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Controlling Lipid Oxidation via a Biomimetic Iron Chelating Active Packaging Material

Fang Tian, Eric A. Decker, and Julie M. Goddard*

Department of Food Science, University of Massachusetts, Chenoweth Lab, 102 Holdsworth Way, Amherst, Massachusetts 01003, United States

ABSTRACT: Previously, a siderophore-mimetic metal chelating active packaging film was developed by grafting poly(hydroxamic acid) (PHA) from the surface of polypropylene (PP) films. The objective of the current work was to demonstrate the potential applicability of this PP-*g*-PHA film to control iron-promoted lipid oxidation in food emulsions. The iron chelating activity of this film was investigated, and the surface chemistry and color intensity of films were also analyzed after iron chelation. In comparison to the iron chelating activity in the free Fe^{3+} solution, the PP-*g*-PHA film retained approximately 50 and 30% of its activity in nitrilotriacetic acid (NTA)/Fe^{3+} and citric acid/Fe^{3+} solutions, respectively (pH 5.0), indicating a strong chelating strength for iron. The ability of PP-*g*-PHA films to control lipid oxidation was demonstrated in a model emulsion system (pH 3.0). PP-*g*-PHA films performed even better than ethylenediaminetetraacetic acid (EDTA) in preventing the formation of volatile oxidation products. The particle size and ζ potential results of emulsions indicated that PP-*g*-PHA films had no adverse effects on the stability of the emulsion system. Attenuated total reflectance Fourier transform infrared spectroscopy (ATR-FTIR) analysis suggested a non-migratory nature of the PP-*g*-PHA film surface. These results suggest that such biomimetic, non-migratory metal chelating active packaging films have commercial potential in protecting foods against iron-promoted lipid oxidation.

KEYWORDS: *Active packaging, lipid oxidation, iron chelating, biomimetic, antioxidant*

INTRODUCTION

Lipid oxidation in emulsified foods (e.g., salad dressings, sauces, desserts, etc.) is a significant issue affecting product shelf life and quality. Metal chelators [e.g., ethylenediaminetetraacetic acid (EDTA)] are commonly used in emulsified products to inhibit the iron-promoted lipid oxidation, yet there is growing consumer and industry interest to remove such synthetic additives from food formulations. We have previously reported on a novel strategy to control iron-promoted lipid oxidation using non-migratory active packaging that chelates iron from oil-in-water emulsions.[1] In that work, the food contact surface of a polymer packaging film was modified by covalent grafting of chelating moieties. The use of carboxylate chelating grafts resulted in a film capable of inhibiting lipid oxidation in foods at pH values of 5.0 and higher.[2] In the interest of preventing iron-promoted oxidative reactions in acid and acidified foods, it was necessary to investigate a chelating ligand capable of forming a stable complex with iron at lower pH values.

Siderophores are small molecules excreted by microorganisms and plants to aid in the uptake of iron in iron-poor environments.[3] Their exceptionally high binding constants and specificity to iron has made them the subject of research developing polymers.[4−13] We have developed a biomimetic active packaging film, in which the chelating moiety [hydroxamic acids (HAs)] is analogous to hydroxamate siderophores [e.g., desferrioxamine (DFO)].[14] The biomimetic metal chelator poly(hydroxamic acid) (PHA) was grafted from a polypropylene (PP) film surface via photoinitiated surface graft polymerization. In surface graft polymerization, the film is first activated by an initiator to ensure that monomers graft from the film surface rather than onto the film by cross-linking. Using such a grafting from technique encourages covalent bond formation to the polymer film surface and prevents migration or delamination of the grafted polymer. This PP-*g*-PHA film (Figure 1A) contains large numbers of siderophore-mimetic HAs as chelating ligands. On the basis of the chelating property of free PHA macromolecules reported in prior work,[8] we hypothesize that the HA/Fe^{3+} complex could be formed on the

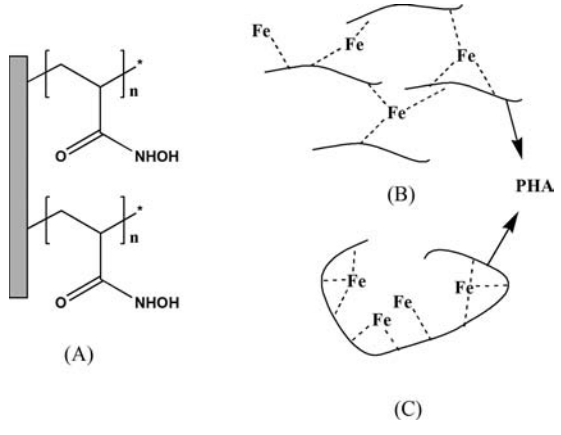

Figure 1. Schematic structure of (A) PP-*g*-PHA and (B) intermolecularly formed HA/Fe complex and (C) intramolecularly formed HA/Fe complex.

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PP-*g*-PHA film surface both intermolecularly (Figure 1B) and intramolecularly (Figure 1C) at a mixture of ligand/iron binding ratio ranging from 1:1 to 3:1.

The iron chelating activity of the siderophore-mimetic PP-*g*-PHA film was preliminarily demonstrated in free iron solution in the prior work.[14] Hydroxamate siderophores (e.g., DFO) have been reported to be able to form very stable ligand/Fe^{3+} complexes with high stability constants (e.g., log *K* of the DFO/Fe^{3+} complex is 30.6).[15] Winston et al.[10] measured the stability constants for free (i.e., not bound to a solid support) PHA polymers with different structures to form PHA/Fe^{3+} complexes at various pH values, and the log *K* values were reported around 29. It is of great interest to understand the chelating strength of the developed PP-*g*-PHA film for iron as well as the stability of the formed PP-*g*-PHA/Fe^{3+} complex. Difficulty was encountered to determine the specific stability constant for PP-*g*-PHA film to chelate Fe^{3+}, because the chelating ligands are bound to the surface of a solid support. To obtain more knowledge with respect to the iron chelating ability of the PP-*g*-PHA film, competition experiments were performed by allowing the active film to compete with other well-known metal chelators (Table 1) for iron in chelated Fe^{3+} solutions.

Table 1. Stability Constant for Well-Known Iron Chelators[15,16]

chelator	Fe^{3+}	Fe^{2+}
citric acid[16]	11.85	3.2
nitrilotriacetic acid (NTA)[16]	15.87	8.84
ethylenediaminetetraacetic acid (EDTA)[16]	25.7	14.3
desferrioxamine (DFO)[15]	30.6	7.2

The objective of this work was to demonstrate the potential applicability of the biomimetic PP-*g*-PHA active packaging films in preventing lipid oxidation in emulsified food products. The chelating strength of the PP-*g*-PHA film for Fe^{3+} was investigated by competition experiments with other well-known metal chelators (NTA, citric acid, EDTA, and DFO). The ability of the film to inhibit lipid oxidation was demonstrated in a stripped soybean oil-in-water emulsion system at pH 3.0, and the impact of the film on the physical and chemical stabilities of the emulsion system was also discussed. Finally, the non-migratory nature of this active film was evaluated by a controlled extraction test.

MATERIALS AND METHODS

Materials. Commercial soybean oil (Wesson, 100% natural vegetable oil) was purchased from the local grocery store. PP (isotactic, pellets) was purchased from Scientific Polymer Products (catalog number 130; Ontario, NY). 2-Propanol, acetone, heptane, methanol, isooctane, 1-butanol, hexane, sodium acetate trihydrate, ferric chloride anhydrous, hydrochloric acid, Brij 35 (a non-ionic surfactant), trichloroacetic acid (TCA), 4-(2-hyddroxyethyl)-1-piperazineethane-sulfonic acid (HEPES), and sodium hydroxide were purchased from Fisher Scientific (Fair Lawn, NJ). Hydroxylamine hydrochloride, 3-(2-pyridyl)-5,6-diphenyl-1,2,4-triazine-*p*,*p*′-disulfonic acid disodium salt hydrate (ferrozine, 98+%), citric acid monohydrate, EDTA, and imidazole (99%) were purchased from Acros Organics (Morris Plains, NJ). Benzophenone (BP, 99%), barium chloride dihydrate, ammonium thiocyanate, cumene hydroperoxide (80%), hexanal (98%), silicic acid (100−200 mesh, 75−150 μm, acid washed), activated charcoal (100−400 mesh), deferoxamine mesylate salt (≥92.5%, DFO), nitrilotriacetic acid trisodium salt (≥98%, NTA), and methyl acrylate (MA, 99%) were purchased from Sigma-Aldrich (St. Louis, MO). All of the chemicals and solvents were used without further purification.

Preparation of PHA-Grafted PP Films. PP pellets were cleaned in isopropanol, acetone, and deionized water sequentially by sonication (2 times for each solvent and 10 min per time). A Carver laboratory press (model B, Fred S. Carver, Inc., Summit, NJ) was used to press cleaned pellets into films at 160 °C with a loaded force of 9000 lbs. PP films (225 ± 25 μm thickness) were cut into 2 × 2 cm coupons cleaned by the same procedure used to clean PP pellets. The cleaned PP films were dried and stored in a desiccator [25 °C and 15% relative humidity (RH)] until further use.

A two-step grafting process was used to introduce a large density of HAs to the surface of PP films to mimic the metal chelating property of siderophores, such as DFO. In the first step, poly(methyl acrylate) (PMA) was grafted from the PP film surface by photoinitiated graft polymerization as previously described.[14] Briefly, BP (5 wt % in heptane) was spin-coated (model WS-400BZ-6NPP/LITE, Laurell Technologies Corporation, North Wales, PA) on each side of PP films, followed by exposure to 90 s of ultraviolet (UV) irradiation (Dymax, model 5000 flood, 320−395 nm, 200 mW/cm^2, Dymax Corporation, Torrington, CT). The BP-functionalized PP (PP−BP) film was submerged in monomer solution (70 wt % MA in acetone), followed by nitrogen purging to remove any oxygen. Vials were subjected to 3 min of UV irradiation to graft PMA from the PP film surface. Soxhlet extraction (150 mL of acetone, 12 h) was used to remove any residual monomer and non-covalently grafted PMA homopolymers from the surface of PMA-grafted PP (PP-*g*-PMA) films.[17]

Ester groups on the surface of PP-*g*-PMA were converted to HAs by reaction with hydroxylamine.[18,19] The hydroxylamine solution was prepared by dissolving hydroxylamine hydrochloride in methanol/water (5:1) solution (0.1 g/mL), adjusting the pH of the solution to 13 by sodium hydroxide, and finally removing sodium chloride precipitate by Buchner filtration for a final methanol/water ratio of 4:1. PP-*g*-PMA films were submerged in hydroxylamine solution in a flask equipped with a reflux condenser, and the hydroxyamidation reaction was conducted at 73 °C for 4 h with stirring. After the reaction, films were washed 3 times in methanol/water (5:1) solution, treated with acidic methanol/water (5:1) solution (0.2 M HCl) for 5 min, rinsed 3 times in methanol/water (5:1) solution, and finally washed 3 times (30 min per time) in deionized water to remove any residual compounds from the inner grafting layer. The obtained PP-*g*-PHA films were dried and stored in a desiccator (25 °C and 15% RH) until further use.

Attenuated Total Reflectance Fourier Transform Infrared Spectroscopy (ATR-FTIR) Analysis. The surface chemistry of control and modified films was analyzed by an ATR-FTIR spectrometer equipped with a diamond ATR crystal (IRPrestige-21, Shimadzu Scientific Instruments, Inc., Kyoto, Japan). Each spectrum was carried out with 32 scans at a 4 cm^{-1} resolution. A background spectrum was conducted with the ATR crystal against air. The representative spectrum of each sample reported in this work was replotted with SigmaPlot 12.0 (Systat Software, Inc., Chicago, IL).

Iron Chelating Assays. The ferric iron (Fe^{3+}) chelating activity of the PP-*g*-PHA film was measured in Fe^{3+} solution with the absence and presence of other well-known metal chelators (i.e., citric acid, NTA, EDTA, and DFO) at pH 5.0. Metal chelator stock solutions (20 mM in 0.05 M sodium acetate/imidazole buffer at pH 5.0) were mixed with Fe^{3+} stock solution (20 mM ferric chloride anhydrous in 0.05 M HCl) at appropriate chelation ratios (2:1 citric acid/Fe^{3+}, 2:1 NTA/Fe^{3+}, 1:1 EDTA/Fe^{3+}, and 1:1 DFO/Fe^{3+}). These chelation ratios were selected on the basis of the reported ligand/metal binding ratio of each chelator. Citric acid and NTA are tridentate and tetradentate metal chelating ligands, respectively, and the ligand/Fe^{3+} ratio of 2 is needed to form the most stable octahedral complex structure. As hexadentate chelating ligands, EDTA and DFO form stable iron complexes with the ligand/Fe^{3+} ratio of 1. The chelated Fe^{3+} stock solutions were then diluted with the sodium acetate/imidazole buffer (0.05 M, pH 5.0) to prepare chelated Fe^{3+} solutions with a final Fe^{3+} concentration of 0.08 mM. Chelated Fe^{3+} solutions (10 mL) with films (native PP, PP-*g*-PMA, and PP-*g*-PHA; 1 × 2 cm) or no film were

prepared for each treatment. Free Fe^{3+} solution (0.08 mM, pH 5.0) with no metal chelators and buffer were used as the positive and negative control treatments, respectively. The chelating reaction was conducted in the dark with shaking for 24 h at room temperature. Chelating activity of the films was determined by the difference of Fe^{3+} concentrations in solutions with films against the corresponding controls (i.e., with no film) as determined by the colorimetric ferrozine assay.[1] Briefly, Fe^{3+} was reduced to Fe^{2+} by hydroxylamine hydrochloride (5 wt %) and TCA (10 wt %). The iron solution (0.5 mL) was mixed with 0.25 mL of reducing agent, followed by the addition of 0.25 mL of ferrozine solution (18 mM in 0.05 M HEPES buffer at pH 7.0). The absorbance of the reaction solution was measured at 562 nm after 1 h of incubation at room temperature with shaking. The Fe^{3+} concentration was determined using a standard curve made of ferric chloride anhydrous.

L**, *a**, and *b** Analyses. The ferrozine assay quantifies the iron concentration based on the assumption that ferrozine will effectively bind all of the iron in a system. Because the binding constants of EDTA and DFO exceed that of ferrozine, an alternate method was required to quantify the concentration of iron chelated by films submerged in EDTA/Fe^{3+} and DFO/Fe^{3+} solutions. HAs develop a characteristic reddish brown color when they chelate iron; therefore, film color was analyzed using a colorimeter. The color coordinates (L^*, a^*, and b^*) of PP-*g*-PHA films after incubation in free or chelated Fe^{3+} solutions (pH 5.0) were analyzed using a colorimeter (ColorFlez EZ, HunterLab, Reston, VA) with a tristimulus absorption filter. L^* represents the lightness, and its value ranges from 0 to 100, corresponding to pure black to pure white. a^* refers to the color change from greenness to redness, when its value changes from negative to positive. b^* is a measure of the color from blueness to yellowness, while it changes from negative to positive.[20] The color intensity [chroma (C^*)] of films was calculated as eq 1. The difference (or the distance) (ΔE^*) between the colors of PP-*g*-PHA films incubated in free Fe^{3+} solution (L_f^*, a_f^*, and b_f^*) and in chelated Fe^{3+} solutions (L_c^*, a_c^*, and b_c^*) was also calculated as eq 2.

$$C^* = [(a^*)^2 + (b^*)^2]^{1/2} \quad (1)$$

$$\Delta E^* = [(L_f^* - L_c^*)^2 + (a_f^* - a_c^*)^2 + (b_f^* - b_c^*)^2]^{1/2} \quad (2)$$

Lipid Oxidation Study. Stripped soybean oil, which primarily contains triacylglycerols, was used to prepare the model oil-in-water emulsion system to conduct the lipid oxidation study. Minor lipid components, such as α-tocopherol, free fatty acids, and pigments, in commercial soybean oil were removed by a chromatographic column separation process.[21] Silicic acid (22.5 g) and activated charcoal (5.625 g) were individually suspended in hexane and packed sequentially in a chromatographic column (35 × 3.0 cm inner diameter) (silicic acid, then charcoal, and then an additional layer of silicic acid). Commercial soybean oil (30 g) was dissolved in hexane (30 mL) and loaded onto the column. The separation was carried out with 270 mL of hexane as the eluent to collect triacylglycerols. A vacuum rotary evaporator (RE 111 Buchi, Flawil, Switzerland) was used to remove the hexane, and the final trace residue was eliminated by nitrogen flushing. The obtained stripped soybean oil was stored at −80 °C for further use.

The oil-in-water coarse emulsion was prepared by dissolving stripped soybean oil (1 wt %) and Brij 35 (0.1 wt %) in sodium acetate/imidazole buffer (0.05 M, pH 3.0), followed by blending for 2 min with a two-speed hand-held homogenizer (Biospec Products, Inc., Bartlesville, OK) at the low-speed setting (7000 rpm). A fine emulsion was obtained by passing the coarse emulsion through a microfluidizer (Microfluidics, Newton, MA) 4 times with the pressure of 9000 psi. Emulsions (1 mL) with PP or PP-*g*-PHA films (1 × 1 cm) were allowed to oxidize in the dark at 25 °C for 24 days. Emulsions with no film as well as emulsions containing EDTA (0.01 mM) were used as negative and positive controls, respectively. The formation of lipid oxidation products (lipid hydroperoxides and hexanal), the particle size, and the droplet charge (ζ potential) of emulsions were measured throughout the incubation period, as described below. All measurements were conducted in triplicate, and the results reported here are representative of two independent studies.

The primary lipid oxidation products, lipid hydroperoxides, were quantified by the method reported by Alamed et al.[22] and Shantha et al.[23] Briefly, emulsion (0.3 mL) was mixed with isooctane/isopropanol (3:1, v/v, 1.5 mL) by vortex (3 times, 10 s/time) and then centrifuged at 2400*g* for 5 min. The upper phase solution containing hydroperoxides (200 μL) was collected and added to a methanol/1-butanol (2:1, v/v, 2.8 mL) solution, followed by the addition of ammonium thiocyanate (3.94 M, 15 μL) and ferrous iron solution (15 μL). The ferrous iron solution was prepared freshly by mixing $BaCl_2$ (dissolved in 0.4 M HCl, 0.132 M) and $FeSO_4$ (0.144 M) solutions at the ratio of 1:1, and the precipitate was removed by centrifugation (1000*g*, 2 min). The reaction solution was incubated at room temperature for 20 min, and the absorbance was quantified by a spectrophotometer at 510 nm. Hydroperoxide concentrations were determined by a standard curve made of cumene hydroperoxide.

The determination of hexanal (a secondary lipid oxidation product) in emulsions was carried out on headspace gas chromatography (GC, Shimadzu GC-2014, Tokyo, Japan) equipped with an autoinjector (AOC-5000, Shimadzu, Tokyo, Japan) and a flame ionization detector (FID), as previously described.[24] Samples were preincubated at 55 °C for 8 min to evaporate volatile compounds from the emulsion to the sample headspace. A divinylbenzene/carboxen/polydimethylsiloxane (DVB/carboxen/PDMS) stable flex solid-phase microextraction (SPME) fiber (50/30 μm, Supelco, Bellefonte, PA) was then exposed to the sample headspace for 2 min to adsorb the volatile compounds, followed by the desorption at 250 °C for 3 min in the injector at a split ratio of 1:7. The separation of volatile compounds was conducted on a fused-silica capillary column (30 m × 0.32 mm inner diameter × 1 μm) coated with 100% poly(dimethylsiloxane) (Equity-1, Supelco). The run time was 10 min for each sample, and the temperatures of the injector, oven, and detector were 250, 65, and 250 °C, respectively. The hexanal concentration was quantified using a standard curve made of emulsions containing hexanal and EDTA (0.2 mM, to prevent oxidation during the analysis). The lag phase of the oxidation product formation in emulsions was defined as the day before the significant increase of their concentration compared to the zero time concentrations determined by the one-way analysis of variance (ANOVA) with Duncan's pairwise comparison ($p < 0.05$).

The particle size distribution and the electrical charge of emulsion droplets were examined using a Zetasizer Nano ZS (Malvern Instruments, Ltd., Worcestershire, U.K.). Emulsions were diluted 100-fold with sodium acetate/imidazole buffer (0.05 M, pH 3.0) before the measurement. The diluted emulsion was equilibrated for 120 s for the measurement of the particle size of emulsion droplets, which was reported as the *Z*-average mean diameter (nm). Two measurements were collected for each sample, and each measurement was an average of 11 readings. For the electrical charge of emulsions, three measurements were performed on each sample (10 readings for each measurement) with the equilibration time of 60 s and the ζ potential (mV) of emulsion droplets was collected.

Stability of Grafted Polymers against Migration. The likelihood of the grafted polymers to migrate from the PP films was assessed using a controlled study, in which MA monomer was exposed to UV irradiation in contact with both native PP and BP-activated PP. The goal of this study was to demonstrate that the photografting procedure resulted in covalently grafted PMA and that PMA homopolymers that may have formed in the monomer solution were effectively removed in the post-treatment rinse steps. Demonstrating that the chelators grafted onto PP films are unlikely to migrate from the films is important, because the objective of this work was to develop a non-migratory active packaging film. ATR-FTIR was performed on films prepared with and without the BP activation step. The absence of PMA on PP films to which MA was photografted without the BP activation step would indicate that PMA homopolymers were effectively removed during rinse steps and, therefore, unlikely to migrate from the films.

Statistical Analysis. The data were presented as means ± standard deviation (SD). Statistical analyses were conducted using SPSS Release

17.0 (SPSS, Inc., Chicago, IL). The significance of differences was determined by the independent sample t test ($p < 0.05$) and one-way analysis of variance (ANOVA) with Duncan's pairwise comparison ($p < 0.05$). The correlation relationship between variables was analyzed by a two-tailed Pearson correlation test.

RESULTS AND DISCUSSION

Iron Chelation by Biomimetic PHA-Active Packaging Films. The ability of the PHA-grafted PP (PP-*g*-PHA) films to chelate iron was quantified by ATR-FTIR, colorimetry, and ferrozine iron binding assay. The effect of iron chelation on the surface chemistry of the PP-*g*-PHA film was analyzed by ATR-FTIR (Figure 2). The spectrum of the PP-*g*-PHA film showed

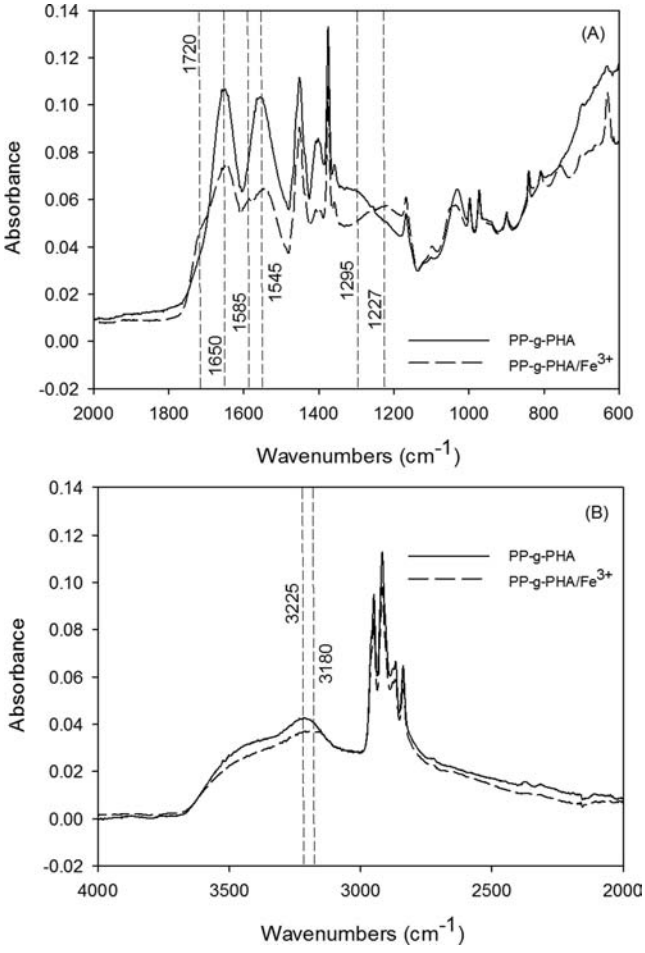

Figure 2. ATR-FTIR spectra of PP-*g*-PHA and PP-*g*-PHA/Fe^{3+} films in the frequency range of (A) 2000−600 cm^{-1} and (B) 4000−2000 cm^{-1}. The PP-*g*-PHA/Fe^{3+} film refers to the PP-*g*-PHA film after 24 h of incubation in the free Fe^{3+} solution (0.08 mM, pH 5.0). Each spectrum is representative of six measurements collected from three independent films.

characteristic absorption bands at 1650, 1545, 1295, and 3225 cm^{-1}, corresponding to C═O, C−NH, CN−H, and O−H of HA groups, respectively. After iron chelation, the absorption bands of C═O and C−NH were split into two bands (1720 and 1650 cm^{-1} for C═O and 1585 and 1545 cm^{-1} for C−NH). The absorption bands of CN−H and O−H all shifted to lower wavenumbers (from 1295 to 1227 cm^{-1} and from 3225 to 3180 cm^{-1}, respectively). The splitting and shifting of the characteristic absorption bands of HA groups confirmed the occurrence of iron chelation on the active film surface and the participation of HA groups in the iron complexation reaction. In both spectra, the broad absorption band in the range of 3650−3340 cm^{-1} was likely a result of the HOH stretch, likely corresponding to water absorption by the highly hydrophilic PHA polymer grafts.[3]

The HA/Fe^{3+} complex exhibits a reddish brown color; in fact, the formation of this characteristic color has been used to develop colorimetric methods for the quantification of various compounds.[25−27] The intensity of this color increases with an increasing concentration of the HA/Fe^{3+} complex.[8] After 24 h of incubation of PP-*g*-PHA films with free and chelated iron solutions, films exhibited different levels of color intensity (Figure 3). This difference in color intensity corresponded with

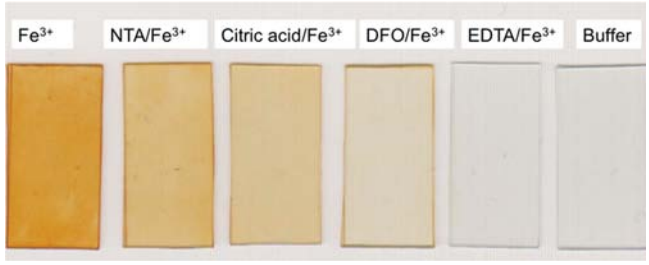

Figure 3. PP-*g*-PHA films after 24 h of incubation in free Fe^{3+} solution, NTA/Fe^{3+} (2:1), citric acid/Fe^{3+} (2:1), DFO/Fe^{3+} (1:1), EDTA/Fe^{3+} (1:1), and sodium acetate/imidazole buffer (0.05 M) at pH 5.0.

the ability of the films to chelate iron from free and chelated Fe^{3+} solutions (i.e., NTA/Fe^{3+}, citric acid/Fe^{3+}, DFO/Fe^{3+}, and EDTA/Fe^{3+}), with sodium acetate/imidazole buffer (i.e., no Fe^{3+}) at pH 5.0 serving as a negative control. The most intense color was present on films incubated with free Fe^{3+} solution, because no chelators were present to compete with PHA chelating moieties on the surface of the films. In the four chelated Fe^{3+} solutions, NTA lost the most Fe^{3+} to PP-*g*-PHA films, followed by citric acid, DFO, and EDTA. It is worth noting that PP-*g*-PHA films acquired Fe^{3+} more easily from NTA than citric acid, which is inconsistent with their reported stability constants to form complexes with Fe^{3+} (Table 1). The stability constant is the equilibrium constant of a chelation reaction, indicating the relative affinity of a particular chelating ligand for a specific metal ion.[28] The reported overall stability constant only considers the concentrations of the chelating ligand in its fully dissociated form and the free metal ion as the factors affecting the complex formation at the optimum pH. For example, the formula to calculate the overall stability constant of NTA for Fe^{3+} is $\log K = \log[\mathrm{FeNTA}]/[\mathrm{Fe}^{3+}][\mathrm{NTA}^{3-}]$. In reality, inconsistencies exist in test models (pH, ionic strength, ligand/Fe^{3+} ratio, etc.), which significantly influence the concentrations of chelating ligands (in their fully dissociated forms) and dissolved metal ions and, therefore, result in different stability constants.[29] The NTA/Fe^{3+} complex was reported to exhibit its maximum stability constant at pH 3,[30] and the citrate/Fe^{3+} complex exhibited various forms of complex species (protonated and hydroxylated) and different stability constant values at different pH values and ligand/Fe^{3+} ratios (reported values range from 1.66 to 22.56).[29,31] In addition, it is also possible that the formation of PP-*g*-PHA film/Fe^{3+}/chelator complexes might occur on the active film surface, because Fe^{3+} has six coordination sites. In this case, the iron is chelated in part by PHA grafted from the film surface and in part by the chelator in solution (i.e., NTA, citric acid,

DFO, and EDTA). Such dual chelation is especially plausible for DFO, because both it and the film use HA groups as the chelating ligands.

The iron chelating activity of native PP, PP-*g*-PMA, and PP-*g*-PHA films in free Fe^{3+}, NTA/Fe^{3+}, and citric acid/Fe^{3+} solutions was quantified by the colorimetric ferrozine assay (Figure 4). Because DFO/Fe^{3+} and EDTA/Fe^{3+} complexes

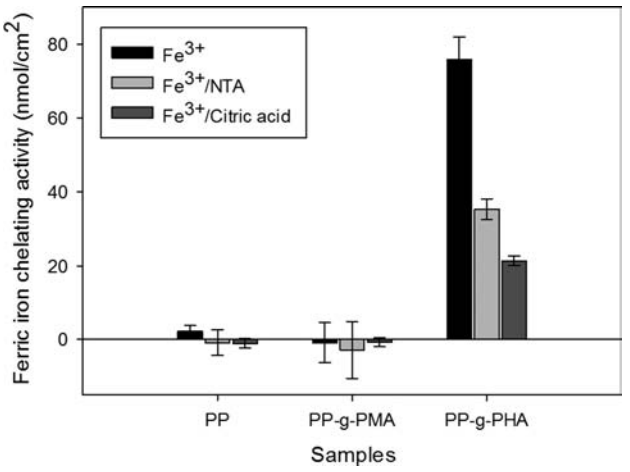

Figure 4. Ferric iron chelating activity of PP, PP-*g*-PMA, and PP-*g*-PHA films in ferric iron solution (pH 5.0) with/without the presence of metal chelators (i.e., NTA and citric acid).

have very high stability constants, the iron concentration of these two chelated Fe^{3+} solutions could not be measured by the ferrozine assay. The native PP and PP-*g*-PMA films showed no detectable iron chelating activity, while PP-*g*-PHA films exhibited significant ability to chelate iron in both free and chelated Fe^{3+} solutions. The absence of chelating activity in PP-*g*-PMA films indicates that the chelation by PP-*g*-PHA films is a result of the specific interaction between HA chelating ligands and iron and not a result of non-specific adsorption or swelling into the grafted layer. In comparison to the iron chelating activity in the free Fe^{3+} solution (75.75 ± 6.13 nmol/cm^2), the PP-*g*-PHA film retained approximately 50% of its activity in the NTA/Fe^{3+} (2:1) solution (35.24 ± 2.80 nmol/cm^2) and 30% in the citric acid/Fe^{3+} (2:1) solution (21.39 ± 1.28 nmol/cm^2). From the iron chelating activity of PP-*g*-PHA films in free Fe^{3+} solution (75.75 ± 6.13 nmol/cm^2), we estimate that the density of HA groups present on this active film is between 76 and 228 nmol/cm^2, assuming a HA/Fe^{3+} ratio of between 1:1 and 3:1. In this experiment, 10 mL of iron solution and 4 cm^2 (1 × 2 cm) of films were used. At the iron concentration of 0.08 mM, 10 mL of iron solutions contain 800 nmol of EDTA or DFO (or 1600 nmol of NTA or citric acid). With the surface area of 4 cm^2, PP-*g*-PHA active films contain between 304 and 912 nmol of grafted HA, as estimated from iron binding assays. It is therefore expected that, in the chelator competition experiment, the amount of soluble chelators (i.e., EDTA, DFO, NTA, or citric acid) was in excess of the amount of chelators grafted onto the PP film surface.

The color coordinates (L^*, a^*, and b^*) of PP-*g*-PHA films after iron chelation in free or chelated Fe^{3+} solutions were analyzed, and the color intensity (C^*) of each film was also calculated (Table 2). The color intensity of the active films was in the order of Fe^{3+} > NTA/Fe^{3+} > citric acid/Fe^{3+} > DFO/Fe^{3+} > EDTA/Fe^{3+} ∼ buffer, which is in agreement with the results visually observed in Figure 3. This trend stayed the same when taking the L^* values of films into consideration in the calculation of ΔE^*. These results were consistent with the results shown in Figure 4, and the color intensity of PP-*g*-PHA films was significantly correlated with their quantified iron chelating activity (r = 0.998; p < 0.05). This correlation suggested that the color intensity (C^*) could be a potential indicator of the ferric iron chelating capacity of HA-containing compounds that cannot be measured using ferrozine or other colorimetric assays.

Controlling Lipid Oxidation by Chelating PHA-Grafted PP Films. The ability of the PP-*g*-PHA films to control lipid oxidation was demonstrated in a stripped soybean oil-in-water emulsion system. Our prior work indicated that the PP-*g*-PHA film had significant ability to chelate Fe^{3+} at pH 3.0, retaining approximately 50% of the chelation capacity at pH 5.0 (75.75 ± 6.13 nmol/cm^2).[14] In this work, we chose pH 3.0 to conduct the lipid oxidation study to establish its effectiveness in preventing iron-promoted lipid oxidation in acidified emulsion systems.

The primary oxidation products (lipid hydroperoxides) of emulsions were determined throughout the storage study (Figure 5). As expected, lipid oxidation occurred rapidly in the control emulsion as well as emulsion stored with native PP films, exhibiting a lag phase of 7 days. Emulsions containing EDTA and no film exhibited a lag phase of approximately 15 days. Interestingly, the lipid hydroperoxide concentration of the emulsion stored with PP-*g*-PHA films increased nearly linearly with storage time and did not exhibit the characteristic exponential increase observed in the other treatments. This linear increase indicated that the PP-*g*-PHA film did not completely inhibit the formation of lipid hydroperoxides;

Table 2. Color Parameters of PP-*g*-PHA/Fe^{3+} Films[a]

treatments	L^* (%)	a^* (%)	b^* (%)	C^*	ΔE^*
Fe^{3+}	31.09 ± 0.07 a	7.22 ± 0.04 a	24.25 ± 0.37 a	25.30	0
NTA/Fe^{3+}	33.90 ± 0.18 b	3.13 ± 0.03 b	14.94 ± 0.18 b	15.26	10.55
citric acid/Fe^{3+}	35.49 ± 0.03 c	1.89 ± 0.01 c	10.56 ± 0.03 c	10.73	15.34
DFO/Fe^{3+}	37.94 ± 0.14 d	0.45 ± 0.03 d	4.99 ± 0.01 d	5.01	21.53
EDTA/Fe^{3+}	38.66 ± 0.08 e	−0.65 ± 0.01 e	−0.24 ± 0.02 e	0.69	26.81
buffer	39.16 ± 0.13 f	−0.59 ± 0.09 e	−0.19 ± 0.19 e	0.62	26.90

[a]PP-*g*-PHA/Fe^{3+} films were prepared by incubating PP-*g*-PHA films in free Fe^{3+}, NTA/Fe^{3+}, citric acid/Fe^{3+}, DFO/Fe^{3+}, and EDTA/Fe^{3+} solutions for 24 h at pH 5.0, and the film incubated in the sodium acetate/imidazole buffer was used as the negative control. Different letters in each column indicate significant differences (p < 0.05). L^*, lightness; a^*, negative to positive refers to greenness to redness; b^*, negative to positive indicates blueness to yellowness; C^*, color intensity; and ΔE^*, color difference (distance) compared to the color of the film obtained from the free Fe^{3+} solution.

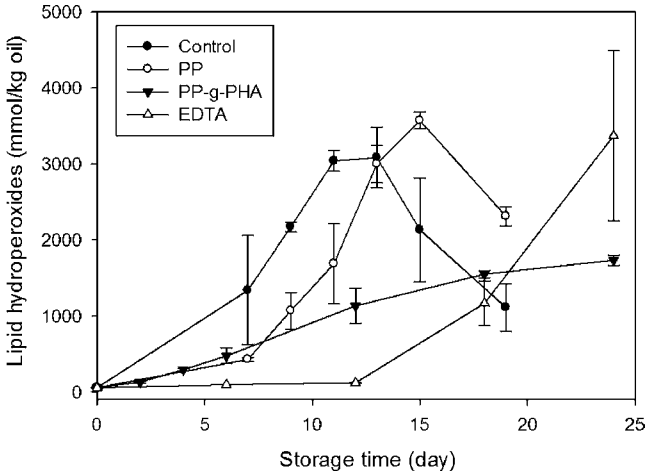

Figure 5. Lipid hydroperoxide concentrations of stripped soybean oil-in-water emulsions (pH 3.0) incubated at 25 °C over 24 days.

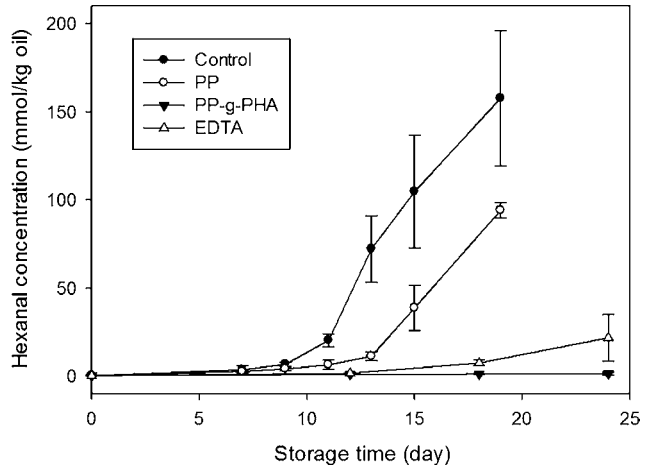

Figure 6. Hexanal concentrations of stripped soybean oil-in-water emulsions (pH 3.0) incubated at 25 °C over 24 days.

however, it is possible that the films effectively prevented the exponential increase of lipid oxidation by inhibiting iron-promoted hydroperoxide decomposition and the subsequent formation of free radicals that could attack additional unsaturated fatty acids. The different antioxidant activity mechanisms between PP-*g*-PHA films and EDTA may be due to differences in form. EDTA was dissolved in the emulsion system and, therefore, was uniformly dispersed in the system and readily available to react with compounds throughout the emulsion system. For PP-*g*-PHA films, HA groups may have experienced steric hindrance from adjacent PHA grafts as well as decreased mobility because of their covalent linkage to a insoluble solid support (the PP film). These factors would likely influence the way in which PHA polymers perform their chelating activity. In addition, because the equilibrium rate of the formation of the HA/Fe^{3+} (3:1) complex is relatively low, PHA needs a longer time to completely bind iron in a manner that decreases its reactivity.[15]

Volatile secondary oxidation products are generated by the decomposition of lipid hydroperoxides, causing the unpleasant rancid smell to oxidized lipid-containing food products. Hexanal is one of the main aldehydes formed by the oxidation of the linoleic acid in soybean oil and was quantified over the storage period (Figure 6). In the control emulsion and emulsion stored with native PP films, the lag phase of hexanal formation was 11 and 13 days, respectively. As expected, the EDTA control (emulsion with EDTA, no film) effectively delayed lipid oxidation, exhibiting a slowly increasing hexanal concentration beginning on day 18, with no apparent exponential phase over the course of the 24 day study. The PP-*g*-PHA film effectively controlled lipid oxidation in the emulsion system, with no formation of hexanal throughout the 24 days storage study. In fact, under the conditions of this study (stripped soybean oil-in-water emulsion system at pH 3.0 stored for 24 days at 25 °C), the siderophore-mimetic PHA-grafted active packaging films reported herein performed better than EDTA in preventing formation of volatile off-odors associated with lipid oxidation. It is interesting to note that, while there was no evidence of hexanal generation in the emulsion with PP-*g*-PHA films throughout the study, lipid hydroperoxides were formed to some degree in the same time period. Ongoing research will investigate the discrepancy observed between the two measures of lipid oxidation to determine if there may be an interaction between the film and hexanal. Nevertheless, the absence of headspace hexanal throughout the study suggests that the developed PP-*g*-PHA films can reduce the level of volatile off-odors resulting from lipid oxidation.

The impact of the PP-*g*-PHA film on the physical and chemical stability of emulsions was investigated by measuring the particle size and ζ potential of emulsion droplets on day 0 and the last day of the storage study (Table 3). The particle size of emulsion droplets remained stable in both control emulsion and emulsion with native PP samples after 19 days of storage incubation. Even though emulsions with PP-*g*-PHA and EDTA exhibited relatively high particle size values on day 24, there was no significant difference between these two samples ($p > 0.05$). High standard deviations in particle size were also noticed in these two samples. The stability of emulsion droplets is mainly balanced by the attractive (van der Waals, etc.) and repulsive (steric, electrostatic, etc.) interactions between droplets.[32] The ζ potential of emulsions maintained around 0 mV for all of the samples throughout the storage period, indicating no electrostatic repulsive force between droplets to prevent them from aggregating. The steric repulsion generated by the hydrophilic head groups from the surfactant (Brij 35) was the main factor to stabilize the emulsion system. The high standard deviations were likely a result of insufficient steric repulsion to oppose the attractive interaction between emulsion droplets.[32] Table 3 also showed that there was no significant difference between the emulsions with PP-*g*-PHA and EDTA in terms of ζ potential, indicating that the PP-*g*-PHA film did not have an unanticipated adverse effect on the physical and chemical stability of this emulsion system.

Table 3. Particle Size and ζ Potential of Emulsions over the Storage Period[a]

samples	particle size (nm)	ζ potential (mV)
original emulsion (day 0)	227 ± 31 a	2.2 ± 2.9 a
emulsion (no film) (day 19)	194 ± 5 a	−0.4 ± 0.9 b
PP (day 19)	217 ± 16 a	−0.2 ± 1.0 b
PP-*g*-PHA (day 24)	333 ± 85 b	−0.1 ± 0.7 b
EDTA (day 24)	310 ± 62 b	−0.6 ± 0.7 b

[a]Values are averages ± standard deviations ($n = 6$). Different letters in each column indicate significant differences ($p < 0.05$).

Stability of Grafted Polymers against Migration. An experiment was performed to determine the likelihood of homopolymers, oligomers, or other small molecules formed during the photografting procedure to migrate from the PP film surface. In this experiment, MA monomer was exposed to UV irradiation in contact with both native PP and BP-activated PP; resulting films are denoted "PP-*g*-PMA control" and "PP-*g*-PMA", respectively. After the post-treatment rinse steps, the surface chemistry of the "PP-*g*-PMA control" film was analyzed by ATR-FTIR and spectra were compared to those of the native PP and the standard "PP-*g*-PMA" film (Figure 7). The

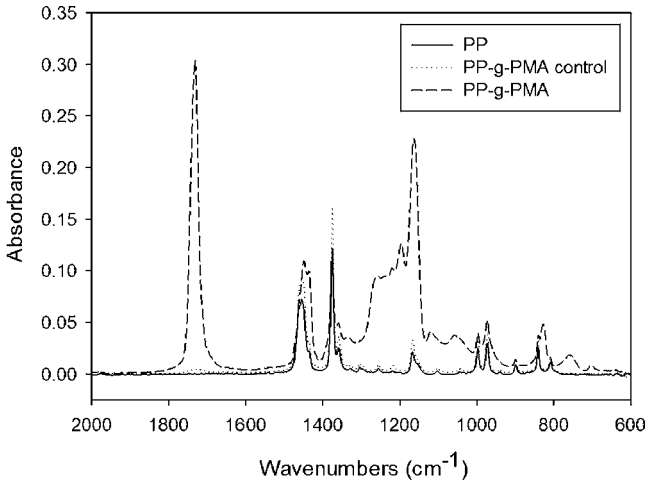

Figure 7. ATR-FTIR spectra of native PP, PP-*g*-PMA control, and PP-*g*-PMA film surfaces. Each spectrum is representative of six measurements collected from three independent films.

standard "PP-*g*-PMA" film showed two characteristic absorption bands at 1740 and 1200 cm^{-1}, corresponding to C═O and C−O of ester groups typical in MA, respectively. No absorption bands were evident at these two wavenumbers in the spectrum of "PP-*g*-PMA control" film, indicating that the PMA homopolymers generated during the surface grafting process could be completely removed by the Soxhlet extraction step. Although not a formal migration study, this study demonstrates that the two-step grafting procedure used in the preparation of PHA-grafted PP films resulted in covalently grafted chelators and that homopolymers and oligomers that may have formed during the photografting step were effectively removed in the post-treatment rinses.

In conclusion, the potential applicability of the siderophore-mimetic PP-*g*-PHA film to control iron-promoted lipid oxidation was demonstrated. In comparison to the iron chelating activity in the free Fe^{3+} solution, the PP-*g*-PHA film retained approximately 50% of its activity in iron solution with the presence of NTA (2:1 NTA/Fe^{3+}) and 30% in the citric acid/Fe^{3+} (2:1) solution, indicating a strong chelating strength for iron. In a model stripped soybean oil-in-water emulsion system at pH 3.0, the PP-*g*-PHA film exhibited strong activity to control lipid oxidation, which performed even better than EDTA in preventing the formation of volatile off-odor oxidation products. Both particle size and ζ potential results of emulsions indicated that the PP-*g*-PHA film did not have an unanticipated adverse effect on the physical and chemical stabilities of the model emulsion system. The control experiment to evaluate the effectiveness of the post-treatment rinsing step to remove the generated non-covalently grafted oligomers and homopolymers suggested a non-migratory nature of the polymeric grafts on the PP-*g*-PHA film surface. These results suggest that such biomimetic metal chelating active packaging film has potential commercial applicability in the food industry for the protection of food products against the iron-promoted lipid oxidation.

AUTHOR INFORMATION

Corresponding Author

*Telephone: 413-545-2275. Fax: 413-545-1262. E-mail: goddard@foodsci.umass.edu.

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Notes

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