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ARTICLE

Immobilization of Caffeic Acid on a Polypropylene Film: Synthesis and Antioxidant Properties

Dario Arrua,[†] Miriam Cristina Strumia,[‡] and Mónica Azucena Nazareno*,[†]

[†]INQUINOA-CONICET, Facultad de Agronomía y Agroindustrias, Universidad Nacional de Santiago del Estero, Avenida Belgrano (S) 1912 (CP 4200), Santiago del Estero, Argentina, and [‡]Departamento de Química Orgánica, Facultad de Ciencias Químicas, Universidad Nacional de Córdoba, Ciudad Universitaria (CP 5000) Córdoba, Argentina

The immobilization of caffeic acid (CA) on a polypropylene (PP) film was successfully performed through the covalent binding of the caffeoyl chloride on a modified polymeric surface of PP films grafted with hydroxyethyl methacrylate as monomer (PP-*g*-HEMA). The different reaction steps were monitored by FT-IR spectroscopy. The synthesized films were characterized by Folin−Ciocalteu method by measuring the available phenolic groups as caffeic acid equivalents linked to the surface. The antioxidant efficiency of the modified polymers was evaluated by typical spectrophometric methods, such as the bleaching of radicals DPPH$^{\bullet}$ and ABTS$^{\bullet+}$, and the inhibition of the enzymatically induced coupled oxidation of linoleic acid and betacarotene. The available phenolic groups on the modified film presented a good correlation with the antiradical activity toward DPPH$^{\bullet}$. Moreover, the polymer synthesized in this work showed a good protective activity against ascorbic acid oxidation in real samples of orange juice.

KEYWORDS: Antioxidant; active packaging; polyphenols; antiradical activity; grafting

INTRODUCTION

Nowadays there is a great interest in the research and development of "active packaging" in both the food and pharmaceutical industries. An active packaging is able to interact directly with the product and/or its surroundings to improve one or more aspects of its quality or safety (*1*). The main aim of active packaging is to have extra functions (related with the quality, safety, and integrity of the product), which could be obtained by incorporating active functional groups on the surface of packaging systems.

Among the main advantages of synthetic polymers utilized in packaging industries such as polypropylene, poly(vinyl chloride), and polyethylene, their excellent physicochemical properties as well as the possibility of their being processed and their low cost can be mentioned. However, these commodities present an inert surface. Therefore, one of the approaches to obtain active packages from synthetic polymers is carried out using surface modification techniques through chemical processes. Photografting reactions are among these techniques and consist of the use of energy for the formation of free radicals on the polymer surface and the further polymerization reaction. Through this process, a polymer with an active surface is obtained as a consequence of the grafting of polymeric chains, where the functionality will depend on the monomers used (*2*−*4*).

Several undesirable alterations that occur in foods, beverages, and pharmaceuticals are due to the oxidation of organic molecules by the action of radical species formed in the product by endogenous as well as exogenous factors. Therefore, the presence of antioxidants is very important to prevent these deleterious reactions and extend the product shelf life (*5*). As the oxidation reactions are a serious problem for the food industry, currently synthetic antioxidants are used, butylated hydroxytoluene (BHT) and butylated hydroxyanisole (BHA) being the most common. However, the use of synthetic antioxidants is increasingly questioned by consumers because of their health risks and toxicity (*6*,*7*). In this sense, the use of antioxidants obtained from natural sources has interesting advantages given their recognized safety when compared to synthetic ones. Among natural phenolic compounds, hydroxy and polyhydroxy derivates of cinnamic acids such as caffeic, coumaric, and ferulic acids have been reported as good free radical scavengers. Their reactivity against this unpaired-electron species is dependent on both the number of phenolic groups present in the molecule and the substitution pattern in the aromatic ring (*8*,*9*). In a comparative study of the antioxidant and antiradical capacities of a series of polyphenols, caffeic acid was among the most active compounds (*10*).

In addition, a direct dissolution of the antioxidant in the food matrix is sometimes inconvenient because it would result in undesirable alteration in the product composition. In this case, the development of polymeric materials containing the active moiety covalently bound to their surface is an interesting alternative. Nowadays, the food industry has increasing interest in the development of polymeric films with antioxidant properties. Using this polymer modified on its surface, the antioxidant groups are directly in contact with the medium where the free radicals species are generated without contaminating the matrix.

*Author to whom correspondence should be addressed (phone +54-385-4509500, int. 1617; fax +54-385-4509525; e-mail nazareno@unse.edu.ar).

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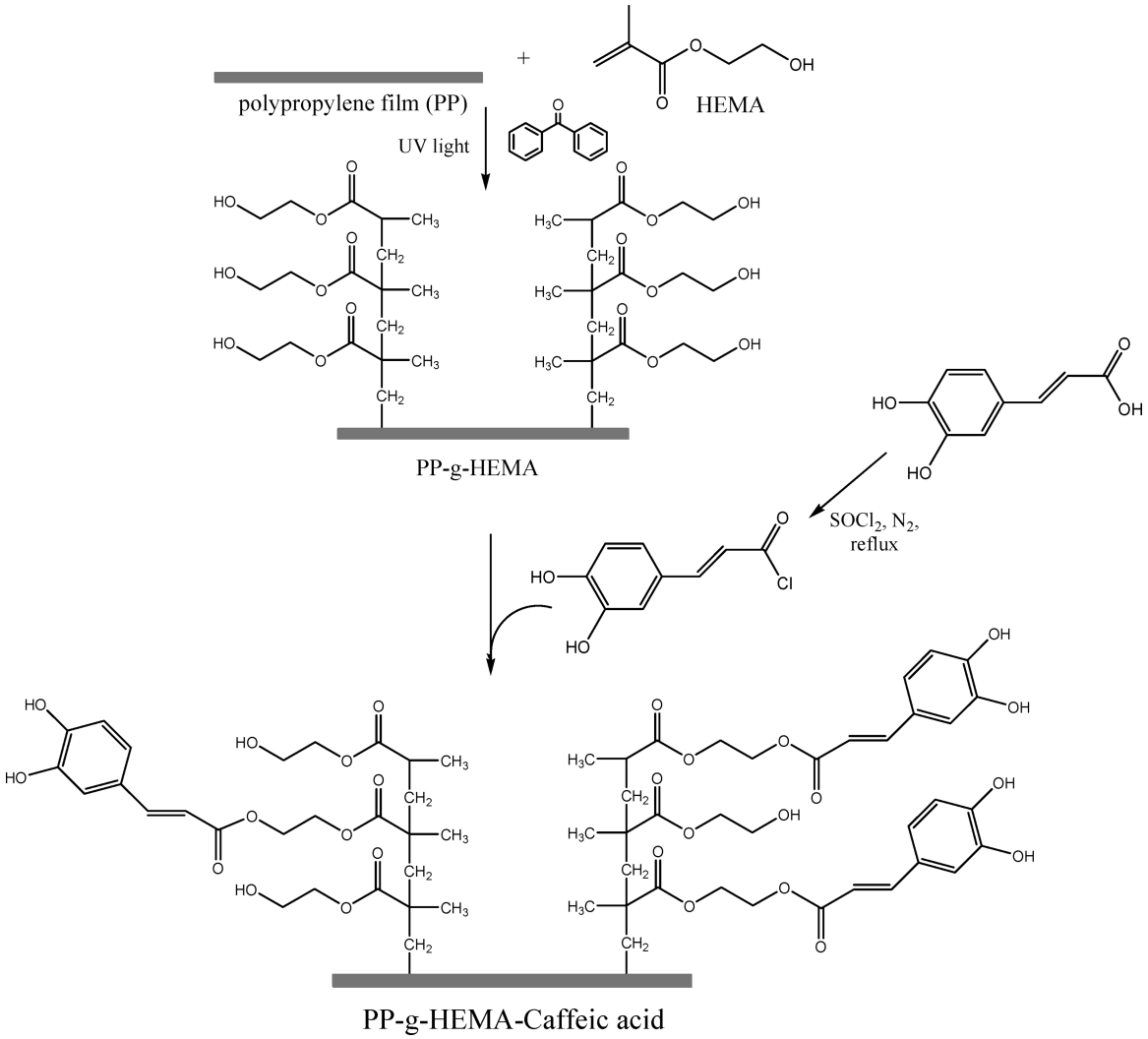

Figure 1. Representation of the sequential reactions steps for caffeic acid covalent immobilization on polypropylene films.

In this sense, Nerín et al. (*11*) have successfully designed antioxidant PP films by incorporating rosemary extracts containing essential oils and phenolic compounds. This complex mixture of antioxidants is fixed to the matrix, but the nature of the interactions among the active compounds and the polymers has not been discussed.

Another interesting approach concerning active polymers that has recently been developed is the covalent binding of antioxidant compounds to natural (*12*, *13*) and synthetic polymers (*14*, *15*) as well as their use as comonomers (*16*). Even though these materials could have interesting uses, they were not presented as a film format.

Taking into account the precedents above-mentioned, the main aim of this work was the binding of a natural antioxidant to a polypropylene (PP) film surface. The functionalization of PP films by introducing a hydroxyethyl methacrylate (HEMA) monomer by UV-induced photografting reactions and the use of the introduced functional groups in further reactions toward the covalent linkage of caffeic acid were investigated. Characterization of the modified film was carried out, and its antioxidant activity and protective ability toward a real food sample were evaluated and compared to the degree of caffeic acid incorporation.

MATERIALS AND METHODS

Chemicals. PP films of 32 μm thickness were kindly provided by Converflex-Arcor (Córdoba, Argentina). All commercially available chemicals were purchased from Sigma-Aldrich (Buenos Aires, Argentina). HEMA, caffeic acid (97%), thionyl chloride, Folin−Ciocalteu reagent, linoleic acid, β-carotene (95%), and lipoxidase preparation, type I-B, from soybean were from Aldrich; benzophenone, 2,2-diphenyl-1-picrylhydrazyl radical (DPPH$^{\bullet}$), and 2,2′-azinobis(3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt (ABTS) were from Fluka. All chemicals were used as received. Tetrahydrofuran (THF) was freshly distilled over benzophenone.

Equipment. The FTIR spectra were performed on films in the transmission mode with a resolution of 8 cm^{-1} and 32 scans using a Nicolet 5 SXC apparatus (Madison, WI). UV−vis absorption spectra were obtained with a Unicam UV2 spectrophotometer (Unicam, Cambridge, U.K.). Quantitative analysis of ascorbic acid in natural juice samples was performed by high-performance liquid chromatography (HPLC). The determinations were carried out using a Lab Alliance series III liquid chromatograph (Lab Alliance, Alvarado, TX) coupled with a Shimadzu diode array detector (Shimadzu Scientific Instruments, Columbia, MD). The column used was a 250 mm × 4.6 mm i.d., 5 μm, SS Wakosil RP-18, with a 4 mm × 4 mm i.d. guard column of the same material (SGE Inc., Ringwood, Australia).

Preparation of Polypropylene Films Containing Caffeic Acid. The covalent immobilization of caffeic acid on PP films was achieved through two subsequent chemical reactions: first, the UV-initiated grafting polymerization of polypropylene films using HEMA as monomer, and, second, the esterification reaction between the hydroxyl groups present in the surface of polymeric films and the caffeoyl chloride. The mentioned reactions are represented in **Figure 1**.

Grafting Copolymerization Reaction. The reactions were carried out following the method used by Costamagna et al. (*2*) to obtain PP-*g*-HEMA films. Briefly, the PP films were cut in 10 cm diameter circular pieces and washed with distilled water and dried under vacuum at room

temperature to a constant weight. Then, the piece of film was placed in a glass Petri dish, and 0.5 mL of 0.2 M solution of the initiator benzophenone in the HEMA monomer and 0.5 mL of water were added. The dish containing the reactive materials was placed in a photoreactor designed in our laboratory and irradiated with UV light (medium-pressure UV lamp; Engenlhard-Hanovia, Slough, U.K.) under a nitrogen atmosphere at room temperature for 15 min. Once the grafting reaction finished, the grafted films were extensively washed with a pH 8 NaOH solution before analysis and finally with distilled water to remove traces of the unreacted monomer and the free homopolymer chains formed. The samples were dried under vacuum at room temperature to constant weight. The grafting yield was determined by gravimetric measurements as the mean of five single experiments using the equation

$$\text{grafting } (\%) = [(W_{gf} - W_{ngf})/W_{ngf}] \times 100 \quad (1)$$

where W_{gf} and W_{ngf} are the dry weights of grafted and nongrafted films, respectively.

Immobilization of Caffeic Acid on PP-*g*-HEMA Films. These reactions were performed under anhydrous conditions with a nitrogen flow. First, 0.4 g of caffeic acid and 0.84 mL of thionyl chloride were dissolved in 30 mL of anhydrous THF. Then, the reaction was allowed to proceed at reflux for 6 h. After that, the THF and thionyl chloride were evaporated under vacuum, and a brown solid was obtained, which correspond to the caffeoyl chloride. Then, 0.300 g of PP-*g*-HEMA, 66 μL of triethylamine, and 30 mL of anhydrous THF were added to the reaction flask containing this acyl chloride. The coupling reaction was carried out at reflux for 10 h. Once the reaction had finished, PP-*g*-HEMA-CA films were washed twice with 50 mL of THF and three times with 50 mL of methanol to eliminate the unreacted agents. Afterward, the modified film was dried under vacuum to constant weight.

Determination of Available Phenolic Groups on the Modified Film. This was determined using the Folin−Ciocalteu method (*17*) with some modifications. In a volumetric flask 100 mg of PP-*g*-HEMA-CA film, 1 mL of Folin−Ciocalteu reagent, and 10 mL of distilled water were placed and stirred. After 3 min, 4 mL of 2% Na_2CO_3 and 10 mL of water were added to a final volume of 25 mL. The reaction was kept at room temperature for 48 h. After that, the absorbance was determined at 760 nm against a control sample of PP-*g*-HEMA film prepared under the same reaction conditions. An experiment in the absence of film was also performed and used as blank for these measurements. To determine the total phenolic content of PP-*g*-HEMA-CA films, a calibration curve was prepared using caffeic acid standard solutions between 5 and 25 μM. The results were expressed as micromoles of caffeic acid per gram of dry polymer. All determinations were performed in triplicate.

Determination of Antiradical Activity of the Synthesized Film. The radical scavenging properties of PP-*g*-HEMA-CA films were evaluated against two different radicals using their well-known bleaching methods: 2,2-diphenyl-1-picrylhydrazyl radical (DPPH$^\bullet$) (*18*) and 2,2′-azinobis-(3-ethylbenzothiazoline-6-sulfonic acid) radical cation (ABTS$^{\bullet+}$) (*19*). All determinations were performed in triplicate.

DPPH$^\bullet$ Bleaching Method. Approximately 40 mg equivalent to 26 cm^2 of PP-*g*-HEMA-CA films was placed in a volumetric flask, containing 25 mL of DPPH$^\bullet$ solution in methanol (initial absorbance at 515 nm equal to 1.00 ± 0.01 AU). The remaining DPPH$^\bullet$ was determined by monitoring absorbance changes at 515 nm for 30 min. The antiradical activity (ARA%) of the polymeric films was calculated according to the equation

$$\text{ARA\%} = 100 \times (1 - A_{ss}/A_0) \quad (2)$$

where A_{SS} is the absorbance of the solution in the steady state and A_0 is the absorbance of DPPH$^\bullet$ solution before the addition of the active film. The absorbance of the system in the steady state was estimated by mathematical fitting of kinetic curves performed with Origin 7.0 software. In the time scale of these experiments, the radical depletion measured as a control assay in the absence of polymers or caffeic acid addition was < 1%. These radical consumption values were negligible and had no effect in the calculations of activity according to eq 2. The antioxidant capacity of PP-*g*-HEMA-CA films was expressed in ARA%/100 mg of film as well as in micromoles of caffeic acid per gram of film. For this purpose, a calibration curve was prepared with a series of standard solutions of caffeic acid. Aliquots of 1 mL of each standard solution was added to 25 mL of DPPH$^\bullet$ solution in methanol to reach final concentrations between 5 and 25 μM. Absorbance changes were monitored at 515 nm for 30 min. Then, the percentage of the radical disappearance was determined according to eq 2, and a calibration curve was obtained by plotting ARA% versus caffeic acid concentration. A control reaction was performed using PP-*g*-HEMA films.

ABTS$^{\bullet+}$ Bleaching Method. ABTS was dissolved in distilled water to yield a 7 mM solution. Radical cation solution was prepared by incubating 1 mL of ABTS solution with 3.42 mL of 2.45 mM potassium persulfate solution for 16 h in the dark at room temperature and subsequently diluted with water to an absorbance of 1.00 ± 0.01 AU at 734 nm. To determine the antiradical capacity of PP-*g*-HEMA-CA films, approximately 40 mg of film was placed in a volumetric flask, containing 25 mL of ABTS$^{\bullet+}$ solution, and the absorbance decrease at 734 nm was evaluated for 30 min. All determinations were performed in triplicate. The ARA% for ABTS$^{\bullet+}$ was calculated according to eq 2. The radical scavenging activity of PP-*g*-HEMA-CA films was expressed in ARA%/100 mg of film as well as in micromoles of caffeic acid per gram of film. For this purpose, a calibration curve was prepared with a series of standard solutions of caffeic acid. Aliquots of 1 mL of each standard solution was added to 25 mL of ABTS$^{\bullet+}$ solution in methanol to reach to final concentrations between 0.1 and 1.5 μM. The absorbance of the system was monitored at 734 nm for 30 min. Then, the percentage of the radical disappearance was calculated according to eq 2, and a calibration curve was obtained plotting ARA% versus caffeic acid concentration. A control reaction was performed using PP-*g*-HEMA films. In the time scale of these experiments, the radical depletion measured as a control assay in the absence of polymers or caffeic acid addition was < 1% and neglected.

Antioxidant Capacity of the Modified Films in the β-Carotene−Linoleic Acid Cooxidation Induced by Lipoxygenase (LOX). The experiments were carried out according to the procedure of Chaillou and Nazareno (*10*) with minor modifications. First, linoleic acid solution was prepared by mixing this compound with 219 mg of Tween 20 and diluting with 25 mM borate buffer (pH 9.0) to a final concentration of 3.6 mg/mL. An aliquot of 1 mL of a saturated stock solution of β-carotene in chloroform was mixed with 1.1 g of Tween 20. Chloroform was removed using a nitrogen stream for 20 min, and the final β-carotene aqueous solution was prepared by adding pH 9.0 borate buffer. Once these solutions were prepared in a volumetric flask containing 50 mg of polymeric film, 0.55 mL of linoleic acid solution, 5.0 mL of β-carotene solution, and 10 mL of borate buffer (pH 9.0) were added. The β-carotene initial absorbance was adjusted to 1.00 ± 0.01 AU. Finally, an aliquot of 1 mL of 1.0 mg/mL LOX solution was added to initiate the reaction, which was followed by monitoring the absorbance at 460 nm for 25 min. As a control assay the same procedure was performed excluding the polymeric film addition. Antioxidant activity (AOA) was calculated as suggested in the literature (*20*) as the percentage inhibition of the β-carotene bleaching by the polymeric film compared to that of the control.

Antioxidant Capacity of Polymeric Films in Orange Juice. The protective ability of the films synthesized against ascorbic acid oxidation in natural orange juice was tested by putting in contact the PP-*g*-HEMA-CA films with juice samples. For this purpose, orange juice was freshly squeezed and subsequently filtered. An amount of 60 mg of dry films equivalent to 39 cm^2 was added in a flask containing 25 mL of the orange juice, and the mixture were stirred at 40 °C for 29 h to allow ascorbic acid decomposition. As a control to determine the natural disappearance of ascorbic acid under the same conditions, an experiment was performed with orange juice without film. The antioxidant capacity of PP-*g*-HEMA-CA films was also compared with that of a caffeic acid solution. In this experiment, an aliquot of a caffeic acid solution containing 0.2930 μmol was added to the 25 mL orange juice system. The depletion of ascorbic acid concentration was monitored by HPLC. The ascorbic acid concentration in the system was determined by taking 1 mL from the orange juice and placing this aliquot in a vial containing 1 mL of a metaphosphoric acid−acetic acid mixture. The sample was filtered and immediately injected in the liquid chromatograph. The injection volume was 20 μL. The mobile phase used was pH 2.5 H_2SO_4 aqueous solution at 1 mL/min at 24 ± 1 °C. The detection wavelength was 254 nm. A calibration curve was performed with authentic samples of ascorbic acid in a concentration range between 15 and 450 μg/mL.

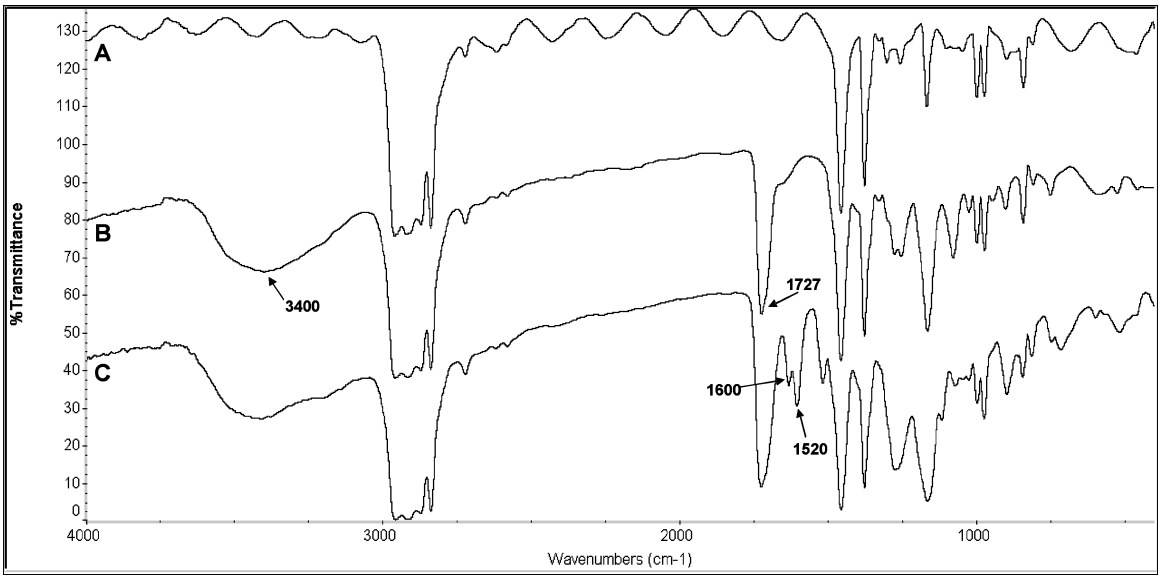

Figure 2. FTIR spectroscopic changes after polypropylene (PP) film modification (grafting with HEMA monomer) and caffeic acid binding on the film surface: (**A**) PP; (**B**) PP-*g*-HEMA; (**C**) PP-*g*-HEMA-CA.

RESULTS AND DISCUSSION

Grafting Copolymerization Reaction. Surface grafting polymerization enables the introduction of specific properties derived from the grafted layer while also preserving the bulk and structural properties of the underlying material. The introduction of functional groups on the polymeric films via grafting polymerization is a versatile approach for the preparation of materials with the controlled incorporation of functional groups (*21*). In this work, the chemical modifications of the PP surfaces were carried out by radical grafting polymerization initiated by UV light using HEMA as the grafting monomer according to the conditions proposed by Costamagna et al. (*4*). The yield of chemical modification on the surface was evaluated by gravimetric measurements, obtaining 11.9% (standard deviation = 0.7%) of grafting yield. For surface modification applications, thick grafting layers are unnecessary and even undesirable because they may change the physical properties of the bulk polypropylene film. The presence of water in the reaction medium promotes the formation of thin grafted layers on the surface because it prevents the diffusion of the monomer inside the polymeric film. Moreover, the grafting agent and the grafted chains are soluble in water, and this fact facilitates the radical reaction propagation on the polymer surface (*4*). In addition, water is inert to the triplet excited state of the benzophenone photoinitiator, so the grafting reaction is not inhibited (*22*). The remaining homopolymer formed as side product was removed by exhaustive washing of the grafted films with a very slightly basic solution (pH 8) and then with distilled water. After these washing processes, the typical signal at 1630 cm^{-1} of the C═C groups from the HEMA monomers was not observed in the IR spectrum of the grafted films, demonstrating the absence of residual monomer. The IR spectra of the PP and PP-*g*-HEMA films are shown in **Figure 2**, spectra **A** and **B**, respectively.

The characteristic PP bands were observed at 2842 and 1458 cm^{-1}, corresponding to the $-CH_2$ and $-CH_3$ bending vibrations, respectively. The PP-*g*-HEMA film spectrum presented typical bands at 3400 and 1727 cm^{-1}, which correspond to the −OH and −C═O stretching vibrations, respectively. Therefore, the surface activation of PP films was successfully performed through UV-initiated grafting polymerization reactions. The free alcohol groups were further used in the subsequent chemical reactions to make covalent linkages with caffeic acid.

Immobilization of Caffeic Acid on PP-*g*-HEMA Films. There are numerous reports about esterification reactions of polyphenolic acids that occurred using carbodiimides as coupling agent (*9*, *15*) and through the previous formation of the acyl chloride of the polyphenolic compound, either with protection of the phenolic group (*23*, *24*) or without it (*25*−*27*). Among the different synthetic methods, two different esterification reactions to immobilize caffeic acid were tried. First, a synthetic method described by Chen et al. (*9*) was performed as a direct reaction between PP-*g*-HEMA films and caffeic acid using *N*,*N*′-dicyclohexyl carbodiimide (DCC) as coupling agent. However, no covalent immobilization of caffeic acid was obtained in this experiment. Second, the esterification reaction of PP-*g*-HEMA film and caffeoyl chloride, previously synthesized from the caffeic acid, was carried out in anhydrous THF, obtaining better final results. The acyl chloride formation from the polyphenolic acid was monitored by FTIR determinations, looking for the band at approximately 1780 cm^{-1}, which corresponds to the stretching vibration of the carbonyl group of the caffeoyl chloride (data not shown). No further changes were observed in this signal after 6 h, indicating that the reaction had been completed. A subsequent reaction to make the covalent linkage of the polyphenolic compound on PP-*g*-HEMA films was carried out in THF as solvent. **Figure 2** shows the FTIR spectra of PP-*g*-HEMA (**Figure 2B**) and PP-*g*-HEMA-CA films (**Figure 2C**), which confirmed the caffeic acid linkage on the PP-*g*-HEMA matrix. By comparison of the two FTIR spectra, in the spectrum of PP-*g*-HEMA-CA new bands at 1600 and 1520 cm^{-1}, corresponding to the aromatic carbon−carbon stretching vibrations, were found. Therefore, to the best of our knowledge, this is the first approach toward the caffeic acid covalent immobilization on polyolefin films.

Determination of Total Phenolic Content. To quantify the available phenolic groups present on the polymeric film surface, the Folin−Ciocalteu method was used. This method has been extensively used in the quantification of phenolic compound content in wines, vegetable extracts (*28*, *29*), foods, and also polymeric materials (*16*). It is based on a colorimetric oxidation−reduction reaction by which the Folin−Ciocalteu reagent is reduced by the phenolic compounds at basic pH. Total phenolic contents determined in the modified films were compared with their free radical scavenging properties. **Table 1** shows that the

available phenolic content in PP-*g*-HEMA-CA films was equivalent to 6.7 μmol of caffeic acid/g of dry film or 0.011 μmol of caffeic acid/cm^2 of dry film.

Determination of Antiradical Activity of the Modified Films. Numerous methods have been developed to evaluate the antioxidant power of natural and synthetic substances and also more complex systems such as food and beverage extracts (*30*, *31*). These methods, based on the disappearance of colored synthetic radicals such as DPPH$^\bullet$ or ABTS$^{\bullet+}$, were chosen because of their simplicity and versatility. They measure the ability of antioxidant compounds for trapping free radicals by donating hydrogen atoms or electrons, producing in consequence the bleaching of the colored radical solutions. The free radical scavenging abilities of PP-*g*-HEMA-CA and PP-*g*-HEMA films were determined by both DPPH$^\bullet$ and ABTS$^{\bullet+}$ bleaching methods and expressed in ARA%/100 mg of film. **Table 1** shows the values obtained by both methods.

The antiradical capacity of the PP-*g*-HEMA-CA film determined in the DPPH$^\bullet$ assay obtained from the caffeic acid calibration curve was equivalent to 6.6 $\pm$ 0.4 μmol of caffeic acid/g of dry film, indicating a good ability to scavenge the radical in methanolic medium. Because this value is very similar to that obtained from the total phenolic content, this activity of the modified films can be ascribed to the presence of active phenolic groups over the polymeric surface.

Compared with other antioxidant polymers previously reported, the activity value reached for PP-*g*-HEMA-CA obtained in this work was of the same order as the obtained for water-soluble antioxidant polymers based on ferulic acid and methacrylic acid as comonomers (*16*). Therefore, the radical scavenging behavior that presents the polyphenolic compound covalently bound to a polymeric film was similar that obtained for soluble polymers in which the antioxidant moiety forms part of the polymeric chain. When the DPPH$^\bullet$ assay was performed with the control polymer PP-*g*-HEMA, a negligible decrease in absorbance at 515 nm was found and ARA% was only of 2.2%/100 mg of film, whereas the inhibition obtained for PP-*g*-HEMA-CA films was 89%/100 mg of film. This difference is shown in **Figure 3A**, which represents the kinetic behaviors of the radical scavenging reaction by the polymeric films. These results showed that the radical-scavenging properties presented for the films prepared in this work can be attributed to the caffeic acid molecule linked to the polymeric surface.

Table 1. Antiradical Capacities against DPPH$^\bullet$ and ABTS$^{\bullet+}$ and Total Phenolic Content by Folin−Ciocalteu Method of PP-*g*-HEMA-CA Films

sample	DPPH$^\bullet$ (ARA%/100 mg)	ABTS$^{\bullet+}$ (ARA%/100 mg)	phenolic content (μmol of caffeic acid/g of dry film)
PP-*g*-HEMA-CA	89 $\pm$ 6	18 $\pm$ 2	6.7 $\pm$ 0.5
PP-*g*-HEMA	2.2 $\pm$ 0.4	2.1 $\pm$ 0.3	2.3 $\pm$ 0.2

The reuse of the antioxidant film was assayed after the DPPH$^\bullet$ assay; therefore, it was washed with water and rinsed with methanol. However, subsequent assays indicated the complete loss of activity. Moreover, several attempts were carried out to recover it by regenerating the active groups. One of them consisted of soaking the already used film in ascorbic acid solutions; however, no positive results were found.

With regard to the ABTS$^{\bullet+}$ assay, the radical scavenging capacity of the PP-*g*-HEMA-CA film was 18%/100 mg of film and equivalent to 0.19 μmol of caffeic acid/g of dry film, this value being markedly lower than that obtained for the DPPH$^\bullet$ test. This difference could be attributed to the different media in both assays. In contrast to the DPPH$^\bullet$ tests, which used methanol as solvent, the aqueous medium used in ABTS$^{\bullet+}$ assays seems to be less compatible with the hydrophobic surface presented on the polymeric films after the esterification reactions. Therefore, the antioxidant molecules immobilized on the polymeric surface are less available to scavenge the radical species presented in the aqueous solution. With respect to the results obtained for the control polymer PP-*g*-HEMA, the value measured of ARA%/100 mg of film was 2.1%, markedly lower than the 18% obtained for PP-*g*-HEMA-CA film. As in the DPPH$^\bullet$ assay, these values indicate that the radical scavenger properties shown for the modified polyolefins can be ascribed to the antioxidant molecule immobilized on the polymeric surface. The kinetic behaviors of ABTS$^{\bullet+}$ bleaching by PP-*g*-HEMA-CA and PP-*g*-HEMA films are shown in **Figure 3B**.

Antioxidant Capacity toward β-Carotene−Linoleic Acid Cooxidation Induced by LOX. The PP-*g*-HEMA and PP-*g*-HEMA-CA polymeric films showed extremely low but similar inhibitions of the β-carotene−linoleic acid cooxidation induced by LOX; the antioxidant activities determined corresponded to 0.01 and 1.54%, respectively. This clearly indicates that the linkage of caffeic acid to the film decreases its protective ability in this system. The antioxidant action against the β-carotene bleaching in the cooxidation process can take place by different mechanisms such as the inhibition of the prooxidant enzyme, which initiates the oxidation as well as the scavenging of peroxyl radicals involved in the propagation step. According to their polarity, such intermediates are located in the micelle interface of this system. In this microheterogeneous medium, where the reagents are distributed in different phases, their reactivity is highly conditioned or limited by their partition in the appropriate phase. In contrast to free caffeic acid in the solution bulk, the caffeic acid

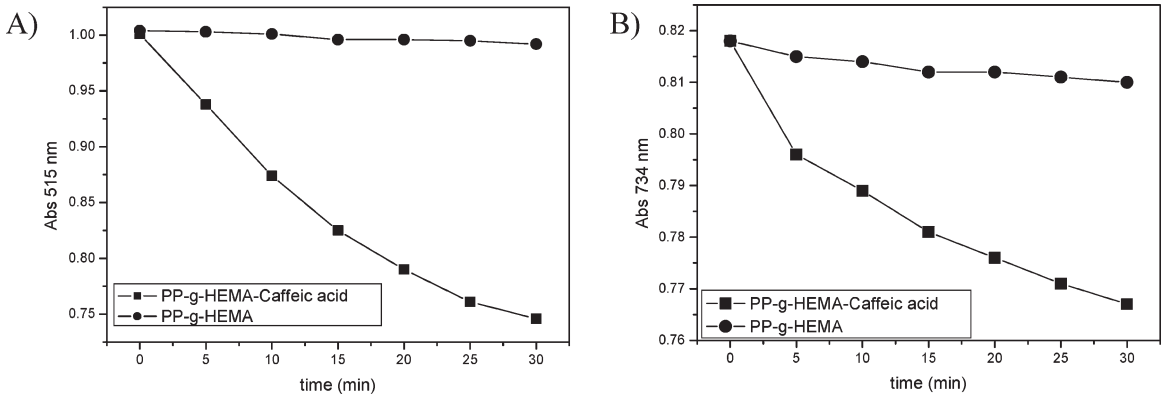

Figure 3. Kinetic behaviors of DPPH$^\bullet$ (**A**) and ABTS$^{\bullet+}$ (**B**) solutions after addition of PP-*g*-HEMA (●) and PP-*g*-HEMA-CA (■) polymeric films.

Table 2. Ascorbic Acid Depletion in Orange Juice Samples after 29 h in Aerobic Conditions at 40 °C

sample	antioxidant addition (μmol of caffeic acid equiv/L of juice)	ascorbic acid retention (%)
orange juice (control)	no addition	37 ± 1
orange juice + PP-*g*-HEMA-CA film	16.0 μmol	74 ± 1
orange juice + CA	11.7 μmol	83 ± 1

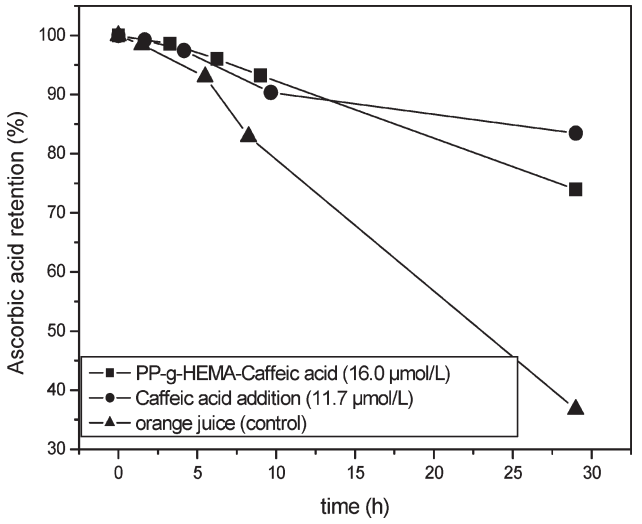

Figure 4. Time dependence of the ascorbic acid retention (%) in 25 mL of orange juice at 40 °C in contact with a caffeic acid containing film (16.0 μmol/L) (■), taking as a reference caffeic acid addition (11.7 μmol/L) (●) and using as a control the juice without additions (▲).

moieties bound to the film are possibly unable to interact with the lipid radicals or to inhibit the enzyme due to their steric hindrance to access to its active site.

Protective Ability of Polymeric Films against Vitamin C Oxidation in Orange Juice Samples. To evaluate the antioxidant properties of the modified films in a real food system, a natural sample of orange juice was exposed to adverse conditions (40 °C for 29 h) to allow ascorbic acid natural depletion as a control experiment. Ascorbic acid degradation during orange juice storage has previously been documented (*32*), the reaction kinetic fitting a zero-order model at 40 °C.

On the one hand, the PP-*g*-HEMA-CA film was assayed with juice samples in the same conditions. The pieces of film added correspond to 16 μmol of caffeic acid equiv/L of orange juice according to Folin−Ciocalteu previous determinations. On the other hand, caffeic acid addition of 11.7 μmol/L of juice was also assayed. Variations in ascorbic acid content of the juices were monitored by HPLC. Ascorbic acid retention values for the three experiments are presented in **Table 2**. The kinetic curves for the ascorbic acid disappearance in the orange juice samples abovementioned are shown in **Figure 4**.

The ability of caffeic acid to reduce the semiascorbyl radical (generated by oxidation of ascorbate) was assessed by Laranjinha and Cadenas (*33*). They demonstrated the reversibility of the reaction shown in eq 3:

$$\text{CA aroxyl radical} + \text{ascorbate} \rightleftarrows \text{CA} + \text{semiascorbyl radical} \quad (3)$$

The retention of ascorbic acid after 29 h was significantly higher for the juice sample in contact with the film than for the untreated juice. However, the highest ascorbic acid protection was obtained for the experiments with caffeic acid added to the juice, although at a lower concentration than the film. This confirms that the synthesized film has protective activity against the oxidation of the vitamin in a natural matrix, but it is less reactive in aqueous media than in alcoholic systems.

In summary, the immobilization of caffeic acid on a grafted PP film was successfully performed through the covalent binding of the caffeoyl chloride on a PP-*g*-HEMA polymer surface. The available phenolic groups on the modified film presented a good correlation with the antiradical activity toward $DPPH^{\bullet}$, although a lower reactivity as $ABTS^{\bullet+}$ scavenger in aqueous medium was observed. Moreover, the polymer synthesized in this work showed protective properties against the oxidation of a juice constituent and, therefore, promising applications as food packaging in different fields. Further studies are in progress searching for higher hydrophilicity of the grafted layer as well as the development of new synthetic pathways toward the immobilization of natural antioxidants.

ABBREVIATIONS USED

LOX, lipoxygenase; FC, Folin−Ciocalteu; $DPPH^{\bullet}$, 2,2-diphenyl-1-picrylhydrazyl radical; GA, gallic acid; LA, linoleic acid; $ABTS^{\bullet+}$, 2,2′-azinobis(3-ethylbenzothiazoline-6-sulfonic acid); AOA, antioxidant activity; ARA, antiradical activity; CA, caffeic acid; HEMA, hydroxyethyl methacrylate; PP, polypropylene; FTIR, Fourier transform infrared; HPLC, high-performance liquid chromatography; THF, tetrahydrofuran; AU, absorbance unit; BHT, butylated hydroxytoluene; BHA, butylated hydroxyanisole; DCC, *N*,*N*′-dicyclohexylcarbodiimide.

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