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Enhancing the Release of the Antioxidant Tocopherol from Polypropylene Films by Incorporating the Natural Plasticizers Lecithin, Olive Oil, or Sunflower Oil

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ABSTRACT: In this work, natural plasticizers-modified polypropylenes intended for food active packaging were developed. Sunflower oil, olive oil, and soy lecithin, without any known harmful effects or toxicity, were employed as natural plasticizers, also implementing the attractiveness of using synthetic plastics on active packaging developments. Their incorporation during the extrusion of polypropylene was tried as a means to obtain polymers with improved diffusion paths, allowing differences in antioxidant release rates for active packaging materials. Thermal and rheological characterization of the films showed that blending natural plasticizers do not significantly modify their thermal properties; however, small variations of viscoelastic properties were observed. Furthermore, the resulting release of tocopherol was highly dependent on the polymer formulation. Furthermore, it was clearly time-controlled by using those natural plasticizers, especially olive oil. Antioxidant activity results also showed that packaged foods are protected against oxidative degradation over time, resulting from the improved release of the antioxidants.

KEYWORDS: *plasticizer, active packaging, antioxidant, release, polypropylene*

INTRODUCTION

Oxidative rancidity is a major cause of food quality deterioration, leading to the formation of undesirable off-flavors as well as unhealthful compounds. The addition of antioxidants to food, and the design of a suitable vacuum or modified atmosphere packaging technology are the two most common solutions to reduce oxidation reactions in sensitive food products. Limitations of the first approach are associated both to the cessation of the protective effect once the active compounds are consumed, and the lack of selectivity to target the food surface. Those limitations can be overcome by an active antioxidant package, which can provide continuous release of the active compound from packaging material to food surface.[1] Thus, antioxidant agents are added to polymeric films aimed of imparting the food with improved chemical stability.

The growing trend to reduce the use of synthetic additives in packaging has led to a focus on their replacement by natural antioxidants, particularly tocopherol, plant extracts, and essential oils. Several works have reported interesting results about antioxidant active packaging developments.[2−7]

Most of those developments base their activity on the mass transport properties of plastic materials (sorption, migration, and permeation) and the release of the antioxidants, depending on several factors, such as the type of polymer or food, among others.[8]

Despite emerging technology and the broad growth of research on active packaging developments, only a few examples of antioxidant-active materials have been commercialized. This could be related to the fact that most of the developments are based on polymers that are not the most commonly used in industrial applications. Despite the increasing demand for biodegradable materials, polyolefins are still the most common in food packaging. Most studies were centered in the incorporation of α-tocopherol in low density polyethylene (LDPE) polymer,[7,9−11] since tocopherols are excellent stabilizers during polymer processing, according to both their stability under processing conditions and their solubility in polyolefins.[12,13] They are also classified as substances generally recognized as safe (GRAS) for intended use in food.

Nevertheless, the use of tocopherols, or other antioxidants, as additives for active polypropylene (PP) materials has been limited by their practically total lack of release into foodstuffs and food-simulating liquids.[14,15] PP materials did not show any interactive tendency toward any of the foods, remaining more or less unaffected in the PP film content of the active agent. In this sense, Castro-López et al.[16] have recently proposed the use of some chain extenders blended into film formulation to improve the capacity of the PP films in releasing tocoferol, intended to improve antioxidant active packaging.

A polypropylene glycol (PPG)−polyethylene glycol (PEG) triblock copolymer was used, resulting in the separation of polymer chains, thus facilitating molecular movement. Zhu et al.[17] also proposed the use of LDPE blended with PP to control the release of tocopherol. The higher the PP content on the blending ratio of LDPE/PP, the slower the tocopherol release becomes, which can be likely due to the more tightly packed structure and higher crystallinity of PP than LDPE.

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Some PPG, PEG, or their copolymers have also been reported as potential plasticizers into film formulations.[18,19] On the basis of their reported role in drug release,[20] plasticizers may also be another alternative to modify polymer properties and, therefore, the mass transport of the active agents. The use of plasticizers within the manufacture of plastic products is not new. Their applications to modify polymer characteristics began in the 1800s. Usually, they have been applied to increase workability, flexibility, and extensibility of polymers. In the present work, they are intended to increase the release rate of an active agent from an hydrophobic polymer matrix: the polypropylene. In relation to both the results obtained in our previous work and the consumer concerns, the main objective of this work is the substitution of previous synthetic plasticizers by natural substances, mainly due to their low toxicity and migration.[21]

Plasticizers cover a wide range of chemical compounds, including esters, hydrocarbons, water, alcohols, glycols, phenols, ketones, and esters. Fatty acids and the correspondent vegetable oils have received substantial attention as plasticizers. The use of sunflower and olive oil is very interesting since their incorporation into food packaging materials is not restricted by a specific migration limit.

Commercial lecithin may also represent another possible plasticizer to be used to enhance release properties. As used by food manufacturers, lecithin commonly refers to a complex mixture of naturally occurring phospholipids in oil. A major source of lecithin is soybean oil. High in choline, it has shown to be good for brain development, heart disease prevention, and more recently, many research scientists have focused on its possible therapeutic uses to counter neurological diseases, such as Alzheimer's or hypercholesterolemia. However, conflicting results on the clinical benefits in these diseases has been reported.[22,23]

The addition of plasticizers to polymers is a well-established technology. The improvement of several original properties of polymers, such as mechanical (decreasing the glass transition temperature, increasing the flexibility) or thermal and chemical properties (modification of chemical reactivity, decreasing temperature of dissolution, etc.) have already been reported.[24] Therefore, plasticizers could be blended during the extrusion of polypropylene to obtain polymers with different arrangements into the diffusion network, which may allow differences in antioxidant release rates from active packaging materials.

The objective of this work was the development of optimized polypropylene formulations with natural plasticizers (soy lecithin, olive oil, and sunflower oil) to reach a convenient release of antioxidants. Their performance was compared with that obtained by two high molecular-weight commercial plasticizers allowed for use in the manufacture of materials into contact with foods: Priplast 3019 and 3114. They were selected as reference materials. Tocopherol was chosen as a natural antioxidant and incorporated into film formulation as Nutrabiol T90, a commercial antioxidant formulated with tocopherol and allowed for food applications. The effects of type and amount of natural plasticizers on the release of tocopherol, the influence of the concentration of antioxidant, and the time conditions were studied. The influence of plasticizers in rheology and, therefore, in processing behavior was also tested.[25,26]

MATERIALS AND METHODS

Materials and Reagents. PP ISPLEN^{R} PP 070 G2M was provided by Repsol YPF. Nutrabiol T90 (NUT) was supplied by BTSA (Madrid, Spain). Irgafos 168 (Tris(2,4-ditert-butylphenyl) phosphate; I168), 2,2′-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) and 2,2-diphenyl-1-picrylhydrazyl (DPPH) 95% free radical were supplied by Sigma (Steinheim, Germany). Commercial plasticizers Priplast 3114 (PRIA) and Priplast 3019 (PRIB) were from Croda Europe Ltd., (Barcelona, Spain). Methanol and ethanol HPLC-gradient for instrumental analysis were supplied by Merck (Darmstadt, Germany). Water was purified using a Milli-Q Ultrapure water-purification system (Millipore, Bedford, MA, U.S.). Sunflower oil (SO), olive oil (OO), and soy lecithin (SL) were bought in a local supermarket.

Polymer Samples Manufacture. Blends 1−13 (shown in Table 1) were carried out using a miniextruder equipped with twin conical corotating screws and a capacity of 7 cm^3 (MiniLab Haake Rheomex CTW5, Thermo Scientific). A screw rotation rate of 40 rpm, temperature of 180 °C, and 1 min of residence time were the parameters used. Materials with different plasticizers and Nutrabiol T90, added as a natural antioxidant, were formulated with PP. Materials were extruded at two different concentrations of Nutrabiol T90 and plasticizers to study their influence on Nutrabiol T90 release. Blends without plastizicers, BLN1 and BLN 2, were also extruded, with the aim of studying the effect of plasticizers as polypropylene chain extenders. Irgafos 168 was incorporated in all cases at 0.2% to protect the polymer during polymer processing steps. The resulting films were approximately 120−130 μm thick.

Table 1. Composition of the Different Polypropylene-Blends Extruded

blend	sample	Nutrabiol T90 (%)	plasticizer	conc. plast (%)[a]
1	**BLN1**	1		
2	**M1SL2**	1	soy lecithin	2
3	**M1SO2**	1	sunflower oil	2
4	**M1OO2**	1	olive oil	2
5	**M1PRIA2**	1	Priplast 3114	2
6	**M1PRIB2**	1	Priplast 3019	2
7	**M1SL5**	1	soy lecithin	5
8	**M1OO5**	1	olive oil	5
9	**M1PRIA5**	1	Priplast 3114	5
10	**BLN2**	2		
11	**M2SL5**	2	soy lecithin	5
12	**M2OO5**	2	olive oil	5
13	**M2PRIA5**	2	Priplast 3114	5

[a]Conc. plast.: concentration of plasticizer onto film blend.

Nutrabiol T90 Characterization. Concentration of delta (δ), gamma (γ), and alpha (α) tocopherol in Nutrabiol T90 was determined by preparing 0.01 g of Nutrabiol T90 in 25 mL of ethanol under magnetic stirring for 10 min. Samples were diluted (1:10), filtered through an AcrodiscR PTFE CR 13 mm, 0.2 μm filters (Waters, Mildford, MA, U.S.) and transferred into HPLC vials.

Tocopherols were quantitatively analyzed by HPLC with a Waters 2695 (Waters, Mildford, MA, U.S.) system with a gradient pump and automatic injector. Chromatographic experiments were carried out using a stainless steel column packed with SunFire C18 (150 mm × 3.0 mm, 3.5 μm) (Waters) kept at 35 °C. Detection was carried out using a photodiode array detector (PDA, model 996 UV) set in the range of 200 to 500 nm (295 nm as output signal), and a fluorescence detector (FL, model 2475) (Waters) with $\lambda_{excitation}$ 295 nm and $\lambda_{emission}$ 325 nm. Output signals were monitored and integrated using a personal computer operated under the Empower software (Waters). A two solvent gradient elution was performed, with a flow rate of 0.5 mL min^{-1} and injection volume of 20 μL. The mobile phase was composed of water (A) and methanol (B). The following gradient

elution profile was used: mobile phase composition started at 30% B and was maintained for 0.5 min. Then, it was linearly increased to 90% B in 2 min, maintained for 1 min, and linearly increased to 100% B in 0.5 min. Finally, it was maintained for 10.5 min and brought back to the initial conditions. The three tocopherol homologues were identified by means of retention time and UV spectrum comparison with corresponding peaks in the standard solution. Total tocopherol was obtained by adding the areas of the three homologues and calculating the total concentration.

Total Extraction of Tocopherols from Films. Losses of tocopherols during the extrusion were determined by the total extraction of tocopherols. A similar procedure to those reported by Koontz et al. and Zhu et al.[11,17] was used. A 0.5-f portion of each film was cut into small pieces of approximately 1 × 1 cm^2 squares and immersed in 20 mL of methylene chloride in a 60 mL flask to loosen the film structure and extract tocopherols from the films. The flasks were agitated at 500 rpm using a shaking incubator (Velp Scientifica, Usmate, Italy) for 24 h. The methylene chloride extracted solution samples were withdrawn, filtered, and quantify by means of HPLC-PDA-FL. The concentration of each tocopherol was determined using a standard curve of tocopherol in methylene chloride.

Release Studies. A study of the release of tocopherols from the films was performed by determining their specific migration into simulant D1 (50% ethanol), assigned for foods that have a lipophilic character (Commission Regulation (EU) No. 10/2011).[27] Double-sided, total immersion migration tests were performed as follows: rectangular strips film pieces (80 ± 0.099 × 3.4 ± 0.26 × 1.5 ± 0.14 mm) and 10 mL of food simulant were placed in glass-stoppered tubes with PTFE closures. Release tests were conducted at 40 °C, and tocopherols were quantified after 1, 5, and 10 days of storage. Twenty days of storage was also tested.

Nutrabiol T90 is a commercial mixture of α, γ, and δ-tocopherols and Nutrabiol T90 released into the food simulant after the contact period was then calculated as the amount (in grams) of each totocpherol released with reference to the amount (in grams) of each homologue loaded into the film formulation. Moreover, for the purpose of comparison, the total tocopherol released was also quantified as the total amount of Nutrabiol T90 released (expressed in grams) per gram of total Nutrabiol T90 added into film formulation. Data from the losses of tocopherols during the extrusion process were also considered and release data were then corrected. Measurements of the stability of the antioxidants under migration conditions were made by storing a solution of Nutrabiol T90 in the simulant D1 in parallel with the migration tests, and results were recalculated.

Thermal Analysis. Differential scanning calorimetry (DSC) measurements (Perkin-Elmer series 7) were performed to analyze the effect of the addition of different plasticizers on the thermal stability of the PP matrix. Thermograms were obtained from −20 to 200 °C with 10 °C/min heating, cooling to −20 °C and holding at this temperature for 2 min, and a second heating process to 200 °C. Melting and crystallization temperatures, T_m and T_c, and enthalpies, ΔH_m and ΔH_c, were calculated from the cooling and the second heating process.

Thermal stability studies were completed by thermogravimetric analyses (TGA) carried out using a thermal analyzer Perkin-Elmer TGA 7. Samples (ca. 10 mg) were heated in 100 μL platinum sample pans from room temperature to 800 °C under a nitrogen atmosphere at 10 °C/min, to determine the degradation temperatures of new formulation materials.

Rheological Characterization. Viscoelastic characterization was performed using a controlled strain rheometer (ARES, TA Instruments) with parallel-plate geometry (25 mm diameter, 1 mm gap) at 180 °C. The complex viscosity (η^*), storage modulus (G'), and loss modulus (G''), of reference PP and its different formulations, were measured as a function of frequency (w). The rheological tests were performed in the linear viscoelastic region (LVE). This LVE region was determined by a strain sweep before testing the viscoelasticity of the composites under a frequency test. The frequency sweep measurements were set up in the frequency range 1 × 10^{-1} to 1 × 10^2 rad/s.

Antiradical Activity of Materials. Antioxidant activities of new materials formulated with natural plasticizers were measured by two different antioxidant assays, ABTS assay and DPPH method. They measure the antioxidant effectiveness by monitoring the inhibition of their corresponding radicals. The ABTS and DPPH assays are based on the bleaching rate of radical cation 2,2′-azinobis(3-ethylbenzothiazoline-6-sulfonate) ($ABTS^{\bullet+}$), and free radicals 2,2-diphenyl-1-picrylhydrazyl ($DPPH^{\bullet}$). Both radicals, $ABTS^{\bullet+}$ and $DPPH^{\bullet}$ are neutralized either by direct reduction via electron transfers or by radical quenching via H atom transfer.[28−30] Although release of antioxidant has only been tested in simulant D1, antioxidant activity was evaluated through both ABTS and DPPH assays, not only because of their simplicity, but also to study the radical scavenging behavior of the developed materials in different environments, because $ABTS^{\bullet+}$ in water and $DPPH^{\bullet}$ is dissolved in ethanol. Extruded materials were hot-pressed on a pressing plate IQAP LAP S.L. model PL15-series1381 (Barcelona), and very thin (approximately 35−45 μm) films were obtained in order to better simulate the real interaction that can occur between a packaging and a food contact product. Approximately 15 mg of each material with dimensions 1 × 3 cm^2 was immersed in 3 mL of $ABTS^{\bullet+}$ and $DPPH^{\bullet}$ radical solutions, and their absorbance was kinetically monitored. Both radical solutions were obtained as follows: (i) 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; (ii) a 2 mM ethanolic solution of radical $DPPH^{\bullet}$ was diluted to an absorbance value of 1 at 517 nm. All experiments were performed in triplicate.

Statistical Analysis. Data were analyzed by a one-way analysis of variance (ANOVA) test using the SPSS statistics software (SPSS Inc., Chicago, IL). Significant differences among the different samples were evaluated and box plot representations were also used to display differences between groups of data.

RESULTS AND DISCUSSION

As mentioned before, the present work is closely linked to a previous study developed by Castro-López et al., 2010,[16] where the following chain extenders, PE-PEG and PPG-PEG-PPG, were used as plasticizers to achieve a suitable release of Nutrabiol T90 from a polypropylene matrix. Added at three levels of concentration, ranging from 1 to 5%, release of tocopherol was then accelerated by the presence of those plasticizers. The diffusivity of tocopherol was increased between 1 and 2 orders of magnitude, and from 1.5 to 4-fold, the release levels were higher. Moreover, the more compatibility of the plasticizer with the matrix, the more the performance of the plasticizer into the release of the antioxidant.

Therefore, on that basis, lecithin, olive oil, and sunflower oil as natural plasticizers were studied and also compared with two more commercial plasticizers (Priplast 3019 and 3114) to modify the film release behavior.

Aiming to compare results with the previous work, a percentage of Nutrabiol T90 ranging between 1 and 2% (Table 1) was selected for the release studies. Higher percentages were discarded, as some tocopherol exudation from PP doped with 5% of Nutrabiol T90 was observed in the previous work.

Characterization of Nutrabiol T90. Thermal stability of Nutrabiol T90 at the PP processing temperature was previously assessed by means of TGA experiments.[31] Results showed that the degradation process does not begin until approximately 250 °C. Therefore, considering that the PP processing temperature is 180 °C, degradation of Nutrabiol T90 at that temperature was ruled out.

The average concentration of each tocopherol (α, γ, and δ tocopherol) in Nutrabiol T90 was 287 ± 20 mg of δ-tocopherol

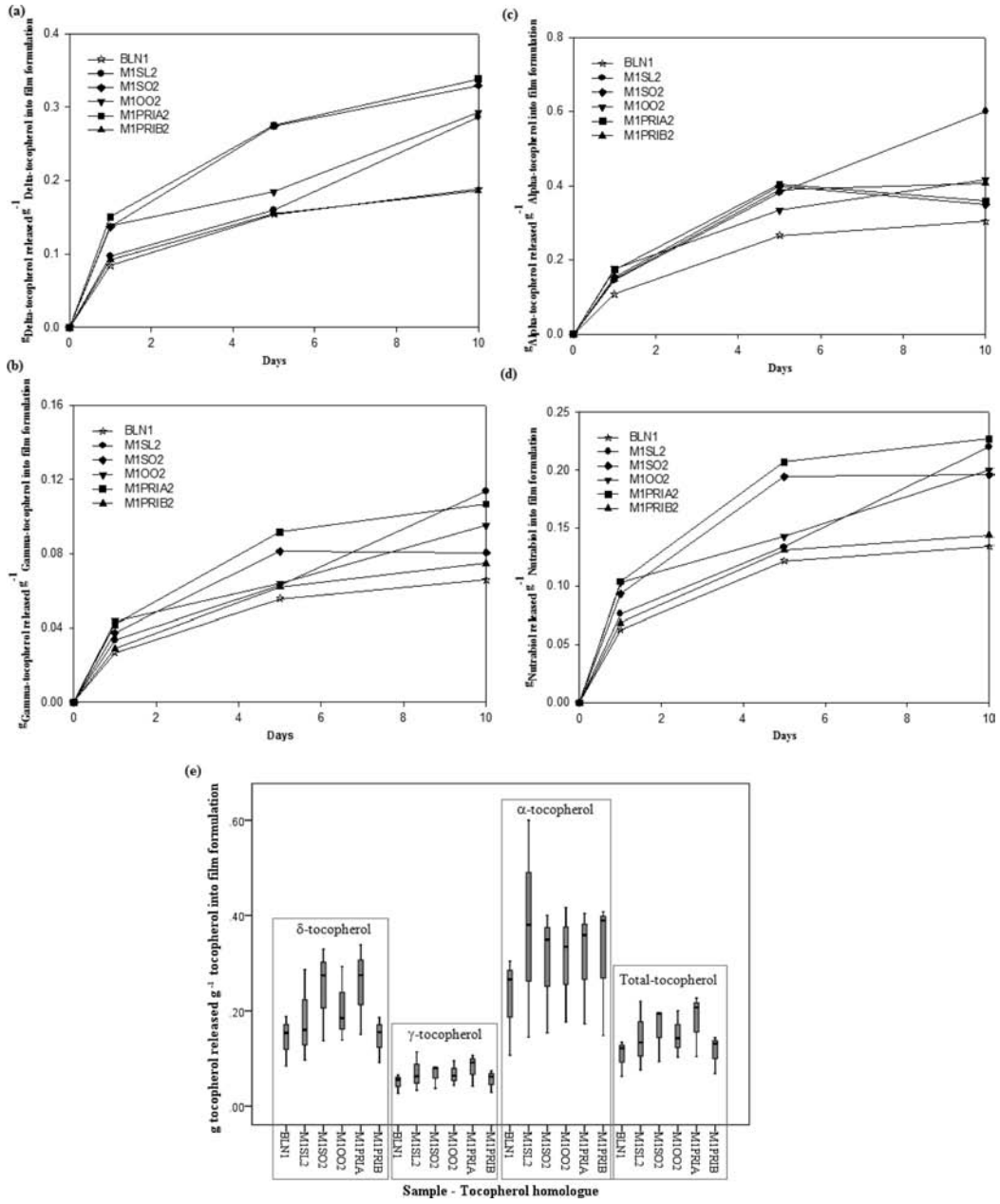

Figure 1. Migration of Nutrabiol T90 from blends 1−6 in contact with simulant D1 at 40 °C for 10 days expressed as g of δ-tocopherol (a), γ-tocopherol (b), α-tocopherol (c) and total-tocopherol (Nutrabiol) (d) per gram of each tocopherol into film formulation. (f) Statistical comparison of release data through box plot (SPSS statictics software).

per gram of Nutrabiol T90; 592 ± 58 mg of γ-tocopherol per gram of Nutrabiol T90 and 112 ± 10 mg of α-tocopherol per gram of Nutrabiol T90.

The amount of each tocopherol remaining in the film after the extrusion process was quantified as 0.76 ± 0.032 g of δ-tocopherol extracted per gram of δ-tocopherol initially added; 0.93 ± 0.050 g of γ-tocopherol extracted per gram of γ-tocopherol initially added and 0.87 ± 0.079 g of α-tocopherol extracted per gram of α-tocopherol initially added. It indicates that between 76% and 93% of the tocopherol added to the film was retained after the extrussion process, which suggests that no significant degradation of the tocopherol added into the plastic film occurred during the extrusion process.

Selection of the Most Suitable Natural Plasticizers by Release Studies. Blends 1−6 (Table 1) were exposed to migration assays in simulant D1 to select two natural substances as plasticizers. Results are exposed in Figure 1 as the influence of the plasticizer on the release of each individual tocopherol homologue (Figure 1a,b,c) and on the release of the total amount of tocopherol (Figure 1d). As the figure shows, the release of Nutrabiol T90 has been positively influenced by the incorporation of plasticizers, which can also be observed when statistical analysis is analyzed (Figure 1e).

The blends with olive oil or lecithin have been delayed release trough time; the major amount of antioxidant has been released in the last five days. However, Nutrabiol T90 has been mainly released by sunflower oil blend in the first step (1−5 days). This result suggests that the different release rate could be related with a possible different influence of each plasticizer into the structure of the polymer matrix, resulting from the

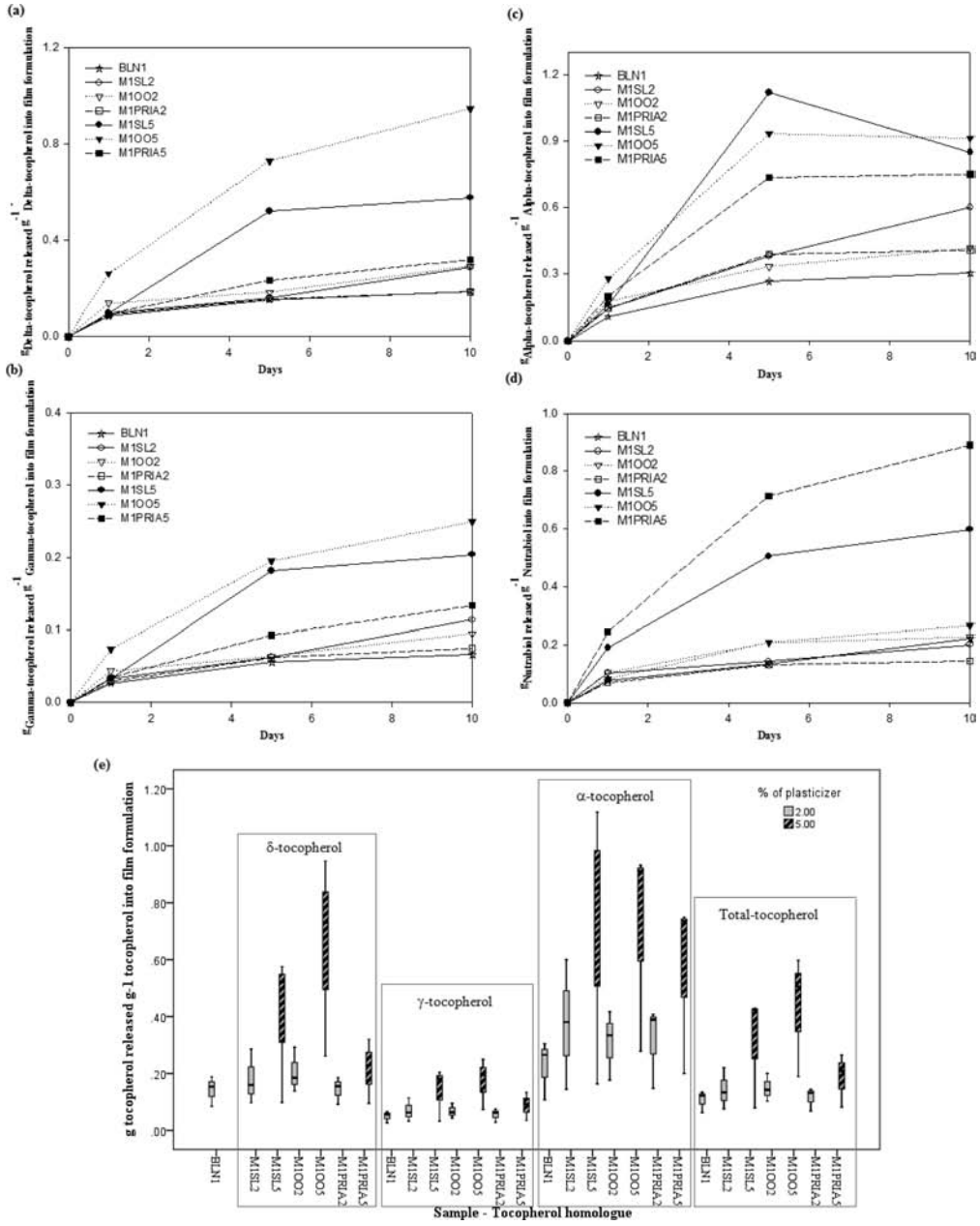

Figure 2. Migration of Nutrabiol T90 from blends with different concentrations of plasticizers (simulant D1, 40 °C, 10 days) expressed as g of δ-tocopherol (a), γ-tocopherol (b), α-tocopherol (c), and total-tocopherol (Nutrabiol) (d) per gram of each tocopherol into film formulation. (f) Statistical comparison of release data through box plot (SPSS statictics software).

similar basic general skeleton of the structure of olive oil and soy lecithin with reference to sunflower oil.

Because of release profile, joined to the healthier benefits of olive oil,[25] soy lecithin, and olive oil were selected as the most interesting natural plasticizers in this work. Statistical analysis (Figure 1e) also showed statistical similarities among those film samples.

Commercial plasticizer Priplast 3114 also had a great activity, being chosen as commercial reference plasticizer for next experimental sections.

Antioxidant release was greatly improved comparing to results obtained when PE-PPG and PPG-PEG-PPG were used (release values between 1 to 2-fold higher when plasticizers are blended compared with release for chain extenders-blends).[16]

Effect of Concentration of Plasticizer and Nutrabiol T90 on the Release of Nutrabiol T90. Once soy lecithin, olive oil, and Priplast 3114 were selected as plasticizers, specific migration tests of blends 1, 2, 4, 5, 7–9 (Table 1) in simulant D1 were performed in order to determine the influence of plasticizers concentration on release. As it was expected, an increase in the amount of released Nutrabiol T90 can be observed when the concentration of plasticizers increased, but in a different rising depending on the type of plasticizer incorporated and the specific tocopherol homologue considered (Figure 2). Average increases from 43% to 66% were observed in the case of soy lecithin and Priplast 3114. In the case of δ- and γ-tocopherol, soy lecithin, and Priplast3114, release increased 44.3 and 19.9%, respectively. Nevertheless, when the concentration of olive oil was enhanced from 2 to 5%,

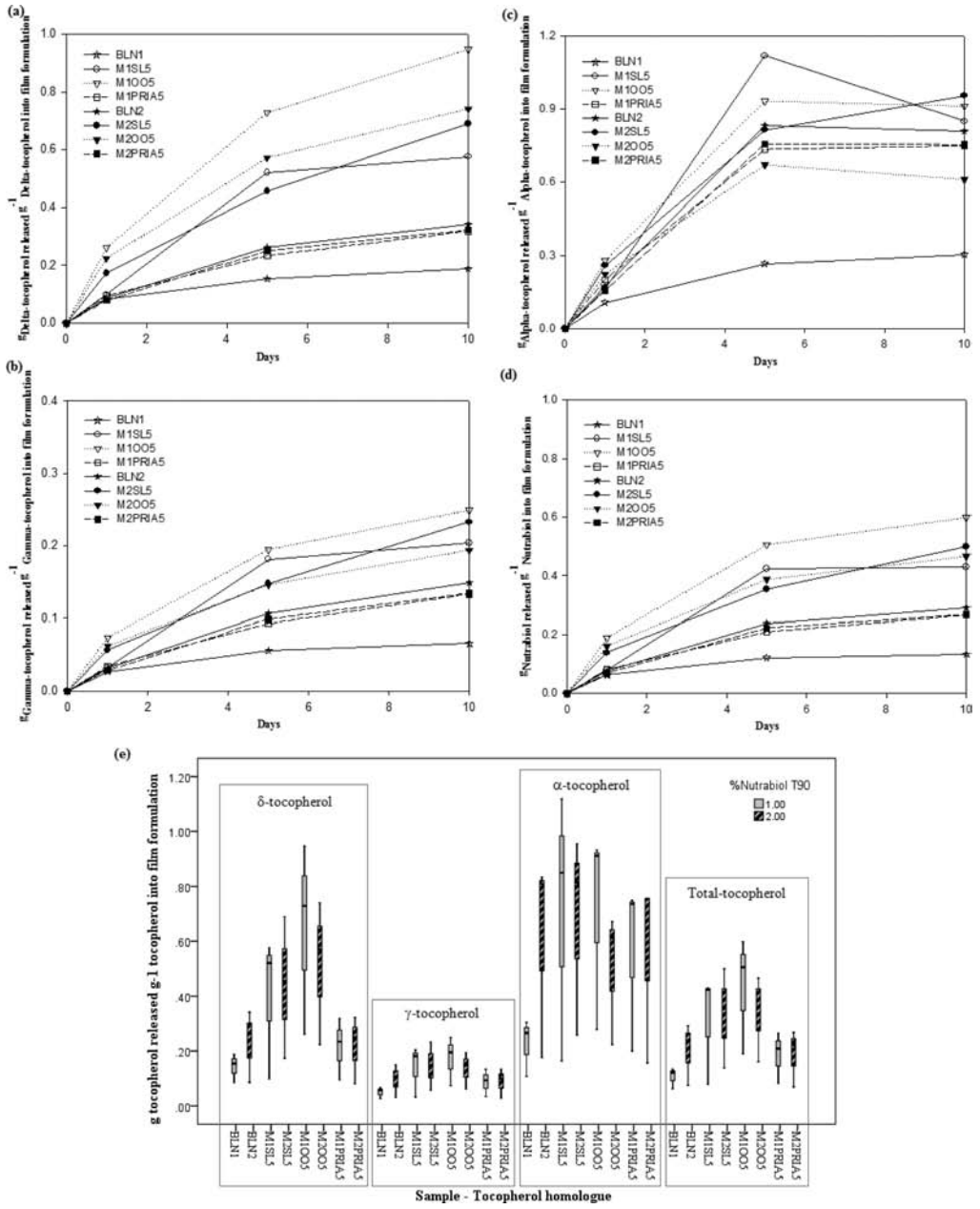

Figure 3. Migration of Nutrabiol T90 from blends with different concentrations of Nutrabiol T90 (simulant D1, 40 °C, 10 days) expressed as g of δ-tocopherol (a), γ-tocopherol (b), α-tocopherol (c), and total-tocopherol (Nutrabiol) (d) per gram of each tocopherol into film formulation. (f) Statistical comparison of release data through box plot (SPSS statictics software).

antioxidant release increased approximately 3-fold. However, an increment of 74% of α-tocopherol was observed when lecithin or Priplast3114 were incorporated versus 50% in the case of olive oil. This could suggest a possible influence of more or less compatibility between each plasticizer and each tocopherol analogue.

Statistical comparison between release data, considering the different samples studied and the two levels of plasticizers concentration (Figure 2e) also confirmed those differences between doping with 2 and 5% of plasticizer.

The release was then greatly improved compared with the results obtained with incorporation of 5% of PPG-PEG-PPG,[16] which released approximately 6%, while in the case of olive oil and soy lecithin, up to 42 and 59%, respectively, were released, when expressed as the amount of tocopherol migrated with reference to the amount present in the film (also considering losses during the extrusion process).

The enhancement of the antioxidant released becomes even more significant when related to the results obtained for reference samples, BLN1, reaching risings of 217 and 302%, when soy lecithin and olive oil were respectively incorporated.

As can be observed in Figure 2, at the end of storage time, the maximum had not yet been reached. Therefore, the release was expected to continue. After 20 days of contact time, release has still continued (data not shown), which seems to indicate a controlled release of the antioxidant with time.

Moreover, fixing the concentration of plasticizers at 5%, a study of the influence of concentration of Nutrabiol T90 incorporated was performed. Materials with 2% Nutrabiol T90, (blends 10−13, Table 1), were also exposed to selected

migration conditions for previous blends (40 °C, 10 days), and results were compared.

Figure 3 shows all of the release extents expressed as the amount of tocopherol released (in grams) per gram of tocopherol initially added and after considering the losses during the extrusion process. Both data for each individual tocopherol homologue and the total tocopherol content were considered. In general, when no plasticizers were added, antioxidant release in proportion to nominal content increased, which was also confirmed by the statistical analysis (Figure 3f). In particular, different release profiles were observed with each plasticizer. M1OO5 (PP-1% of Nutrabiol T90-5% of olive oil) clearly showed higher antioxidant release levels than the corresponding sample with 2% of Nutrabiol T90 (M2OO5). When soy lecithin blends are considered, it is clearly observed that during the first five days, a more gradual and slower increase on the release of Nutrabiol T90 is produced from samples with 2% of Nutrabiol T90 (M2SL5 vs M1SL5). Nevertheless, from that moment, the behavior is reversed and at 10 days, the amount released from sample M2SL5 is somewhat higher than that from M1SL5.

Concerning samples blended with commercial plasticizer Priplast 3114, both samples with 1 and 2% of Nutrabiol T90 (M1PRI5 and M2PRI5) showed similar release profiles along the entire period of contact time. No statistical differences were observed (Figure 3e) confirming those release studies.

The different behavior observed by changing the amount of antioxidant could be suggesting that, at least, two effects may be influencing the release of the process. Adding tocopherol into the films generally produces changes into their physical properties, which is commonly related with a plasticizing effect of the tocopherol in the films;[11,17] nevertheless, the blending of the natural plasticizers could also be changing the level of compatibility between PP and tocopherol. Therefore, a mixing effect over the release could be produced, giving place to those conflicting effects observed on the release.

Figures 2 and 3 show that, controlling Nutrabiol T90 and plasticizer concentration, different release rates and extents can be achieved, which have to be considered for future food conservation based in active food packaging.

Comparison between individual tocopherol homologues released (Figures 1a–c; 2a–c, and 3a–c) suggested that higher amounts of δ- and α-tocopherol with reference to the amount of each one initially added are released. It could suggest a different level of compatibility between matrix-plasticizer and each tocopherol homologue as a function of the stoichiometry of the latter. Nevertheless, the same release profile is observed in all cases regarding the type and amount of plasticizer and amount of Nutrabiol T90 blended into film formulations.

Materials M1SL5, M1OO5, M2SL5, and M2OO5 were selected as the most interesting for future food packaging applications. These materials and their respective references without plasticizers (samples BLN1 and BLN2) and with the synthetic plasticizer Priplast 3114 (samples M1PRI5 and M2PRI5) were characterized in following sections.

Thermal Analysis. The blends containing plasticizers were characterized by DSC to check for effects on the polymer thermal stability and crystallinity caused by the addition of the plasticizers. Results obtained from cooling and second heating processes are included in Table 2.

Because the compounds were melt-blended with the polymer at high temperatures, thermogravimetric analyses were also performed to determine the degradation temperature (T_{deg}) of the new materials and the thermal stability of the plasticizers.

Table 2. Thermal Properties Information of Developed Materials Obtained From TGA and DSC Analyses

	cooling		second heating	
material	T_c (°C)	$\Delta H_{c\ correg}$ (J g^{-1})	T_m (°C)	$\Delta H_{m\ correg}$ (J g^{-1})
BLN1	113.1 ± 0.2	29.6 ± 1.1	163.8 ± 0.5	25.0 ± 0.8
M1LS5	113.1 ± 0.2	31.1 ± 0.2	163.9 ± 0.7	25.8 ± 0.3
M1OO5	112.4 ± 0.1	30.0 ± 0.3	163.4 ± 1.1	24.9 ± 0.2
M1PRI5	113.3 ± 0.5	29.8 ± 1.3	164.9 ± 1.2	23.8 ± 1.4
BLN2	113.1 ± 0.2	30.1 ± 0.4	164.0 ± 0.2	24.1 ± 0.1
M2LS5	112.8 ± 0.3	29.8 ± 0.2	163.6 ± 0.2	24.1 ± 0.2
M2OO5	112.1 ± 0.1	29.8 ± 0.9	162.7 ± 0.5	24.0 ± 0.3
M2PRI5	113.3 ± 0.1	30.2 ± 0.6	164.4 ± 0.8	25.9 ± 1.6

During the cooling process, the polymer showed a crystallization exotherm with a maximum at approximately 113 °C and similar values for all samples. During the second heating, samples also presented similar features, temperatures, and enthalpies were in agreement with values reported in the literature for pure PP.[6] Presence of Nutrabiol T90 and the plasticizers did not produce significant changes on polymer crystallinity.

Concerning thermogravimetric analysis, samples blended with 1% of Nutrabiol T90 were analyzed, showing T_{deg} above 460.7 °C. Regardless of the plasticizer used, all samples showed similar T_{deg} to that of the blank sample (487.8 °C). Therefore, these findings reveal that the studied samples show similar thermal behavior under degradation conditions.

Rheological Properties. Rheological properties of the blended samples were investigated to evaluate the viscoelastic properties of the materials. Understanding the rheological behavior is also a very important aspect from the polymer processing perspective. Figure 4a shows complex viscosity versus frequency. It shows the onset of the Newtonian plateau for BLN1 at low frequencies, followed by a mildy shear-thinning behavior. This behavior is basically replicated for all of the formulations. The viscoelastic properties of polymers in the molten state are generally decreased in presence of a

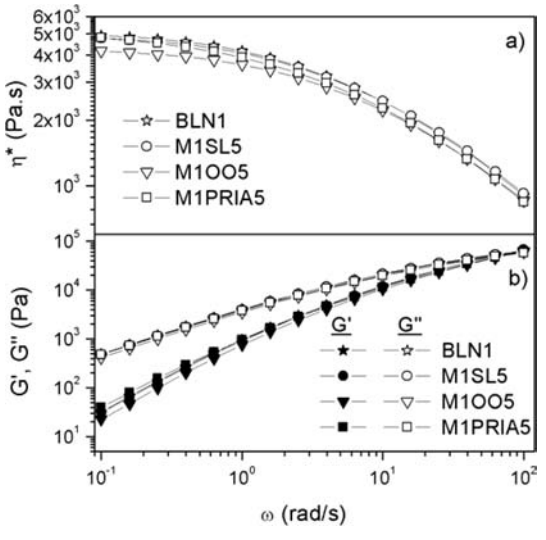

Figure 4. Dynamic viscosity (a) and dynamic moduli (b) versus frequency of PP and different formulations.

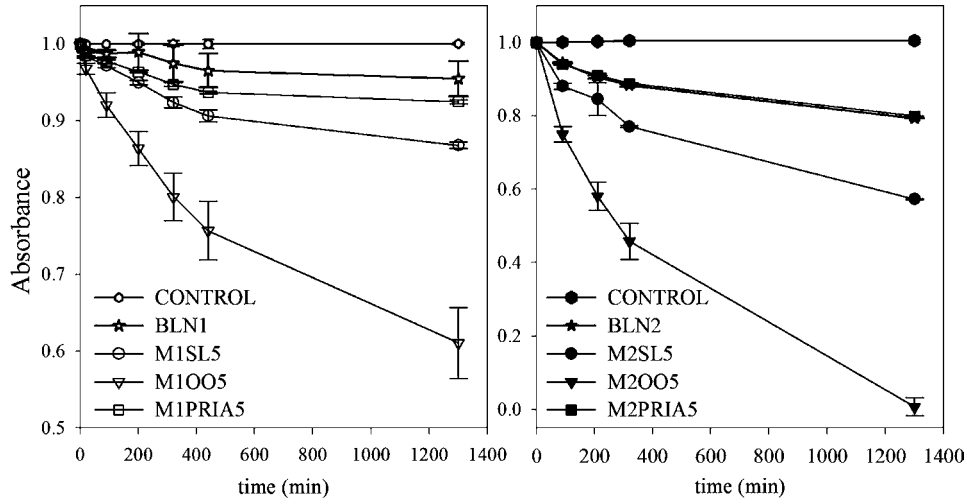

Figure 5. Radical scavenging activity of $ABTS^{\bullet+}$ radical of developed materials with 1%Nutrabiol T90/5%plasticizer (a) and 2%Nutrabiol T90/5% plasticizer (b).

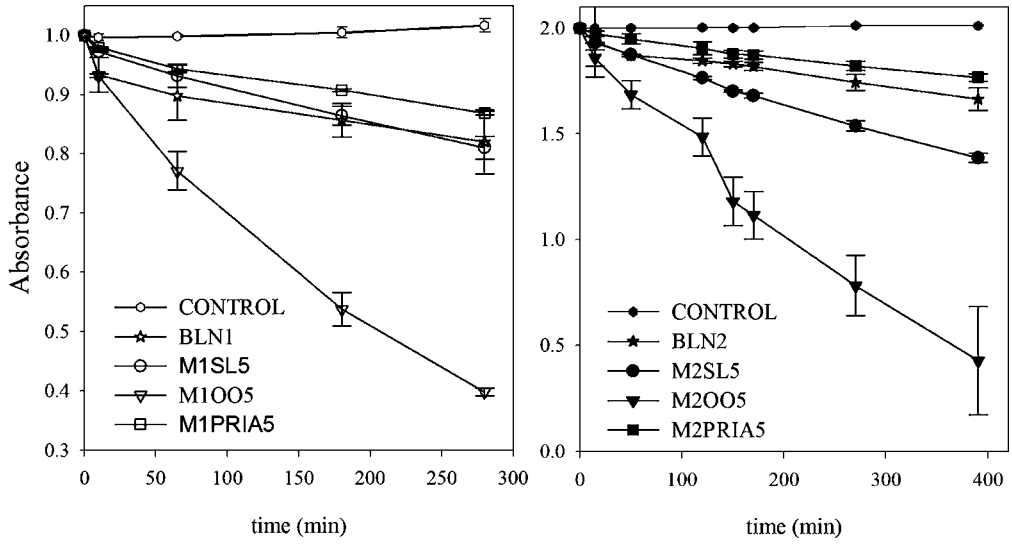

Figure 6. Radical scavenging activity of $DPPH^{\bullet}$ radical of developed materials with 1%Nutrabiol T90/5%plasticizer (a) and 2%Nutrabiol T90/5% plasticizer (b).

plasticizer.[25,26] In this case, the addition of plasticizers does not dramatically change either the viscosity (Figure 4 a) or the storage modulus (G') (Figure 4b) with respect to PP; only minor effects are observed upon addition of Priplast 3114 and olive oil plasticizers. Accordingly, if a higher release of plasticizer is desired, then it could be possible to add higher concentrations of the latter without any deleterious effect on viscoelastic properties.

Furthermore, no effect in loss modulus (G'') was observed with the addition of plasticizers in comparison with PP, indicating that the elastic part is more affected than the viscous part. Finally, in all frequency ranges, G'' is higher than G', showing liquid-viscoelastic behavior.

Antiradical Scavenging Activities Results. ABTS and DPPH assays measure the ability of materials for trapping free radicals by donating hydrogen atoms or electrons, producing in consequence the bleaching of the colored radical solutions. As blends were hot pressed and exposed to European legislation parameters, these results refer to the antioxidant activity that a 1L package (made with these films) would provide to the packaged product.

The main mechanism of the antioxidant action of the materials developed is through the release of the antioxidant compounds in the food product that is being protected, and in this case, the liquid environment where radicals were dissolved. Therefore, the release and the antioxidant activity were related to the solubility of the antioxidants in the solvent.

Control solutions of radicals $ABTS^{\bullet+}$ and $DPPH^{\bullet}$ were carried out to monitor their stabilities. As Figures 5 and 6 show, it was found that the order of radical scavenging activity of the materials developed was in accordance with the antioxidant release values obtained in previous assays, showing the great antioxidant activities of the samples with olive oil incorporated. According with the results obtained from release studies, reference materials without plasticizers, BLN1 and BLN2, exhibited very low radical scavenging activities, followed by the samples with commercial plasticizer Priplast 3114 incorporated. When compared with release data (Figure 3), the same statement is concluded. Olive oil performed well, favoring the

net amount of antioxidant released during a longer contact time, with increased tocopherol release levels among 2-fold and 4-fold higher from those olive oil films than the correspondent blanks (blends without plasticizers) were observed. Percentage of tocopherol release up to 50% and 59% were obtained after 5 and 10 days of contact time, respectively. Moreover, arousing higher tocopherol availability to be released, has also meant achieving high radical scavenging capacity.

Due to the better solubility of tocopherols in ethanol, $DPPH^{\bullet}$ scavenging activities results were quite a bit higher for all materials, and differences in antioxidant activities between samples increased as well. As Figures 5 and 6 show, samples with incorporated olive oil presented the highest bleaching rate, and thus, higher activity, especially the sample M1OO5 with the highest levels compared to the others with double the content of antioxidant.

Similar values are reached when release and antioxidant activity data of tocopherols from olive oil and the rest of the samples are compared. Increments are calculated in release data as the amount of tocopherol released from olive oil samples versus the amount released from the other samples. The same increments are calculated with antioxidant activity data. Increments ranging from 1.5 to 4-fold higher for tocopherol from olive oil samples are observed in both cases.

Differences between release and antioxidant activity data from olive oil samples could be expected based on the reported presence of other antioxidants in olive oil, particularly phenolic compounds or carotenoids.[32] Moreover, I168, an antioxidant added to protect films during the extrusion, could also be released. Its presence was checked after the extrusion process and release contact time following the same methods described for tocopherol. As was expected, very low amounts remained after the extrusion process (0.23% of the initial added amount). Levels of migration well under 1×10^{-04} g of I168 per each gram initially added were observed in all of the cases studied. Therefore, those results suggest that good correlation between release and antioxidant active data can be established.

In general, natural plasticizers containing materials, mainly in the case of olive oil, presented better characteristics for the production of an all-purpose active package. The main conclusion is that by controlling Nutrabiol T90 and plasticizer concentration, different antioxidant activities can be achieved; therefore, a suitable food packaging material can be designed depending on the concentration of antioxidant needed for food product conservation.

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Notes

The authors declare no competing financial interest.

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ABBREVIATIONS USED

(ABTS),2,2′-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid); (DPPH),2,2-diphenyl-1-picrylhydrazyl; Conc. Plast,concentration of plasticizer onto film blend; (Tdeg),degradation temperature; (DSC),differential scanning calorimetry; (FL),fluorescence detector; (Irgafos 168, I168),Irgafos 168 Tris(2,4-ditert-butylphenyl) phosphate; (LVE),linear viscoelastic region; (LDPE),low density polyethylene; (NUT),Nutrabiol T90; (OO),olive oil; (PDA),photodiode array detector; (PP),polypropylene; (PEG),polyethylene glycol; (PPG),polypropylene glycol; (PRIB),Priplast 3019; (PRIA),Priplast 3114; (SL),soy lechitin; (GRAS),substances generally recognized as safe; (SO),sunflower oil; (TGA),thermogravimetric analyses

REFERENCES

(1) Miltz, J.; Passy, N.; Mannecheim, C. H. Trends and Applications of Active Packaging Systems. In *Foods and Packaging Materials Chemical Interactions*; Ackermann, P., Jägerstad, M., Ohlsson, T.,Eds.; The Royal Society of Chemistry: Cambridge, 1995; pp 201, 210.

(2) Granda-Restrepo, D. M.; Soto- Valdez, H.; Peralta, E.; Troncoso-Rojas, R.; Graciano-Verdugo, A. Z. Migration of α-Tocopherol from an Active Multilayer Film into Whole Milk Powder. *Food Res. Int.* **2009**, *42*, 1396−1402.

(3) López-de-Dicastillo, C.; Gómez-Estaca, J.; Catala, R.; Gavara, R.; Hernandez-Muñoz, P. Active Antioxidant Packaging Films: Development and Effect on Lipid Stability of Brined Sardines. *Food Chem.* **2012**, *131*, 1376−1384.

(4) López de Dicastillo, C.; Nerin, C.; Alfaro, P.; Catalá, R.; Gavara, R.; Hernández-Muñoz, P. Development of New Antioxidant Active Packaging Films Based on Ethylene Vinyl Alcohol Copolymer (EVOH) and Green Tea Extract. *J. Agric. Food Chem.* **2011**, *59*, 7832−7840.

(5) Nerin, C.; Tovar, L.; Djenane, D.; Camo, J.; Salafranca, J.; Beltran, J. A.; Roncales, P. Stabilization of Beef Meat by a New Active Packaging Containing Natural Antioxidants. *J. Agric. Food Chem.* **2006**, *54*, 7840−7846.

(6) Pereira de Abreu, D. A.; Paseiro Losada, P.; Maroto, J.; Cruz, J. M. Natural Antioxidant Active Packaging Film and Its Effect on Lipid Damage in Frozen Blue Shark (*Prionace glauca*). *Innov. Food Sci. Emerg. Tech.* **2011**, *12*, 50−55.

(7) Vermeiren, L.; Devlieghere, F.; Van beest, M.; De Kruijf, N.; Debevere, J. Developments in the Active Packaging of Foods. *Trends Food Sci. Technol.* **1999**, *10*, 77−86.

(8) Barbosa-Pereira, L.; Cruz, J. M.; Sendón, R.; Rodríguez-Bernaldo de Quirós, A.; Ares, A.; Castro-López, M.; Abad, M. J.; Maroto, J.; Paseiro-Losada, P. Development of Antioxidant Active Films Containing Tocopherols to Extend the Shelf Life of Fish. *Food Control* **2013**, *31*, 236−243.

(9) Graciano-Verdugo, A. Z.; Soto-Valdez, H.; Peralta, E.; Cruz-Zárate, P.; Islas-Rubio, A. R.; Sanchez-Valdes, S.; Sánchez-Escalante, A.; González-Méndez, N.; González-Rios, H. Migration of α-Tocopherol from LDPE Films to Corn Oil and Its Effect on the Oxidative Stability. *Food Res. Int.* **2010**, *43*, 1073−1078.

(10) Wessling, C.; Nielsen, T.; Giacin, J. R. Antioxidant Ability of BHT and α-Tocopherol Impregnated LDPE Film in Packaging of Oatmeal. *J. Sci. Food Agric.* **2001**, *81*, 194−201.

(11) Koontz, J. L.; Marcy, J. E.; ÓKeefe, S. F.; Duncan, S. E.; Long, T. E.; Moffit, R. D. Polymer Processing and Characterization of LLDPE Films Loaded with A-Tocopherol, Quercetin, And Their Cyclodextrin Inclusion Complexes. *J. Appl. Polym. Sci.* **2010**, *117*, 2299−2309.

(12) Laemer, S. F.; Zambetti, P. F. α-Tocopherol (Vitamin E)—The Natural Antioxidant for Polyolefins. *J. Plast. Film Sheet.* **1992**, *8*, 228−249.

(13) Al-Malaika, S.; Goodwin, C.; Issenhuth, S.; Burdick, D. The Antioxidant Role of α-Tocopherol in Polymers. II. Melt Stabilizing Effect in Polypropylene. *Polym. Degrad. Stab.* **1999**, *64*, 145−156.

(14) Wessling, C.; Nielsen, T.; Leufven, A.; Jagerstad, M. Retention of α-Tocopherol in Low-Density Polyethylene (LDPE) and Polypropylene (PP) in Contact with Foodstuffs and Food Simulating Liquids. *J. Sci. Food Agric.* **1999**, *79*, 1635−1641.

(15) Chen, X.; Lee, D. S.; Zhu, X.; Yam, K. L. Release Kinetics of Tocopherol and Quercetin from Binary Antioxidant Controlled Release Packaging Films. *J. Agric. Food Chem.* **2012**, *60*, 3492−3497.
(16) Castro López, M. M.; Dopico-García, S.; Ares-Pernas, A.; López-Vilariño, J. M.; Gonzalez-Rodriguez, M. V. Effect of PPG-PEG-PPG on the Tocopherol Controlled Release from Intended for Food Packaging Applications. *J. Agric. Food Chem.* **2012**, *60*, 8163−8170.
(17) Zhu, X.; Lee, D. S.; Yam, K. L. Release Property and Antioxidant Effectiveness of Tocopherol-Incorporated LDPE/PP Blend Films. *Food Addit. Contam.* **2012**, *29*, 461−468.
(18) Kowalczyk, M.; Pluta, M.; Piorkowska, E.; Krasnikova, N. Plasticization of Polylactide with Block Copolymers of Ethylene Glycol and Propylene Glycol. *J. Appl. Polym. Sci.* **2012**, *125*, 4292−4301.
(19) Kulinski, Z.; Piorkowska, E.; Gadzinowska, K.; Stasiak, M. Plasticization of Poly(L-lactide)with Poly(propylene glycol). *Biomacromolecules* **2006**, *7*, 2128−2135.
(20) Siepmann, F.; Sielpmann, J.; Walther, M.; MacRae, R. J.; Bodmeier, R. Polymer Blends for Controlled Release Coatings. *J. Controlled Release* **2008**, *125*, 1−15.
(21) Adeodato-Vieira, M. G.; Altenhofen da Silva, M.; Oliveira dos Santos, L. Natural-Based Plasticizers and Biopolymer Films: A Review. *Eur. Polym. J.* **2011**, *47*, 254−263.
(22) Canty, D. J.; Zeisel, S. H. Lecithin and Choline in Human Health and Disease. *Nutr. Rev.* **1994**, *52* (10), 327−329.
(23) Higgins, J. P.; Flicker, L. Lecithin for Dementia and Cognitive Impairment. *Cochrane Database Syst. Rev.* **2003**, *3*, CD001015.
(24) Wypych, G. Expectations from Plasticizers. In *Handbook of Plasticizers*; William Andrew Publishing: Toronto (Canada), 2004, 1−4.
(25) Xu, Y.; You, M.; Qu, J. Melt Rheology of Poly(lactic acid) Plasticized by Epoxidized Soybean Oil. *Wuhan Univ. J. Natl. Sci.* **2009**, *14* (4), 349−354.
(26) Nanda, M.; Tripathy, D. K. Rheological Behavior of Chlorosulfonated Polyethylene Composites: Effect of Filler and Plasticizer. *J. Appl. Polym. Sci.* **2012**, *126*, 46−55.
(27) Commission Regulation (EU) No 10/2011 of 14 January 2011 on plastic materials and articles to come into contact food.
(28) Okada, Y.; Okada, M. J. Scavenging Effect of Water Soluble Proteins in Broad Beans on Free Radicals and Active Oxygen Species. *J. Agric. Food Chem.* **1998**, *46*, 401−406.
(29) Prior, R. L.; Wu, X. L.; Schaich, K. Standardized Methods for the Determination of Antioxidant Capacity and Phenolics in Foods and Dietary Supplements. *J. Agric. Food Chem.* **2005**, *53*, 4290−4302.
(30) Sanchez-Moreno, C. Review: Methods Used to Evaluate the Free Radical Scavenging Activity in Foods and Biological Systems. *Food Sci. Technol. Int.* **2002**, *8*, 121−137.
(31) Barbosa-Pereira, L.; Cruz, J. M.; Sendón, R.; Rodríguez-Bernaldo de Quirós, A.; Ares, A.; Castro-López, M.; Abad, M. J.; Maroto, J.; Paseiro-Losada, P. Development of Antioxidant Active Films Containing Tocopherols to Extend the Shelf Life of Fish. *Food Control* **2013**, *31*, 236−243.
(32) Dimitrios, B. Sources of Natural Phenolic Antioxidants. *Trends Food Sci. Technol.* **2006**, *17*, 505−512.