12, Table 1) are shown in Table 4.

As can be seen, including PPG-PEG-PPG in film formulation generally meant a significant improvement in the amount of compounds released from processed films, especially from film samples doped with 5% of green tea in simulant A and from samples doped with 2 and 5% of green tea in simulant D_1.

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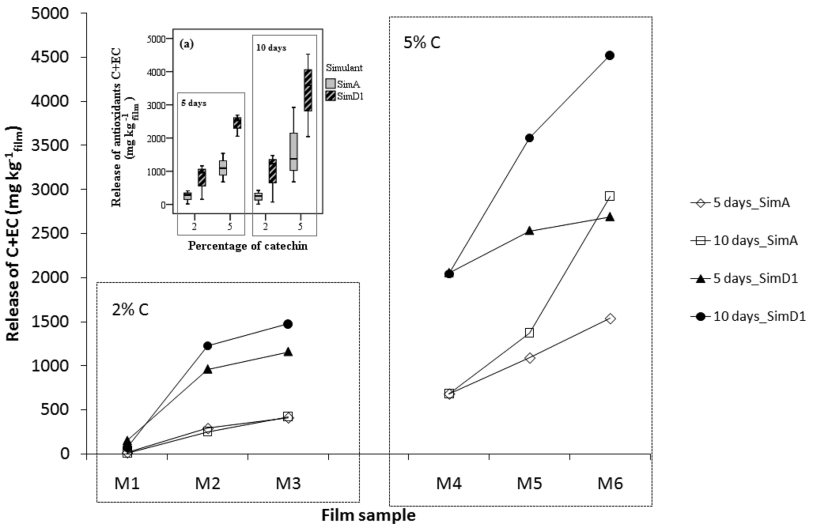

Figure 1. Release profiles of catechin from extruded films containing PP, catechin, and PPG-PEG-PPG (M1−M6, Table 1) into food simulants A and D_1 at 40 °C over 10 days. Box plots were drawn to graphically represent and compare numerical data sets by using SPSS statistics software and included as graph (a).

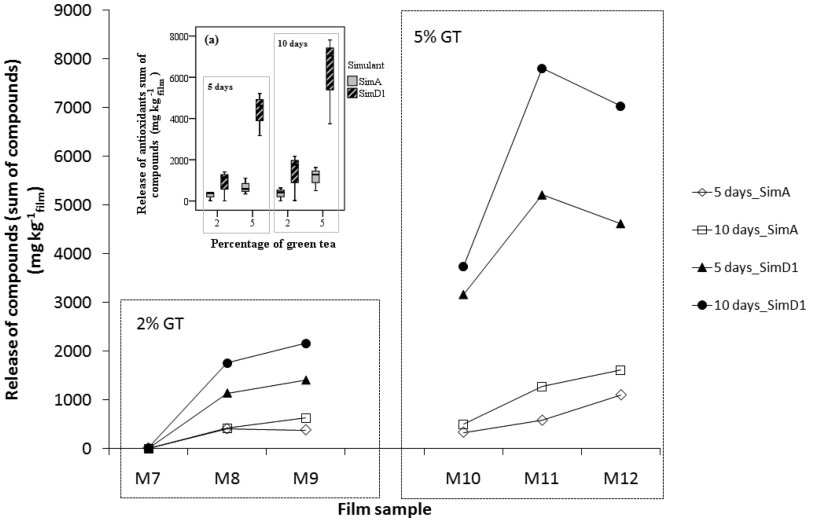

Figure 2. Release profiles of the studied compounds from green tea expressed as sum of catechins, gallic acid, caffeine, and quercetin (mg kg^{-1}_{film}) from extruded films containing PP, green tea, and PPG-PEG-PPG (M7−M12, Table 1) into food simulants A and D_1 at 40 °C over 10 days. Box plots were drawn to graphically represent and compare numerical data sets by using SPSS statistics software and included as graph (a).

Differences in the release behavior were observed among the individually studied green tea compounds (Table 4). In general, the two main compounds that were released into the simulants were gallic acid and caffeine, followed by catechins, which can be explained by the much smaller molecular size of the former that facilitates their release. That difference is also more evident in simulant A. Moreover, catechins not released from films without plasticizer showed significant levels of migration, from those films into both simulants A and D_1. Average release levels from samples with 2% of green tea between 10- and 20- fold higher than from film samples without plasticizer were then observed. When the samples with 5% of green tea (M10−M12) were considered, increased release values between 2- and 12-fold were observed.

However, release of quercetin was only observed into simulant D_1 from both samples doped with 2 and 5% of green tea. The low solubility of quercetin into aqueous media (<5 mg L^{-1}) can explain the lack of its release into simulant.

Furthermore, increasing the amount of plasticizer in the film formulation from 2 to 5% has meant an increase in the amount of compound released from 0.6- to 4-fold depending on the film considered.

Because the extent of the release depends on the compatibility of the active substance with the polymeric matrix and the simulant, the higher the solubility in the simulant, the higher the release. As could be observed, higher release was displayed from all of the films into simulant D_1 than into simulant A (between 2- and 15-fold higher), which could be attributed to the higher solubility of the studied compounds as the higher the ethanolic content of the simulant. Thus, the higher water solubility of caffeine explains the higher release into simulant A. With regard to gallic acid, the high amount released compared with its low amount in green tea sample, especially when simulant D_1 is the extraction solvent, may also indicate a possible contribution as a result of the degradation of other catechins, in which case the ester bond of the gallates

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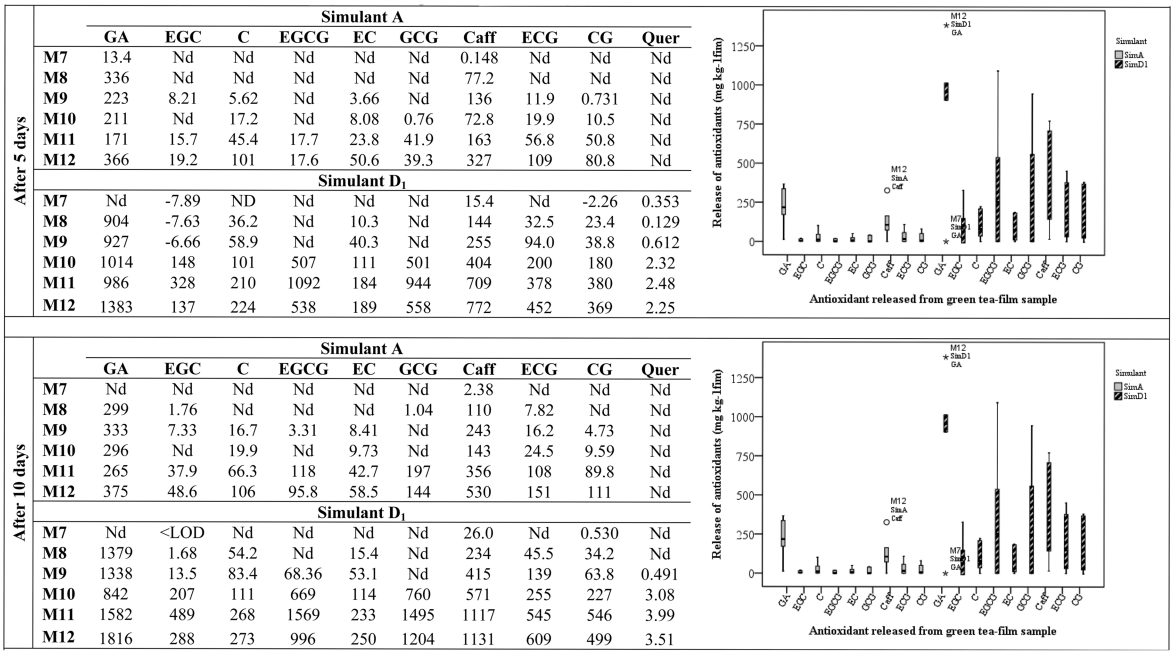
Table 4. Release of Each Catechin, Gallic Acid, Caffeine, and Quercetion from PP/PPG-PEG-PPG/Green Tea Films (M7−M12, Table 1) into Simulants A and D_1 at 40 °C after 5 and 10 Days of Contact[a]

		GA	EGC	C	EGCG	EC	GCG	Caff	ECG	CG	Quer
After 5 days						Simulant A					
	M7	13.4	Nd	Nd	Nd	Nd	Nd	0.148	Nd	Nd	Nd
	M8	336	Nd	Nd	Nd	Nd	Nd	77.2	Nd	Nd	Nd
	M9	223	8.21	5.62	Nd	3.66	Nd	136	11.9	0.731	Nd
	M10	211	Nd	17.2	Nd	8.08	0.76	72.8	19.9	10.5	Nd
	M11	171	15.7	45.4	17.7	23.8	41.9	163	56.8	50.8	Nd
	M12	366	19.2	101	17.6	50.6	39.3	327	109	80.8	Nd
						Simulant D_1					
	M7	Nd	-7.89	ND	Nd	Nd	Nd	15.4	Nd	-2.26	0.353
	M8	904	-7.63	36.2	Nd	10.3	Nd	144	32.5	23.4	0.129
	M9	927	-6.66	58.9	Nd	40.3	Nd	255	94.0	38.8	0.612
	M10	1014	148	101	507	111	501	404	200	180	2.32
	M11	986	328	210	1092	184	944	709	378	380	2.48
	M12	1383	137	224	538	189	558	772	452	369	2.25
After 10 days						Simulant A					
	M7	Nd	Nd	Nd	Nd	Nd	Nd	2.38	Nd	Nd	Nd
	M8	299	1.76	Nd	Nd	Nd	1.04	110	7.82	Nd	Nd
	M9	333	7.33	16.7	3.31	8.41	Nd	243	16.2	4.73	Nd
	M10	296	Nd	19.9	Nd	9.73	Nd	143	24.5	9.59	Nd
	M11	265	37.9	66.3	118	42.7	197	356	108	89.8	Nd
	M12	375	48.6	106	95.8	58.5	144	530	151	111	Nd
						Simulant D_1					
	M7	Nd	<LOD	Nd	Nd	Nd	Nd	26.0	Nd	0.530	Nd
	M8	1379	1.68	54.2	Nd	15.4	Nd	234	45.5	34.2	Nd
	M9	1338	13.5	83.4	68.36	53.1	Nd	415	139	63.8	0.491
	M10	842	207	111	669	114	760	571	255	227	3.08
	M11	1582	489	268	1569	233	1495	1117	545	546	3.99
	M12	1816	288	273	996	250	1204	1131	609	499	3.51



[a]Data expressed as mg of compound per kg of film. *D* estimated by eq 2, under Release Studies; *D* for M7−M12, calculated for each individual compound; Nd, not detected. Box plots were drawn to graphically represent and compare numerical data sets by using SPSS statistics software.

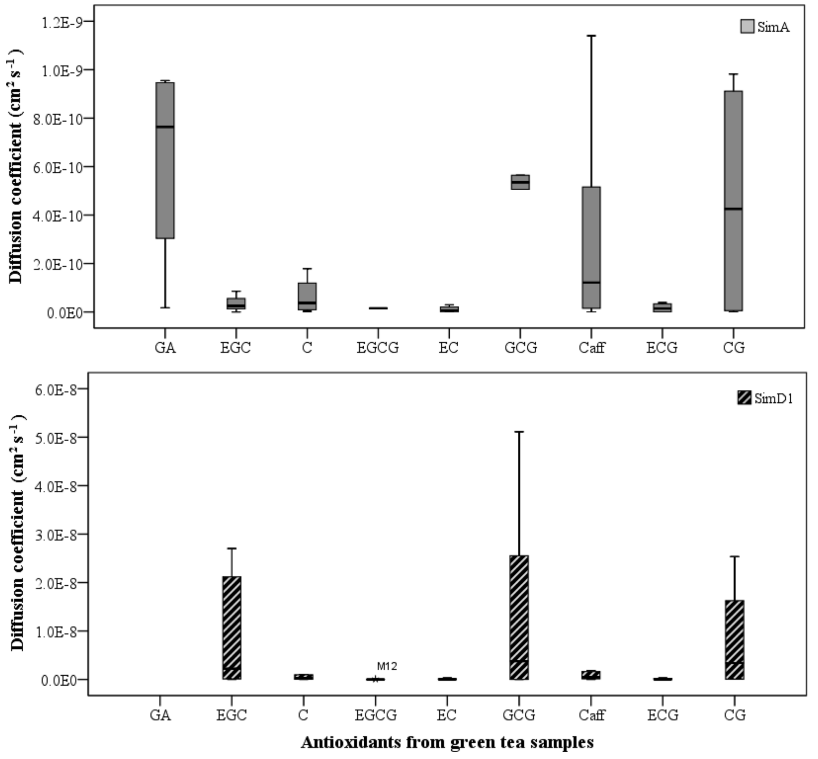


Figure 3. Estimation of the diffusion coefficients (*D*, $cm^2\ s^{-1}$) for the release of catechins, gallic acid, and caffeine from PP/PPG-PEG-PPG/green tea films into stimulants A and D_1 at 40 °C. Data are graphically represented and compared as box plot representations.

could break during the manufacturing process, resulting in an increase of gallic acid concentration.[26] With regard to catechins, its release is more evident in simulant D_1 with higher ethanolic content due to their higher solubility in ethanol than in water. According to release data (Table 4) and the amount of each compound in green tea sample (Table 2), it seems to be a relationship between amount released and the sample green tea major components in green tea sample. Finally, the low solubility of quercetin in water resulted in its nonrelease into simulant A. Nevertheless, the presence of 50% of ethanol in simulant D_1 slightly increased its release. Very low amounts

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were, however, released (between 100 and 300 times less than catechins).

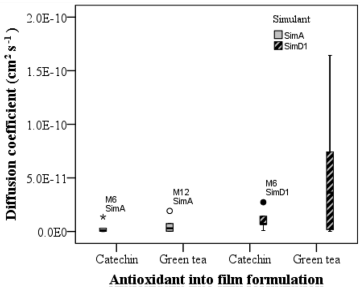
Statistical comparison between data released into different simulant (Figures 1a and 2a and Table 4) also confirmed those results.

Contact time also influenced release levels. Higher time led to release of the studied compounds from the film samples.

Diffusion coefficients (Figure 3; Table 5), estimated by eq 2 (section Release Studies) and statistically compared through

Table 5. Estimation of Diffusion Coefficient (D, $cm^2\ s^{-1}$) for the Release of Catechin from PP/PPG-PEG-PPG/Catechin or Green Tea Films to Simulants A and D_1 at 40 °C

Sample	Simulant A	Simulant D_1
M1	2.3E-14	8.1E-13
M2	3.1E-12	6.8E-12
M3	0.3E-12	6.7E-12
M4	1.2E-12	7.0E-12
M5	0.6E-12	1.4E-11
M6	1.4E-11	2.7E-11
M7	8.4E-14	6.7E-14
M8	6.9E-13	1.3E-11
M9	5.1E-12	5.9E-11
M10	3.0E-13	1.8E-12
M11	7.4E-12	7.4E-11
M12	1.9E-11	1.6E-10



[a] D estimated by eq 2 under Release Studies; D for M7−M12, calculated as sum of catechins, gallic acid, caffeine, and quercetin. Box plots were drawn to graphically represent and compare numerical data sets by using SPSS statistics software.

box plot representations, also confirmed that the release of the studied compounds was accelerated by the presence of the plasticizer. PPG-PEG-PPG increased the diffusivity of the studied compounds between 1 and 3 orders of magnitude. Moreover, the diffusivity values of catechin and green tea extract into simulant D_1 were slightly higher than in simulant A, which may be related to their different ethanolic contents and its effect over the polymer matrix.[28] Comparing these values of diffusivity with those previously obtained for other compounds, namely, tocopherols, from similar polymer matrices,[36] higher D values were obtained for catechins, which may be attributed to their higher solubility in water and, therefore, in simulants A and D_1 than for tocopherols. When compared to the diffusivity of catechins from other matrices, namely, EVOH or PLA[26,28,29] with a much more polar nature than PP, the data from the present work showed how the incorporation of PPG-PEG-PPG gives place to a diffusivity similar to that from those polymer matrices ($D \sim 1 \times 10^{-10}$ to 1×10^{-12} vs $D \sim 1 \times 10^{-9}$ to 3×10^{-11} for release of catechin from EVOH matrices into simulants A and D_2 (95% ethanol)[26,28] and $D \sim 5 \times 10^{-10}$ from PLA matrices into simulant D_2 (95% ethanol) with no release into simulant A or D_1).[29]

Migration of PPG-PEG-PPG. Introducing the plasticizer into film formulation also means adding a new potential migrant that can have a potential influence on the food or food simulant with which it is in contact.

Figure 4 shows the percentage of PPG-PEG-PPG migrated into simulants A and D_1 at 10 days of contact. The migration of PPG-PEG-PPG depended on its initial amount in the film formulation, on the storage time, and on the food simulant with which it was in contact.

According to experimental data, higher migration of plasticizer was observed from polymer samples formulated with higher concentration.

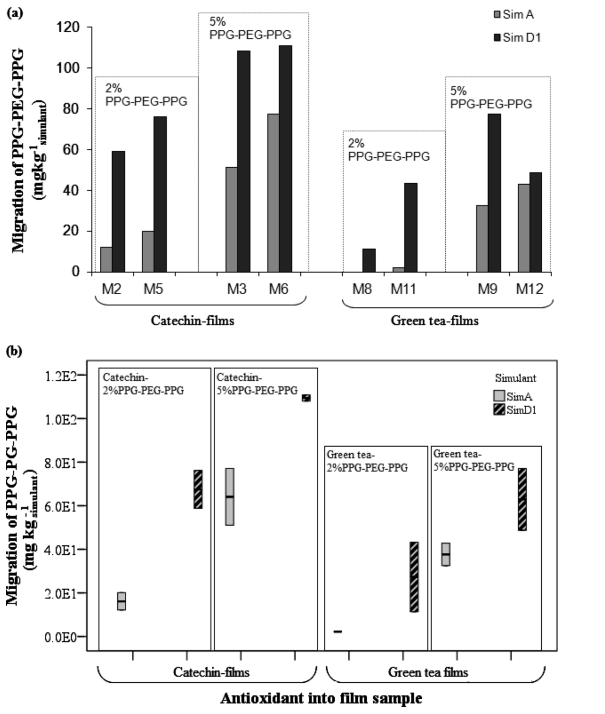


Figure 4. (a) Migration of PPG-PEG-PPG from catechin- and green tea-containing films into simulants A and D_1 at 40 °C and 10 days. (b) Box plots were drawn to graphically represent and compare numerical data sets by using SPSS statistics software.

Statistical differences in the migration of the plasticizer were also observed among films doped with catechin and green tea (Figure 4). Data showed higher migration levels from catechin−film samples.

On the other hand, the effect of time on the migration behavior can be explained as increased time resulting in slightly higher percentages of PPG-PEG-PPG. Moreover, simulant D_1 presented higher levels of migration.

Nevertheless, as PPG-PEG-PPG is not included on the European Union list of authorized monomers, other starting substances, and macromolecules,[39] and having a molecular mass higher than 1000 Da and being capable of forming the main structural component of the plastic material, it complies with the requirements of the regulation.

Antioxidant Activity. Figure 5 presents the antioxidant activity of food simulants exposed to catechin- and green tea-containing films, expressed as equivalent of gallic acid.

Comparison of antioxidant activity data with release data (Figures 1 and 2) reveals the same profile in both studies. Therefore, antioxidant activity was shown to be proportional to the antioxidant concentration in each simulant. Thus, an increase in the initial amount of catechin or green tea and/or the amount of PPG-PEG-PPG led to higher release and higher antioxidant activity. The effect of the type of simulant was also noticeable (Figure 5b), presenting around an average of 4 times higher antioxidant activity in simulant D_1 than in simulant A, which can be attributed to the higher solubility of the studied compounds in ethanol than in water. Moreover, the effect of contact time was also evident.

Likewise, in release studies, antioxidant activity data showed a higher antioxidant capacity of the catechin-containing films than of the green-tea containing films. It can be associated with the different content of each film in the studied compounds: 2

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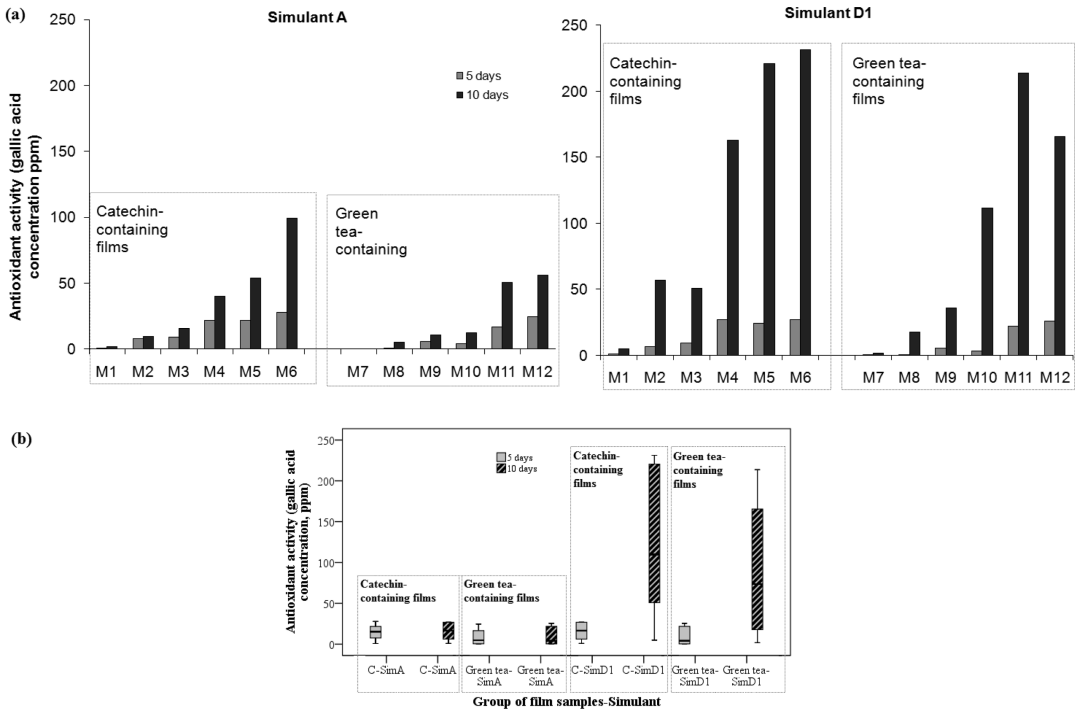

Figure 5. (a) Antioxidant activities of simulants A and D_1 in contact with catechin-containing and green tea-containing films (M1−M12, Table 1) over 5 and 10 days of contact at 40 °C. (b) Graphical representation and statistical comparison of antioxidant activity data through box plot (SPPSS statistics software). Data were measured by ABTS assay and expressed as gallic acid concentration (ppm).

or 5% of pure catechin or green tea, respectively. However, in the latter, according to green tea extract quantification (Table 2), catechins, gallic acid, caffeine, and quercetin make up only 71% of its weight, and each compound results in a lesser amount of each compound able to be released than in the catechin-containing films.

Therefore, antioxidant active films, based on polypropylene polymer formulations modified with PPG-PEG-PPG as a plasticizer, and natural antioxidants were successfully developed. By modifying a PP matrix with PPG-PEG-PPG, release of catechins, gallic acid, caffeine, and quercetin was clearly favored. By increasing the amount of active agent, the amount of plasticizer, the contact time, and/or the ethanolic content of the food simulant, release has been improved. Moreover, adding an antioxidant individually or as a component of a natural sample mixed with other antioxidants of different structures and polarities resulted in a potential system to be used in active packaging likely for a controlled release of those antioxidants to a wide range of foods where they maintain their antioxidant capacity.

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