JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY

Ascorbic Acid-Containing Whey Protein Film Coatings for Control of Oxidation

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A formulation for the whey protein isolate film or coating incorporating ascorbic acid (AA-WPI film or coating) was developed. Tensile and oxygen-barrier properties of the AA-WPI film were measured. Antioxidant effects of the AA-WPI coating on roasted peanuts were studied by comparing the values of peroxide (PO), thiobarbituric acid reactive substance (TBARS), and free-radical-scavenging activity, determined with noncoated peanuts and peanuts coated with WPI with and without ascorbic acid during storage at 21% relative humidity (RH) and 23, 35, and 50 °C. The incorporation of AA reduced elongation of WPI films. The oxygen-barrier property of the WPI film was significantly improved by incorporation of AA. The AA-WPI coating retarded lipid oxidation in peanuts significantly at 23, 35, and 50 °C. The AA-WPI coated peanuts were more red than noncoated peanuts at all storage temperatures.

KEYWORDS: Whey; ascorbic acid; peanuts; oxidation; oxygen permeability

INTRODUCTION

Antioxidant edible film or coating technology has recently emerged as a potential means to inhibit lipid oxidation of foods and thus to extend their shelf life. Natural antioxidants are favored for incorporation into the films and coatings and those include ascorbic acid, α-tocopherol, plant extracts, and Maillard reaction products (*1*). Antioxidant edible films and coatings could provide recyclable package and continued protection of foods after opening.

Whey protein isolate (WPI) can form edible and biodegradable films and coatings that inhibit migration of oxygen, carbon dioxide, aromas, and oil and moisture; enhance appearance of food; improve mechanical integrity; and carry food additives without imparting negative influence on foods where they are applied (*2*−*6*). Whey-protein-based edible coatings exhibited the effectiveness against lipid oxidation during the storage of frozen king salmon (*7*), dry roasted peanuts (*6*, *8*), and walnuts (*9*) because of their excellent oxygen-barrier properties (*4*, *10*). However, like any polymer material, whey protein coatings allow some permeation of oxygen that eventually produces rancidity in foods. Retarding lipid oxidation in foods by a WPI coating could be improved by incorporating an antioxidant in the coating. The antioxidant activity from the incorporated antioxidants would be prolonged at the surface of coated foods because edible films can slow antioxidant diffusion from the surface.

Ascorbic acid (AA) is widely used as a food ingredient because of its reducing and antioxidant properties in addition to its function as an essential nutrient. Ascorbic acid has been shown to effectively scavenge superoxide, hydrogen peroxide, hypochlorite, the hydroxyl radical, peroxyl radical, and singlet oxygen (*11*). Controlling lipid oxidation in peanuts using WPI coatings has been reported (*6*, *8*). However, no study has been reported about antioxidant effects of an AA-incorporated WPI coating (AA-WPI coating) on foods.

Roasted peanuts are highly susceptible to lipid oxidation because of a high content of polyunsaturated fatty acids. Lipid oxidation in peanuts makes them unacceptable because of rancidity formation, which is a concern for the shelf life stability of many confections containing peanuts (*6*, *8*). Peanuts have been used as a model food for studying effects of whey protein coatings on lipid oxidation of foods (*6*, *8*, *12*).

Potential changes in tensile and oxygen-barrier properties of WPI films and coatings by the incorporation of AA into them must be investigated when the antioxidant effects are studied because the tensile properties relate to coating enhancement of mechanical integrity of foods and oxygen-barrier property relates to pertinence of the AA-WPI coating as an antioxidant edible coating. Thus, the objectives for the research were to (1) develop AA-WPI films and coatings suitable for food application, (2) study the effects of the incorporation of AA on film tensile and oxygen-barrier properties, and (3) evaluate the effects of AA-WPI coatings on lipid oxidation and color of roasted peanuts, used as a model food, during storage at 23, 35, and 50 °C.

MATERIALS AND METHODS

Materials. Whey protein isolate (WPI) was supplied by Davisco Foods International (Le Sueur, MN). Glycerol, used as a plasticizer to improve film and coating flexibility, hydrogen chloride (HCl) and

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10.1021/jf062698r CCC: $37.00
Published on Web 03/17/2007

sodium hydroxide (NaOH), used to adjust pH of film-forming or coating solutions, and potassium acetate ($KC_2H_3O_2$), used to adjust relative humidity (RH) inside desiccators (21%) for peanut storage study, were purchased from Fisher Scientific Inc. (Fair Lawn, NJ). L-ascorbic acid was purchased from Sigma-Aldrich (St. Louis, MO). Roasted peanuts (split-blanched dry-roasted, Runner variety) were obtained from Hershey's Foods (Hershey, PA). The size of the peanut kernel was medium. Received peanuts were kept at 2 °C until use. The peanuts contained 48−52% fat, 22−30% protein, 3−5% sugar, and less than 2% moisture.

Formulation Development for AA-WPI Films and Coatings. Either 5 or 10% (w/w) WPI solution was prepared in sterile deionized water. The same weight of glycerol as that of WPI was added to the WPI solution. The solution was maintained at 90 °C for 30 min in a water bath and was cooled on ice. Once cooled, ascorbic acid (AA) was slowly added to the solution to prepare a film-forming or coating solution. After mixing for 1 h, the pH of the film-forming or coating solution was adjusted to 2.5, 3.4, or 4.3, and the solution was degassed under vacuum. The concentrations of AA tested in the film-forming solution were 0.01, 0.05, 0.1, 0.15, 0.2, 0.25, and 0.3 M. The AA-WPI film was cast by pipetting the film-forming solution onto Teflon plates (15.5-cm diam), resting on a leveled granite surface. The volume of the film-forming solution pipetted was determined to produce a 0.1-mm-thick transparent film. The pipetted film-forming solution was dried for 40 h at 23 ± 2 °C/35 ± 5% RH. Dried films were peeled intact from the casting surface and were stored in a chamber controlled at 23 ± 2 °C/50 ± 2% RH until use. A control film-forming solution or control film was prepared with the same way to make an AA-WPI film-forming solution or film except for adding AA. The formulation was determined first by appearance and viscosity of the solution and second by oxygen-scavenging activity of the formed film. The attributes of appearance were turbidity and film formability of the solution, and color (yellowness) and roughness of the film formed with the solution. Viscosity of the film-forming solution was measured by a Brookfield viscometer (LV type, Brookfield, Stoughton, MA). Oxygen-scavenging activity was determined by a dual head space analyzer (Model 650, Mocon, Minneapolis, MN). Films were cut into 4.1-cm-diam circles (0.1-mm thickness), and the disks were placed in ethylene vinyl alcohol copolymer (EVOH) packages containing 200 cm^3 air. The amount of oxygen scavenged by the film at 22 °C was measured for 5 days.

Tensile and Oxygen-Barrier Properties of AA-WPI Films. The tensile properties elastic modulus (EM), tensile strength (TS), and percent elongation at break (*E*) and the oxygen permeability (OP) properties of AA-WPI films were measured and compared to those of control films. A film-forming solution (pH 3.4) for AA-WPI films was prepared by adding sorbitan monolaurate (Span 20) (0.15% (w/w)) into the AA-WPI solution, prepared following the formulation determined previously. Span 20, which did not change the pH of the solution, was added since it is used to improve coverage efficiency when the AA-WPI (pH 3.4) solution is used for coating of peanuts (*13*). Two controls were prepared: control #1, formed with the same procedure used to form AA-WPI films, but not adding AA and Span 20 and not adjusting pH, and control #2, formed with the same procedure used to form AA-WPI films, but not adding AA. The pH values of the film-forming solutions of controls #1 and #2 and AA-WPI film were 6.9, 3.4, and 3.4, respectively. An instron (Model 1122, Instron, Canton, MA) was used to determine EM, TS, and *E* according to a standard method (D882-01, The American Society for Testing and Materials (ASTM)) (*14*). An Ox-Tran 2/20 ML modular system (Mocon, Minneapolis, MN) was used to determine OP of films at 23 °C and 50 ± 1% RH according to ASTM standard method D 3985 (*15*). The OP was measured every 30 min continuously for 323 h. The oxygen flow rate was 0.3 cm^3/s.

Antioxidant Effects of AA-WPI Coating on Roasted Peanuts. Roasted peanuts were placed on pins, which allowed dipping of the peanuts into coating solution, either with or without AA. The peanuts were dipped for 5 s and then were drained for 10 s. The dipping and draining was repeated. The coating solutions were formulated as described previously. The AA-WPI coating solution contained Span 20. The pins placed on peanuts were attached to expanded polystyrene boards. The coating solutions were maintained at 5 °C by an ice bath during coating. After the coatings were dry, the peanuts were removed from the pins and were transferred into desiccators which were conditioned with potassium acetate ($KC_2H_3O_2$) to 21% RH. The 21% RH was selected to match the water (a_w) of roasted peanuts (a_w = 0.21). The desiccators containing peanuts coated with WPI films with and without AA were incubated for 112 and 105 days, respectively, each at 23, 35, and 50 °C. Noncoated control peanuts were separately incubated for 70 days in desiccators, also at 21% RH and 23, 35 and 50 °C. Light was excluded by covering all desiccators with aluminum foil and placing them in incubators that blocked light during storage. The antioxidant properties of the AA-WPI coating on peanuts were determined by analyzing lipid oxidation of peanut samples and antioxidant capacity remaining in the AA-WPI-coated samples during storage. Peroxide (PO) values and thiobarbituric acid reactive substance (TBARS) values of samples were determined as measured for lipid oxidation, following the methods of Mate et al. (*6*) and Nepote et al. (*16*), respectively. Free-radical-scavenging activity of samples was measured to determine antioxidant capacity, following the methods of Shyamala et al. (*17*).

The color of peanut samples was also measured during storage. A colorimeter (CR-200, Minolta, Osaka, Japan) was used to determine Hunter *L*, *a*, and *b* values with C as a light source and 2″ as an observer.

Equilibration of RH and Oxygen Content for Storage Study. Constant RH values were maintained by saturated potassium acetate ($KC_2H_3O_2$) solution in the bottom of the desiccators. RH was monitored by a hygrometer (Thermo-Hygro, Fisher Scientific Inc., Fair Lawn, NJ). A preliminary study was conducted to determine time required for RH equilibrium between desiccator atmosphere and coated peanuts by monitoring weight change of coated peanuts. Results from the preliminary experiments showed that coated peanuts were at equilibrium after 3 days (data not shown). Thus, actual storage study time began after 3 days from placing the peanut samples in the desiccators. All desiccators were flushed with nitrogen every day to prevent lipid oxidation during the equilibration period. Air was replenished in the desiccators at the start of storage time. The amount of coating added to the peanuts, determined to calculate coating thickness, was obtained by measuring the difference in weight between 10 peanuts before coating and after coating followed by RH equilibrium. Creation of a slight vacuum in the desiccators during the storage time was expected, since the peanuts were consuming oxygen by lipid oxidation. Thus, the desiccators were opened every 3 days during storage to resupply the headspace with air to maintain the oxygen level. When this opening day coincided with a sampling day, oxygen was resupplied only by opening during sampling on that day. No RH change was observed in the desiccator from these openings.

Film Thickness. A micrometer (No. 2804-10, Mitutoyo, Kawasaki, Japan) was used to determine film thickness to the nearest 0.00254 mm (0.0001 in). Measurements were randomly taken at five different locations on each type of film at each replication and the mean value was used in calculations for the coefficients.

Statistical Analysis. The numbers (*n*) of each treatment sample were four for all experiments except for mechanical properties and color (Hunter *L*, *a*, *b*). Eight repeated measurements in each replication were performed to determine mechanical properties and color in duplicate. EM, TS, *E*, OP, and color were analyzed by one-way analysis of variance (ANOVA) using the Minitab 13.31 (Minitab, Inc., State College, PA). Data from the storage study were analyzed by the general linear models (GLM) procedure and Duncan's multiple range tests, with examination for significant differences ($\alpha = 0.05$) at each storage interval for individual treatments using the SAS software program (Version 8.1; Statistical Analysis System Institute Inc., Cary, NC).

RESULTS AND DISCUSSION

Formulation of AA-WPI Films and Coatings. Between 5 and 10% (w/w) as the WPI concentration in the film-forming or coating solution, 10% was chosen, because 10% produced a more viscous solution than 5% and was thought to yield a better coverage of coating on peanuts.

Dipping was used as a coating method in this research. Viscosity of coating solution increased from 47.0 ± 2.7 to 172.3 ± 8.6 mPa·s as the concentration of AA in the 10% WPI film-

Table 1. Tensile Properties of Whey Protein Isolate Films, with and without Ascorbic Acid[a]

film	tensile properties[b]		
	EM (MPa)	TS (MPa)	*E* (%)
control #1[c]	25.05 ± 6.25 a[f]	1.983 ± 0.066 a	128.4 ± 7.6 a
control #2[d]	26.18 ± 3.28 a	1.952 ± 0.025 a	119.8 ± 6.2 a
AA-WPI[e]	24.67 ± 1.09 a	1.921 ± 0.029 a	95.5 ± 10.8 b

[a] Concentration of ascorbic acid (AA) in the WPI films on a dry basis was 150 mg/g film. [b] Values are mean ± standard deviation, $n = 16$. [c] Control #1 prepared without AA and Span 20 at pH 6.9. [d] Control #2 prepared with Span 20 at pH 3.4 without AA. [e] Span 20-added AA-WPI film (pH 3.4). [f] Means in a column followed by different letters are significantly different ($p < 0.05$).

forming or coating solution increased from 0 to 0.2 M. This range of viscosity was adequate for dipping. However, the solutions prepared with 0.25 and 0.3 M of AA were too viscous to be applied for peanut coating by dipping. The oxygen % in the EVOH package headspace decreased from 21.0 ± 0.6 to 19.5 ± 0.4, 19.1 ± 0.3, and 19.0 ± 0.4 for 5 days with the films prepared with 0.2, 0.25, and 0.3 M AA, respectively. No significant scavenging activity was observed with the films prepared with 0.01, 0.05, 0.1, and 0.15 M AA ($p > 0.05$).

At low pH, pH 2−5, L-ascorbic acid (AH_2) is expected to be predominant among AH_2, ascorbic radical (AH^-), and dehydroascorbic acid (A^{2-}) in the coating solution. At pH 6−8, AH_2 oxidizes to AH^- and further to A^{2-}. Both AH^- and A^{2-} have antioxidant activity scavenging radicals, such as superoxide, hydrogen peroxide, hypochlorite, hydroxyl radical, peroxyl radical, and singlet oxygen, because they can be oxidized to work as an antioxidant (*11*). However, AA-WPI films and coatings may be desired to contain AH_2 initially before being applied with peanuts or other foods at neutral pH or higher. Once they are applied to foods at a high pH, the pH of the films and coatings would gradually increase and this increase triggers the release of hydrogen radicals, which work against lipid oxidation. Thus, the films and coatings were prepared at a low pH (≤4.3). Another reason to choose a low pH range was because A^{2-} decarboxylates at pH 7, whereas it has fair stability in acid at pH 2−5 (*11*). A^{2-} involves the Maillard browning reaction. Strecker degradation between A^{2-} and an amino acid may initiate this browning reaction (*18*). A^{2-} forms brown products even in the absence of amino compounds (*19*). Depending on pH drip by the addition of AA, whey proteins precipitated in the solution. The ranges of isoelectric points (pH) for whey proteins are 4.2−8.3 (*11*). About 83% proteins (α-lactalbumin, β-lactoglobulin, albumin (BSA)) of whey proteins have pI in the range of 4.2−5.5 (*20*, *21*). To obtain a clear film, the pH of the solution was adjusted to either 2.5 or 3.4. The pH 3.4 was solely obtained by the addition of 0.2 M AA without additional pH adjustment because no obvious advantage in using pH 2.5 against using pH 3.4 was observed in this study. Thus, pH 3.4 was selected as the pH of the film-forming or coating solution to minimize the number of preparation steps.

The 10% (w/w) WPI, 0.2 M AA, pH 3.4 formulation produced a coating solution that was not different in appearance from the WPI coating solution without AA (control). The formulation could also produce a clear film that scavenged oxygen. Thus, this formulation was applied to the following studies of AA-WPI films and coatings in this research.

Tensile and Oxygen-Barrier Properties of AA-WPI Films. No significant differences in EM and TS among controls #1 and #2 and AA-WPI films were observed ($p > 0.05$) (**Table 1**). The tested properties were not altered by the addition of Span 20 and pH adjustment, which can be observed by comparing the values of controls #1 and #2. The incorporation of AA, however, reduced *E* of WPI films, which can be observed by comparing the values of control #2 and AA-WPI. Since edible coatings are supported by foods, *E* may not be as important as EM and TS in this research.

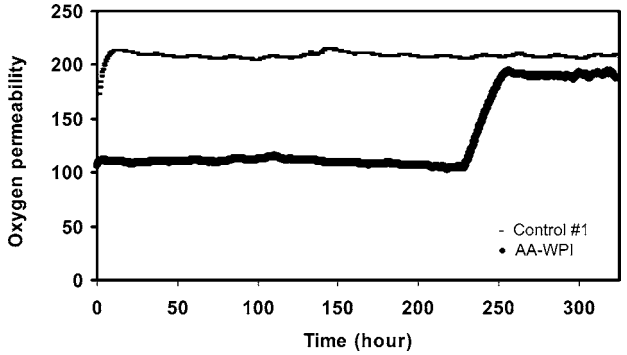

Figure 1. Changes in oxygen permeability (OP) of the whey protein films with and without ascorbic acid (AA-WPI and control, respectively) with time.

Oxygen-barrier properties of WPI films have been reported to be excellent (*2*, *10*). An example of the difference in OP between control #1 and the AA-WPI film is shown in **Figure 1**. All replications ($n = 4$) showed the same trend illustrated in the figure. The OP of the AA-WPI film was significantly smaller than that of the control for 323 h ($p < 0.05$) (**Figure 1**), indicating enhancement of the oxygen-barrier property. The AA-WPI film reached a steady-state condition after 2−4 h and then possessed constant OP for 228−235 h. The OP increased after 235 h and then reached another steady state beginning at 248−253 h (**Figure 1**). The increase after 235 h might be related to a loss of the oxygen-scavenging activity of the film. The secondary OP steady state was still smaller than OP of the control. This result suggests the presence of aggregate domains of AA in the film. Aggregate domains would be more strongly associated than the amorphous protein domains of the film. Such AA aggregate domains could provide greater resistance to the diffusion of oxygen, thereby reducing overall film OP. Thus, incorporation of AA in WPI films may have two positive effects: (1) scavenging oxygen that permeates into the WPI film and (2) reducing film OP. These results suggest that the low OP of whey protein films can potentially be complemented by incorporation of AA, and this concept could be applied to preserve lipid-oxidation-sensitive food products, including peanuts.

Antioxidant Effects of AA-WPI Coating on Roasted Peanuts. The changes in PO values and TBARS values for each kind of peanut samples stored at 23, 35, and 50 °C are shown in **Figures 2** and **3**, respectively. The AA-WPI coating (0.06-mm thickness) retarded lipid oxidation in peanuts significantly ($p < 0.05$), which is clearly seen when the PO values of AA-WPI coated peanuts and those of noncoated peanuts are compared at all temperatures (**Figure 2**). The WPI-only coating also reduced lipid oxidation in peanuts during early storage at 23 °C and throughout the storage at 50 °C. The PO values of WPI-coated peanuts at 35 °C for 70 days were >30, which agrees with those previously reported (*6*). Edible quality of roasted peanuts is lost when PO value is ≥42−47 meq/kg oil (*22*). Thus, all the WPI-only coated peanut samples stored at 23 °C would be acceptable for consumption, whereas only AA-WPI coated samples would possess edible quality throughout accelerated storage at both 35 and 50 °C.

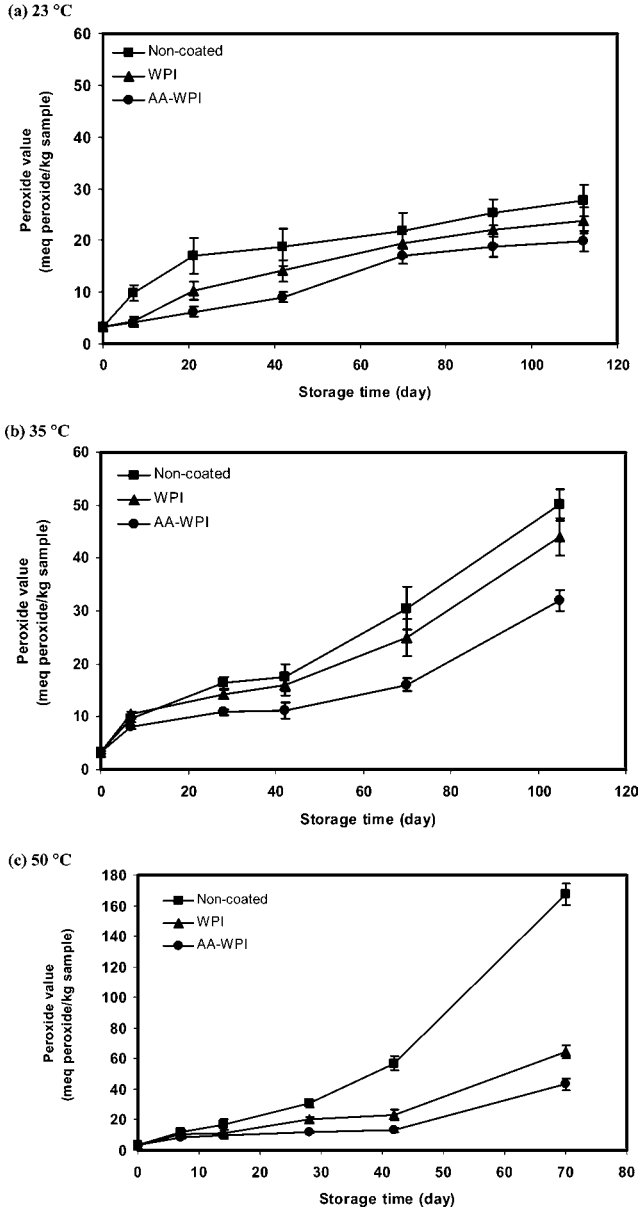

Figure 2. Peroxide (PO) value changes of roasted peanuts without coating (■), coated with whey protein isolate (WPI) only (▲), and coated with ascorbic acid (AA)-containing WPI (●) during storage at 23 (**a**), 35 (**b**), and 50 (**c**) °C. $n = 4$.

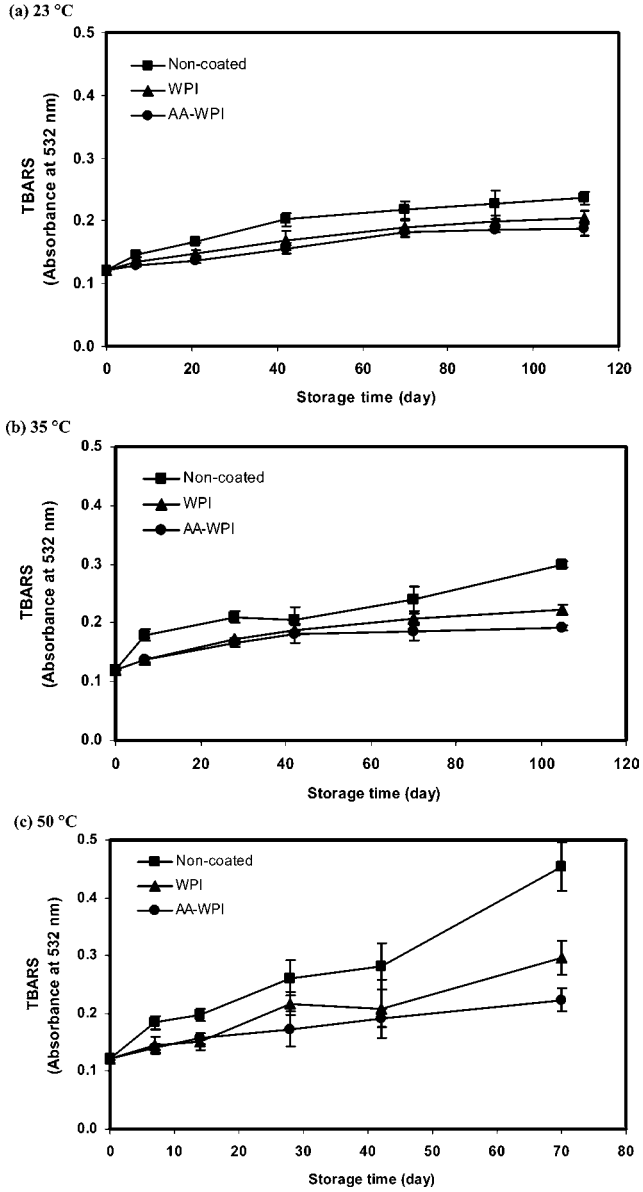

Figure 3. Thiobarbituric acid reactive substance (TBARS) value changes of roasted peanuts without coating (■), coated with whey protein isolate (WPI) only (▲), and coated with ascorbic acid (AA)-containing WPI (●) during storage at 23 (**a**), 35 (**b**), and 50 (**c**) °C. $n = 4$.

The TBARS values from the samples coated with AA-WPI were significantly smaller than those from the noncoated samples at each sampling time during storage at all three temperatures ($p < 0.05$), indicating significant reduction in lipid oxidation in peanuts by the AA-WPI coating (**Figure 3**). The WPI-only coating also reduced the oxidation during storage at three temperatures. The values from the coated peanuts with and without AA were not significantly different during the storages ($p > 0.05$), except for the values obtained on day 105 at 35 °C and day 70 at 50 °C. Thus, effect on inhibition of lipid oxidation by addition of AA to the WPI coating was observed with TBARS at longer storage periods.

PO value has been measured as an indicator of the formation of hydroperoxides, which are predominant products of initial lipid oxidation (*6*). Reactants for the thiobarbituric acid (TBA) reaction include malonaldehyde, alkanals, alkenals, and alkadienals, which are later products of lipid oxidation (*23*). A good correlation ($R^2 = 82.9\%$) between PO values and TBARS values was found (**Figure 4**). This indicates that oxidation in the roasted peanuts involves the formations of hydroperoxides and aldehydes and that data from either test can be used to measure the progress of lipid oxidation in the peanuts. Both PO and TBARS values from this storage study indicate that AA-WPI coating was effective in retarding lipid oxidation in peanuts stored at 23, 35, and 50 °C.

Free-radical-scavenging activities deceased with storage time at all storage temperatures (**Figure 5**). About 67% free-radical-scavenging activity out of the initial 70% was lost during 70 days storage at 50 °C. The activity loss might cause abrupt increases in the PO and TBARS values after 70 days storage at 50 °C.

A continuous coating was not achieved in this study because of the presence of a pinhole in each peanut that was formed when removing the pin used for holding the peanut during coating. A continuous coating, which can be formed by a commercial coating process, could further reduce oxygen uptake

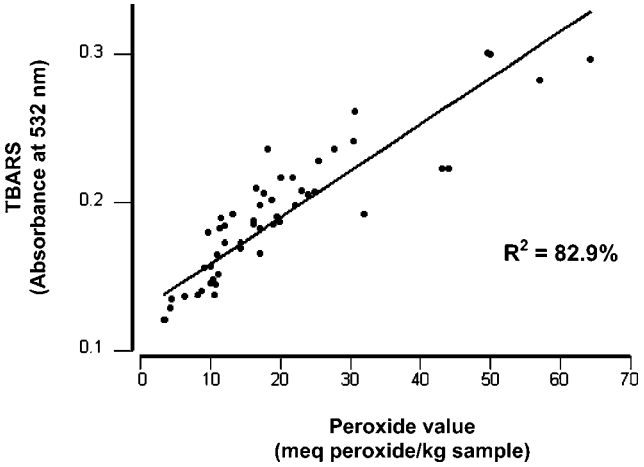

Figure 4. Linear regression plots of the peroxide (PO) values versus the thiobarbituric acid reactive substance (TBARS) values.

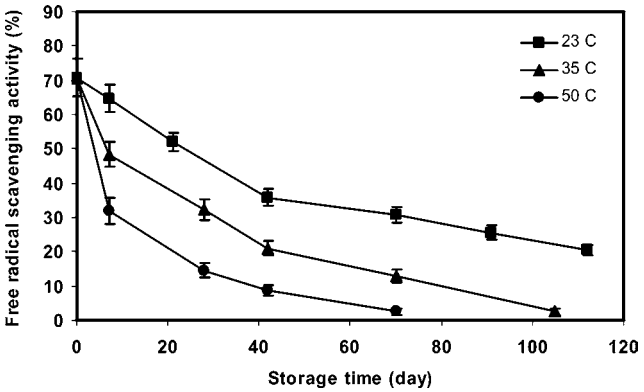

Figure 5. Free-radical-scavenging activities of the roasted peanuts coated with the whey protein isolate incorporating ascorbic acid, stored at 23 (■), 35 (▲), and 50 °C (●). $n = 4$.

of peanuts. Thus, the development of lipid oxidation in peanuts would be more delayed than that reported in this research.

Incorporating AA into WPI films and coatings has potential to further extend stability of foods beyond what would be achieved with the oxygen barrier of WPI films and coatings. Additional information on the migration of AA in the film or coating matrix and food systems will allow prediction of food shelf life as influenced by the WPI film or coating thickness and the amount of AA to be incorporated into the films or coatings.

Effects of AA-WPI Coating on Color of Roasted Peanuts during Storage. Whiteness or lightness (Hunter *L*) values were not significantly different across all three samples during storage at 23 °C for 70 days ($p > 0.05$) (data not shown). The AA-WPI coated peanut samples were darker than WPI-only coated or noncoated peanuts on days 91 and 112 at 23 °C and throughout storage at 35 and 50 °C ($p < 0.05$). No significant differences between *L* values of noncoated peanuts and WPI only coated peanuts were observed at all three temperatures ($p > 0.05$).

The AA-WPI coated peanuts were generally more reddish than noncoated peanuts during storage at all storage temperatures (**Figure 6**). The reddish color of the AA-WPI coating may be attributed to red pigment formed during the Maillard browning reaction between dehydroascorbic acid, which is produced when AA reacts with oxygen in the atmosphere and scavenges free

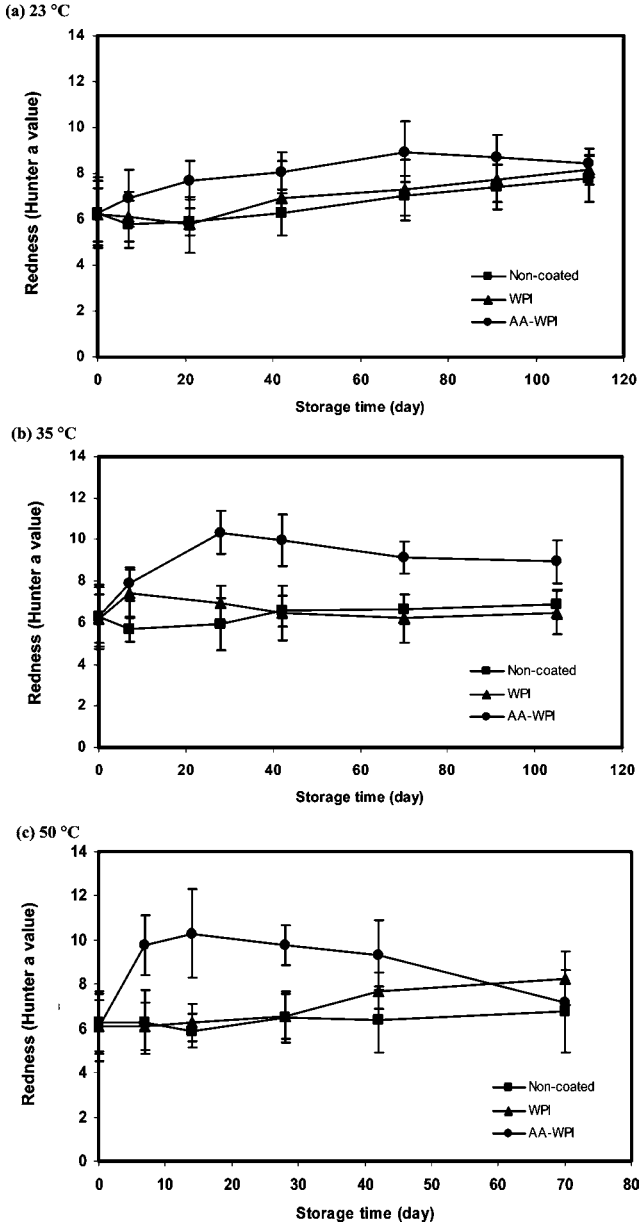

Figure 6. Redness (Hunter *a* value) changes of roasted peanuts without coating (■), coated with whey protein isolate (WPI) only (▲), and coated with ascorbic acid (AA)-containing WPI (●) during storage at 23 (**a**), 35 (**b**), and 50 (**c**) °C. $n = 16$.

radicals, and proteins in the whey protein (*11*). Redness (Hunter *a*) values of noncoated peanuts and WPI-only coated peanuts were not significantly different during storage at all three temperatures ($p > 0.05$).

Yellowness (Hunter *b*) values of all three types of samples were not significantly different from one another during storage at 23 and 35 °C ($p > 0.05$) for 70 days (data not shown). A difference was found between noncoated and AA-WPI samples on day 112 at 23 and 35 °C. At 50 °C, a difference in *b* values was observed between noncoated peanuts and AA-WPI coated peanuts at day 14 and later. AA-WPI coated peanuts were less yellowish than noncoated peanuts. The WPI coating alone did not affect the yellowness of peanuts during storage at any tested temperatures ($p > 0.05$).

Overall, the AA-WPI coated peanuts were slightly more red than the other types of peanuts during storage, but this did not detract from their appearance.

In conclusion, the AA-WPI coating had a significant effect on inhibiting lipid oxidation in peanuts with little change in color. Addition of AA also has little effect on mechanical properties of whey protein films and coatings. Thus, incorporating AA into whey protein films and coatings has potential to further extend stability of foods beyond what would be achieved with the oxygen barrier of whey protein films and coatings.

ACKNOWLEDGMENT

We thank group members of the UC Davis Biopolymer Film Laboratory for their support, especially Jody Renner-Nantz for her help in coating peanuts.

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Received for review September 20, 2006. Revised manuscript received December 20, 2006. Accepted January 10, 2007. This research was supported by University of California under UC Discovery Grant: bio03-10379, which included matching funds from the California Dairy Research Foundation (CDRF). We thank DAVISCO Foods International for their support for this research.

JF062698R