Biomimetic Polyphenol Coatings for Antioxidant Active Packaging Applications

Maxine J. Roman¹, Eric A. Decker^{1,2}, Julie M. Goddard¹

¹Department of Food Science, University of Massachusetts, 102 Holdsworth Way, Amherst, MA
01003, USA

²Bioactive Natural Products Research Group, Department of Biochemistry, Faculty of Science,
King Abdulaziz University, P. O. Box 80203 Jeddah 21589 Saudi Arabia

14

15 **ABSTRACT**

16 Oxidative instability of food, pharmaceutical, and consumer products can be promoted by trace
17 metals, especially iron and copper, with subsequent propagation of free radicals. Plant-derived
18 phenolic compounds that contain catechols are reported to have free radical scavenging, metal
19 chelating and surface adhesion properties upon polymerization. The objective of this study was
20 to synthesize biomimetic polyphenol coatings for development of antioxidant active packaging
21 materials. Two synthetic routes were explored to apply polyphenol coatings to the surface of
22 polypropylene by *in situ* polymerization of a mixture of catechol and catechin and oxidative
23 polymerization with laccase and in alkaline saline. Both polyphenol coatings demonstrated
24 potent metal chelating and radical scavenging capacity, which suggest potential antioxidant
25 capacity. Dual functionality of polyphenol coatings as potent antioxidants and anchors makes
26 them a promising candidate for active packaging coatings that can inhibit metal-promoted
27 oxidative degradation.

28

29 **KEYWORDS**

30 Catechol; catechin; antioxidant active packaging; metal chelator; radical scavenger; biomaterial

31

32 A major challenge to food, consumer products, and pharmaceutical industries is retaining
33 stability of oxidation-sensitive bioactive compounds, such as unsaturated fatty acids, carotenoids,
34 flavonoids, vitamins, and drugs. Oxidative stability of such compounds is determined by the
35 propagation of free radicals. In dispersions containing bioactive compounds, trace amounts of
36 metals, especially iron and copper, are key prooxidants that contribute to product instability as
37 they promote hydroperoxide decomposition that catalyzes free radical chain reactions [1]. Since
38 it is difficult to fully remove trace metals from raw materials and the processing environment,
39 metal chelators, such as ethylenediaminetetraacetic acid (EDTA), and free radical scavengers,
40 such as butylated hydroxytoluene (BHT), are often added to formulations as antioxidants to
41 stabilize these products. In an effort to reduce use of synthetic additives due to consumer
42 perception of toxicity risk, there has been increasing interest in alternative methods of product
43 preservation using antioxidants obtained from natural sources. Directly substituting natural
44 antioxidants into product formulations is challenging because they tend to be less potent than
45 synthetic additives and therefore must be added in larger amounts that may change a product's
46 organoleptic properties (i.e. color, flavor, viscosity). In order to overcome this challenge,
47 researchers have explored incorporation of natural antioxidants into active packaging coatings.
48 Antioxidant active packaging coatings may be applied to the product contact surface of common
49 packaging materials by non-covalent or covalent attachment of antioxidants that are applied with
50 or without a carrier polymeric resin [2]. These coatings are designed to either scavenge
51 prooxidants from the product or slowly release antioxidants from the packaging material.

52 The majority of research on antioxidant active packaging coatings has been focused on
53 application of free radical scavengers on the surface of packaging materials. Contini, *et al.* [3]
54 spray deposited citrus extract on plasma pretreated polyethylene terephthalate trays that inhibited
55 oxidative degradation of cooked meats. Garces, *et al.* [4] patented an antioxidant active varnish
56 for use on packaging materials composed of polymeric resin blended with plant extracts that is

designed to scavenge free radicals in packaging headspace [5]. Arrua, *et al.* [6] covalently attached a polymer containing caffeic acid to the surface of polypropylene and demonstrated its ability to scavenge free radicals and inhibit degradation of vitamin C in orange juice. Recently, antioxidant active packaging coatings by graft polymerization of metal chelating polymers, such as poly(acrylic acid) and poly(hydroxamic acid), have been developed that exhibit functionality across a broad range of pH values and viscosity conditions and in the presence of competing ions [7-11]. Metal chelating active packaging coatings extended the lag phase of lipid oxidation in soybean oil-in-water emulsions and demonstrated improved performance for metal chelating polymers with high iron affinity [12,13].

Among free radical scavenging antioxidants derived from natural sources, plant-derived phenolic compounds that contain catechols are reported to have high affinity for iron (e.g. catechol $\log \beta^{\text{Fe(III)}}=43.76$, catechin $\log \beta^{\text{Fe(III)}}=47.4$) [14,15]. In addition to their antioxidant capacity, phenolic compounds that contain catechols have been researched as biomimetics of mussel adhesive proteins making them ideal candidates for coating technologies [16-18]. With this in mind, the reported work seeks to design biomimetic catechol-based polyphenol surface coatings for the development of antioxidant active packaging materials that provide dual antioxidant functionality, both by scavenging free radicals directly and by removing trace transition metals from the system.

A facile method of coating plant phenols onto material surfaces is through oxidative polymerization. Polymerized plant phenols have been noted to have adhesive properties on many materials relevant to packaging due to their structural similarities to the widely researched mussel adhesive proteins. Jeon, *et al.* [19] demonstrated that plant phenols can be polymerized onto a variety of material surfaces, including aluminum, glass, polyethylene terephthalate, and polypropylene, with the assistance of the oxidase enzyme, laccase. Barrett, *et al.* [20] prepared polyphenol coatings from plant phenols by polymerization in an alkaline aqueous buffer, similar to reported methods for the preparation of polydopamine coatings [17]. However, there has yet to be an investigation of the ability of such polyphenol coatings to function as both free radical

scavengers and metal chelators for antioxidant applications. The objective of this study was to synthesize a biomimetic polyphenol coating for development of antioxidant packaging materials with both metal chelating and free radical scavenging character. Polyphenol coatings were applied to the surface of polypropylene by *in situ* polymerization of a mixture of catechol and catechin (2.5 mg/ml catechol; 2.5 mg/ml catechin) via enzymatic polymerization with laccase (1 mg/ml in 100 mM sodium acetate buffer, pH 5 and methanol (9:1)) or oxidative polymerization in alkaline saline (100 mM bicine, 600 mM sodium chloride, pH 8). Ability of the polyphenol coatings to chelate copper and iron ions and scavenge free radicals was demonstrated.

Polymerization of the catechol and catechin by laccase (pH 5) and in alkaline saline (pH 8) was immediately observed by a change in the color of the reaction solution to a dark brown color, characteristic of polyphenol formation. Deposition of the polymerized phenolic coating onto the surface of polypropylene was identified by similar generation of a dark brown color on the polypropylene film after 24 h in the reaction solution (Figure S1). It is important to note, that for the oxidative polymerization reaction conducted at pH 8 without laccase, preliminary experiments revealed that a minimum of 200 mM sodium chloride was necessary for the polyphenol to be deposited on the surface, with optimum surface deposition at 600 mM sodium chloride (data not shown). Salt ions may shield charges on polyphenols formed under alkaline conditions to allow for better interactions with the hydrophobic polypropylene surface. Previous work in agreement with our findings was conducted by Sileika [21] who found that alkaline saline enabled improved surface deposition of polymerized phenolic compounds compared to pure water.

Surface chemistry of the polyphenol coated materials was characterized by ATR-FTIR spectroscopy (Figure 1). Laccase assisted polyphenol coatings exhibited a strong O-H absorbance band (3000-3680 cm^{-1}), two C=C absorbance bands (1590, 1518 cm^{-1}) attributed to benzene rings on catechol and catechin, and several C-O absorbance bands that may be attributed to crosslinks of catechol and catechin or catechol ligands (1050-1290 cm^{-1}). These results are in agreement with reported spectra collected from laccase-assisted polymerization of catechol,

catechin, and their combination [19]. The polyphenol coating produced by alkaline saline polymerization of catechol and catechin exhibited a similar ATR-FTIR spectrum, with slight differences in the intensity and number of absorbance bands for C=C (1606, 1575, 1537 cm^{-1}) and C-O bonds (1050-1290 cm^{-1}), which suggests that this polyphenol coating may have a different structure than the polyphenol coating formed by laccase assisted polymerization. Oxidation of a phenol by laccase typically involves loss of a single electron that results in the formation of a cationic radical, whereas alkaline oxidation results in the formation of an anionic radical. Different reactive radicals may influence the structure of the polyphenol formed by subsequent polymerization reactions. Further characterization of polyphenol molecular structure is necessary to confirm such differences as well as hypothesize polymerization reaction pathways and will be the subject of future work. The number of available catechol groups on the surface was assessed by Folin Ciocalteu assay (Figure 1B). Laccase assisted polyphenol coatings contained more than twice the number of available phenolic groups than alkaline saline polyphenol coatings (86.7 ± 12 nmol catechol eqv. cm^{-2} and 33.1 ± 7.3 nmol catechol eqv. cm^{-2} , respectively), while uncoated PP contained an insignificant amount of phenol groups (0.791 ± 0.14 nmol catechol eqv. cm^{-2}).

Figure 1. (a) Representative ATR-FTIR spectra and (b) total phenol content (n=4) of native PP and polyphenol coated PP.

To determine the effect of coating preparation method on surface morphology, surface scanning electron microscopy (SEM) images of coated and uncoated materials were taken (Figure 2). Uncoated PP exhibited a smooth, uniform surface (Figure 2A). SEM micrographs confirmed the deposition of laccase assisted and alkaline saline polyphenol coatings onto the surface of polypropylene films. Laccase assisted polyphenol coatings significantly changed the surface morphology of the polypropylene to a rough surface with aggregates of polyphenols throughout the coating (Figure 2B). The coated surface also exhibited cracks throughout most likely caused by dehydration of the hydrophilic surface prior to imaging. The alkaline saline polyphenol coating was smoother than the laccase assisted polyphenol coating and did not have any visible cracking (Figure 2C). Compared to the uncoated PP material, both polyphenol coated surfaces were rougher with presence of polyphenol aggregates. These observations are consistent with previous research on *in situ* polymerization of dopamine on glass and aluminum [22], which suggests that the hydrophobic surface properties of polypropylene did not significantly affect coating morphology. Changes in the surface morphology of coated PP materials were evident across the surface, demonstrating uniform application of the polyphenol coatings. Laccase assisted polyphenol coatings were approximately twice the thickness of alkaline polyphenol coatings (laccase assisted coating: 1087 ± 45.3 nm, alkaline coating: 506.5 ± 32.1 nm). This disparity between coating thickness corresponds with differences in the coatings available phenolic groups (Figure 1B).

Figure 2. Representative SEM micrographs of (a) native PP, (b) laccase-assisted polyphenol coated PP, and (c) alkaline saline polyphenol coated PP (10,000x magnification).

The antioxidant efficacy of the polyphenol coated materials was characterized by screening both metal chelating and free radical scavenging capacity. The ability to chelate ferrous, ferric, and cupric ions was measured at pH 3-5 to reflect a pH range of relevant oxidation prone food and consumer products (Figure 3). Polyphenol coatings prepared by both synthetic routes exhibited significant ferrous ion chelating capacity that was pH dependent. Ferrous chelating capacity decreased with decreasing pH value, with optimum ferrous ion chelation at pH 5. In contrast, polyphenol coated materials exhibited optimum *ferric* ion chelating capacity at pH 4. The decline of ferric ion chelating capacity at pH 5 may be attributed to loss of ferric ion solubility at increasing pH values and/or oxidation of catechol hydroxyl groups by ferric ion that would result in the formation of quinones that have a low affinity for iron [23]. Lack of the ability of ferrous ions to convert hydroxyls to quinones could also help explain why chelating capacity of ferrous ions at pH 4 and 5 were higher than ferric ion chelating capacity. There was no significant difference in ferric and ferrous ion chelating capacity at pH 3. Despite significant differences in total phenol content (Figure 1B) and coating thickness, there was no significant difference in iron chelating capacity of the laccase assisted polyphenol coating and alkaline saline polyphenol coating. Similarities in iron chelating capacity suggests that chelating ligands of the polyphenolic coatings may be most active at the surface. Both polyphenol coated materials demonstrated lower copper chelating capacity than iron chelating capacity. Optimum copper chelation was demonstrated at pH 5 and decreased with decreasing pH values, with no significant difference between uncoated material and polyphenol coated material at pH 3. Similar to ferric ion, cupric ion may oxidize the catechol ligands to hinder

effective chelation of metal ions. Ability to chelate ferrous ion, ferric ion, and cupric ion suggests that the reported polyphenol coated films may inhibit oxidative degradation reactions.

Figure 3. (a) Ferrous ion, (b) ferric ion, and (c) cupric ion chelating capacity (n=4) of native PP and polyphenol coated PP at different pH values (3.0, 4.0, and 5.0).

Free radical scavenging capacity of polyphenol coated films was assessed by oxygen radical absorbance capacity (ORAC, Figure 4). For this assay, the efficacy of an antioxidant is

quantified by its ability to protect fluorescein from oxidative degradation by scavenging peroxy radicals generated by AAPH [24]. Polyphenol coated films were able to decrease the overall rate of fluorescein degradation, which is indicative of radical scavenging capacity. Based on area under the curve, laccase assisted polyphenol coating was estimated to have 22.51 nmol Trolox eq cm⁻² and alkaline saline polyphenol coating was estimated to have 22.62 nmol Trolox eq cm⁻². As observed in the metal chelating capacity measurements, total phenol content did not appear to influence the free radical scavenging capacity of the coated films. Compared to the Trolox positive control, which demonstrates free radical scavenging behavior typical of soluble antioxidants, neither polyphenol coated materials exhibited a distinct lag phase for the induction of fluorescein oxidation (Figure 4). The lack of lag phase may be due to diffusion limitations of surface immobilized antioxidants to quench peroxy radicals or possible higher redox potential of surface immobilized polyphenols compared to Trolox (E°' = 480 mV) [24]. Nevertheless, both coatings demonstrated potent free radical scavenging capacity, which may contribute to their overall antioxidant capacity when applied to packaging materials.

Figure 4. (a) Radical scavenging capacity of polyphenol coated PP (n=4) demonstrated by delayed decay of fluorescent probe in oxygen radical scavenging capacity (ORAC) assay. (b) Approximate radical scavenging capacity (Trolox eqv.) estimated by area under the curve.

Phenolic compounds that are derived from plants represent a promising source for antioxidants that may be used to hinder degradation of oxidation-sensitive bioactive compounds.

Application of such phenolic compounds in active packaging coatings may prevent their interference with the organoleptic properties of food and consumer products, while still delivering antioxidant protection of bioactive compounds. Recent research has demonstrated that compounds that bear structural similarities to mussel adhesive proteins, such as catechol-based plant phenols, have functional adhesive properties useful for the development of coating technologies. This proof of principle study demonstrated that polyphenol coatings can be applied by *in situ* laccase assisted polymerization or alkaline saline polymerization of catechol and catechin possess dual antioxidant capacity, exhibiting both metal chelating and radical scavenging properties. These findings warrant further investigation for the development of antioxidant polyphenol coatings for active packaging applications in food, consumer products, and pharmaceutical industries.

ACKNOWLEDGEMENTS

We would like to acknowledge the Peter Salmon Graduate Fellowship (Dept of Food Science, UMass Amherst) and Northeast Alliance for Graduate Education and the Professoriate Fellowship for their support. This project was supported by Agriculture and Food Research Initiative Grant no. 2012-67017-30157 from the USDA National Institute of Food and Agriculture, Improved Processing Technologies (A1351).

223

224 References

- 225 1. Waraho, T.; McClements, D.J.; Decker, E.A. Mechanisms of lipid oxidation in food
226 dispersions. *Trends Food Sci. Technol.* **2011**, *22*, 3-13.
- 227 2. Bastarrachea, L.J.; Wong, D.E.; Roman, M.J.; Lin, Z.; Goddard, J.M. Active packaging
228 coatings. *Coatings* **2015**, *5*, 771-791.
- 229 3. Contini, C.; Katsikogianni, M.; O'Neill, F.; O'Sullivan, M.; Boland, F.; Dowling, D.;
230 Monahan, F. Storage stability of an antioxidant active packaging coated with citrus extract
231 following a plasma jet pretreatment. *Food Bioprocess Tech.* **2014**, *7*, 2228-2240.
- 232 4. Garces, O.; Nerin, C.; Beltran, J.; Roncales, P. Antioxidant active varnish. *European Patent*
233 *EP1477159-A1* **2003**.
- 234 5. Pezo, D.; Salafranca, J.; Nerín, C. Determination of the antioxidant capacity of active food
235 packagings by in situ gas-phase hydroxyl radical generation and high-performance liquid
236 chromatography–fluorescence detection. *J. Chromatogr. A* **2008**, *1178*, 126-133.
- 237 6. Arrua, D.; Strumia, M.C.; Nazareno, M.A. Immobilization of caffeic acid on a polypropylene
238 film: Synthesis and antioxidant properties. *J. Agric. Food Chem.* **2010**, *58*, 9228-9234.
- 239 7. Tian, F.; Roman, M.J.; Decker, E.A.; Goddard, J.M. Biomimetic design of chelating
240 interfaces. *J. Appl. Polym. Sci.* **2015**, *132*, 41231.
- 241 8. Roman, M.J.; Tian, F.; Decker, E.A.; Goddard, J.M. Iron chelating polypropylene films:
242 Manipulating photoinitiated graft polymerization to tailor chelating activity. *J. Appl. Polym.*
243 *Sci.* **2014**, *131*, 39948.
- 244 9. Tian, F.; Decker, E.A.; McClements, D.J.; Goddard, J.M. Influence of non-migratory metal-
245 chelating active packaging film on food quality: Impact on physical and chemical stability of
246 emulsions. *Food Chem.* **2014**, *151*, 257-265.
- 247 10. Ogiwara, Y.; Roman, M.J.; Decker, E.A.; Goddard, J.M. Iron chelating active packaging:
248 Influence of counter-ions and ph value on effectiveness of soluble and immobilized
249 hydroxamate chelators *Food Chem.* **2015**, *196*, 842-847.
- 250 11. Roman, M.J.; Decker, E.A.; Goddard, J.M. Performance of nonmigratory iron chelating
251 active packaging materials in viscous model food systems. *J. Food Sci.* **2015**, *80*, 1965-1973.
- 252 12. Tian, F.; Decker, E.A.; Goddard, J.M. Control of lipid oxidation by nonmigratory active
253 packaging films prepared by photoinitiated graft polymerization. *J. Agric. Food Chem.* **2012**,
254 *60*, 7710-7718.
- 255 13. Tian, F.; Decker, E.A.; Goddard, J.M. Controlling lipid oxidation via a biomimetic iron
256 chelating active packaging material. *J. Agric. Food Chem.* **2013**, *61*, 12397–12404.

14. Avdeef, A.; Sofen, S.R.; Bregante, T.L.; Raymond, K.N. Coordination chemistry of microbial iron transport compounds. 9. Stability constants for catechol models of enterobactin. *J. Am. Chem. Soc.* **1978**, *100*, 5362-5370.
15. Elhabiri, M.; Carrër, C.; Marmolle, F.; Traboulsi, H. Complexation of iron (iii) by catecholate-type polyphenols. *Inorganica Chimica Acta* **2007**, *360*, 353-359.
16. Waite, J.H. Surface chemistry: Mussel power. *Nat. Mater.* **2008**, *7*, 8-9.
17. Lee, H.; Dellatore, S.M.; Miller, W.M.; Messersmith, P.B. Mussel-inspired surface chemistry for multifunctional coatings. *Science* **2007**, *318*, 426-430.
18. Sileika, T.S.; Barrett, D.G.; Zhang, R.; Lau, K.H.A.; Messersmith, P.B. Colorless multifunctional coatings inspired by polyphenols found in tea, chocolate, and wine. *Angewandte Chemie International Edition* **2013**, *52*, 10766-10770.
19. Jeon, J.-R.; Kim, J.-H.; Chang, Y.-S. Enzymatic polymerization of plant-derived phenols for material-independent and multifunctional coating. *J. Mater. Chem. B* **2013**, *1*, 6501-6509.
20. Barrett, D.G.; Sileika, T.S.; Messersmith, P.B. Molecular diversity in phenolic and polyphenolic precursors of tannin-inspired nanocoatings. *Chem. Commun.* **2014**, *50*, 7265-7268.
21. Sileika, T.S. Functional biomaterials inspired by natural polyphenols. NORTHWESTERN UNIVERSITY, 2014.
22. He, L.; So, V.L.L.; Xin, J.H. Dopamine polymerization-induced surface colouration of various materials. *RSC Advances* **2014**, *4*, 20317-20322.
23. Nkhili, E.; Loonis, M.; Mihai, S.; El Hajji, H.; Dangles, O. Reactivity of food phenols with iron and copper ions: Binding, dioxygen activation and oxidation mechanisms. *Food Funct.* **2014**, *5*, 1186-1202.
24. Bisby, R.H.; Brooke, R.; Navaratnam, S. Effect of antioxidant oxidation potential in the oxygen radical absorption capacity (orac) assay. *Food Chem.* **2008**, *108*, 1002-1007.

GRAPHICAL ABSTRACT