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Lipid-Based Edible Coatings Improve Shelf Life and Sensory Quality Without Affecting Ascorbic Acid Content of White Bell Peppers (*Capsicum annuum* L.)

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Areas in the world without stable power need postharvest techniques that increase shelf life without refrigeration. The effects of candelilla wax coatings on bell peppers were followed during postharvest ambient storage. U.S. Fancy and No. 1 grade white bell peppers 'Dove' and 'Ivory' with no surface blemishes were washed and sanitized then coated or not on the day of harvest with formulations containing 8%, 15.2%, or 16% candelilla wax and stored for up to 21 days at 25 °C and 75% to 80% relative humidity. Uncoated peppers lost 15% of their original weight during 12 days of storage, whereas coated peppers only lost 5% of their original weight during the same period. Coatings delayed color change (yellowing), but did not significantly affect ascorbic acid content ($P > 0.05$). The coated peppers received higher sensory scores from panelists (n=15) in terms of firmness, color, glossiness, and overall preference. The concentration of candelilla wax in the coatings used in this study seemed to be adequate for gas exchange as determined by measurements of internal gas concentrations and the activities of alcohol dehydrogenase (EC 1.1.1.1) and pyruvate decarboxylase (EC 4.1.1.1) enzymes.

The most common problems that reduce marketability and consumer acceptance of bell pepper fruit after harvest are flaccidity, shriveling, wilting, and decay (Lerdthanangkul and Krochta, 1995; Xing et al., 2011). These problems are directly related to the loss of water during storage. Reduction of water loss should improve shelf life of bell peppers. In order to reduce water loss through the cuticle, a film or coating can be applied to the surface of peppers; an ideal film should be able to reduce water loss without blocking gas exchange between the tissue and surroundings. Elevated levels of CO_2 and reduced levels of O_2, beyond optimal conditions to maintain normal aerobic respiration, lead to hypoxia and physiological disorders. Proper maintenance of gaseous equilibrium in a postharvest environment is the basis for modified atmosphere packaging (MAP) or controlled atmosphere (CA) storage, which have been shown to extend the longevity of most horticultural commodities (Kester and Fennema, 1986). The consensus optimum modified atmosphere for mature, unripe bell peppers (i.e., atmosphere limits that do not result in injury during extended storage at 7 °C to 10 °C) is 2% O_2 plus 5% CO_2 (Saltveit, 1997).

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Different types of edible coatings in conjunction with temperature and environmental control have been reported to improved shelf life of on various fruits and vegetables such as deciduous fruits (Smith et al., 1987), minimally processed carrots (Avena-Bustillos et al., 1994a), and zucchini (Avena-Bustillos et al., 1994b), citrus fruits (Hagenmaier and Baker, 1994), tomatoes (Park et al., 1994), and apples (Lau and Meheriuk, 1994). In addition, application of edible lipid coatings also improves the appearance of vegetables (Baldwin et al., 1997), specifically bell peppers (Conforti and Ball, 2002; El Ghaouth et al., 1991; Lerdthanangkul and Krochta, 1996; Xing et al., 2011). However, many areas in the world are without stable power and would benefit from postharvest techniques that increase shelf life without refrigeration (World Bank, 2014).

Green bell peppers individually wrapped in plastic film showed marked reduction of water loss and softening, which extended shelf life (Ben-Yehoshua et al., 1983; Brackett, 1990; Gonzalez and Tiznado, 1993; Hughes et al., 1981; Miller et al., 1986; Otma, 1989). However, plastic film has its limitations for commercial use due to proliferation of aerobic microorganisms as a result of water condensation caused by temperature fluctuation (Brackett, 1990; Miller et al., 1984). Moreover, disposal of plastic films generate solid waste problems.

Edible coatings are commonly made from polysaccharides (pectins, gums, starches, and cellulose derivatives), proteins (gelatin, casein, wheat gluten, and corn zein), and lipids (acetoglycerides, waxes, fatty acids, and resins) or combinations of the above (Baldwin et al., 1997; Kester and Fennema, 1986). These ingredients are Generally Regarded As Safe (GRAS) when applied according to recommended levels and thus the resulting coatings do no require EPA registration. The application of edible coatings and films as well as temperature and relative humidity management has received attention worldwide for improvement of shelf life of fruits and vegetables (Lerdthanngkul and Krochta, 1995; Xing et al., 2011).

Information on changes of nutritional composition and overall quality as perceived by consumers after application of these coatings on vegetables is limited, especially during storage at room temperature. In addition, the effects of a particular coating may vary from vegetable to vegetable; thus, results on other vegetables or other peppers with other colors cannot be applied directly to white bell peppers. Therefore, the objectives of this study were to: 1) select appropriate lipid-based edible coatings for white bell peppers; 2) evaluate quality based on physical properties, shelf life, and consumer acceptance of coated peppers after ambient storage; and 3) compare the ascorbic acid content of peppers as affected by coating during storage.

Materials and Methods

Preliminary tests and selection of coating materials. Coating formulations were prepared from the combination of the following ingredients: refined candelilla and carnauba waxes NC#3 light flakes (Strahl & Pitsch Inc., West Babylon, NY), beeswax (Koster Keunen, Inc., Sayville, NY.), Palmac 760 (82% oleic, 12% linoleic, 6% palmitic acids) [Apperson Chemicals, Jacksonville, FL], Hystrene 9016 (92% palmitic, 7% steric, 1% myristic acids) [Humko Chemical Div., Witco Corp., Memphis, TN], antifoaming agent (FG-10, a polydimethylsiloxane, Dow Corning Corp., Midland, MI), gelatin (Rousselot 275A30, Sanofi Bio-Industries, Mississauga, Ontario, Canada), and soy protein isolate (Supro 620, Protein Technologies International, St. Louis, MO).

Coating microemulsions were made by the water-to-wax method (Hagenmaier and Baker, 1994). The protein ingredients were added after formulating the emulsions. Performance of coatings was evaluated based on spreadability, foaming, appearance, ease of application, and ability to prevent weight loss of peppers. All four coating formulations (beeswax-carnauba, candelilla-beeswax, candelilla, and candelilla-gelatin) satisfactorily delayed water loss in the peppers, but appearance (glossiness) was not the same: some were dull, greasy looking, and much more noticeable than others as evaluated by experienced panelist (n=15). Candelilla wax base was rated the best. The candelilla wax formulation was then slightly reformulated to increase spreadability and reduce foaming for use in this study (Table 1).

Peppers. White bell peppers, 'Dove' and 'Ivory', were grown in Alabama or Florida following current recommendations for fertilization and pest control. Fruit were hand harvested and graded according to Dangler and Whigham (1993). Peppers of the Fancy and US#1 grades were then transported on the day of harvest in perforated paper boxes from the field to the Nutrition and Food Science Lab at Auburn University or the Postharvest Horticulture Lab at the University of Florida (internal atmosphere measurements only). Unblemished peppers were washed, disinfected with chlorinated water (0.7% Clorox solution), and dried at ambient temperature.

Table 1. Treatment groups and formulations of coatings.

Treatment	Formulation
A	16% candelilla wax, 2.5% oleic acid, 0.8% palmitic acid, 0.3% gelatin, 200 ppm antifoam, morpholine, ammonia, and water (total solids 19.6%)
B	15.2% candelilla wax, 0.9% oleic acid, 1.5% palmitic acid, 0.7% soy isolate, 200 ppm antifoam, morpholine, ammonia, and water (total solids 18.3%)
C	1:1 dilution of coating A
D	No coating

Coating experiment: Unblemished peppers were randomly assigned into four treatment groups A (n=25), B (n=25), C (n=25), D (n=25). Groups A, B, and C were coated with coating A, B, and C, respectively, and group D was left uncoated as a control (Table 1). Coating materials were applied by fully submersing peppers once into the appropriate coating for 20 s. Peppers were then left to air dry on an even plastic surface. To assess that the appropriate film thickness did not result in anaerobic stress (hypoxia), the internal atmosphere of the fruit (O_2 and CO_2) and the activities of the enzymes alcohol dehydrogenase (ADH; EC 1.1.1.1) and pyruvate decarboxylase (PD; EC 4.1.1.1) were determined (see below). Three replications of experiments were completed during the months of June to August.

Storage. Dried and coated peppers from all treatments were stored in open boxes under accelerated storage condition at 25 °C with 75% to 80% relative humidity (RH) in normal indoor lighting for 20 d or until coated peppers lost more than 7% of their original weight.

Objective measurements. For each replication of the experiment, color measurements were taken non-destructively using a spectrophotometer (model CM-2002 on SCE mode, Minolta, Japan) on each of 25 peppers in each treatment. An average of three L, a,and b values per pepper were taken at 3-d intervals. Chroma (C) was calculated as $C = (a^2 + b^2)^{1/2}$. Weight loss was calculated as percentage of water loss by monitoring fresh weight change of peppers at 3-d intervals until completion of the study. Internal pepper O_2 and CO_2 concentrations were measured daily using a CO_2/Oxygen MAP gas analyzer (Model 900141, Bridge Analyzers, Inc., Alameda, CA) via sampling needles that were inserted through rubber sleeve stoppers (Size No: 11.5 with 10.70 mm sleeve length and 5.16 to 6.73 mm diameter tapered plug; Fisher Scientific, Inc., Pittsburg, PA) that were inserted through holes bored through the pericarp wall with a 6-mm diameter cork borer.

Sensory evaluation. At 3-d intervals, three peppers from each treatment were randomly selected and assigned a random three-digit number. The various treatment groups were placed next to each other in a compartmentalized box. Fifteen experienced panelists were asked to score samples based on firmness, glossiness, color, and overall preference throughout the course of the experiment using a paper form with a five-point hedonic scale, with a score of one (1) being the worst and five (5) being the best.

Alcohol dehydrogenase and pyruvate decarboxylase activities, which are related to anaerobic stress (hypoxia), were determined 7 d after coating. Since the enzyme analysis is very expensive, only the uncoated and coated peppers with high concentrations of coatings were used for detection of hypoxia condition. Three peppers from treatments A, B, and D were diced, frozen in liquid nitrogen and freeze-dried for enzyme assay according to the procedure described by Chen and Chase (1993). Protein content was determined by the Bio-Rad protein assay kit using bovine serum albumin as a standard (Bradford, 1976). The enzyme assay was carried out only on two replications of the experiments.

Ascorbic acid analysis. Initial total ascorbic acid content of peppers was determined from two independent composites (10 to 12 pepper fruit) of each cultivar. Total ascorbic acid levels were determined by blending the samples in a metaphosphoric-acetic acid extractant (AOAC International, 1995) to prevent ascorbic acid oxidation. The extracts were analyzed for total ascorbic acid by the microfluorometric method (AOAC International, 1995). At 14 d after coating, ascorbic acid content was determined in coated and uncoated peppers (n=12 each).

Statistical Analysis: A completely randomized design with three to five replications was used. Data were analyzed using ANOVA and Duncan's multiple range test at $\alpha = 0.05$ (SAS, 1985).

Results and Discussion

Coating significantly ($P < 0.05$) reduced water loss of bell peppers. Although the effects of the three coatings (A, B, and C) on water loss were not significantly different ($P > 0.05$), coating A reduced water loss the most. Coatings A and C were not significantly different in preventing water loss or in panelists' overall preference. Overall, the coated peppers had lost 5% of their original weight by day 12; whereas, the uncoated peppers lost 15%. Normally, peppers are considered not marketable after losing more than 7% of their original weight (Burton, 1982). The appearance of peppers after extended storage of 21 d at the ambient conditions of 25 °C and 75% to 80% RH is shown in Fig. 1.

Coatings delayed pepper color change as the uncoated peppers had undergone significantly more color change than the coated peppers ($P < 0.05$) after 14 d of storage at room temperature (Table 2). However, there were no significant differences in color change among the different types and concentrations of coatings.

Ascorbic acid content of peppers was not significantly ($P < 0.05$) different among treatments after 14 d of accelerated storage. Ascorbic acid content of control peppers (D) after 14 d storage appeared to increase slightly; this may have been due to the concentration effect of the peppers in the uncoated control (D) having lost more moisture than the coated peppers (Table 2). This study suggests that coating at appropriate levels does not affect the ascorbic acid content of the peppers.

No physiological disorders were observed in the coated peppers with various concentrations of coating materials. The internal concentrations of O_2 and CO_2 equilibrated at about 20% and 0.8%, respectively, in uncoated peppers during ambient storage, and at

Table 2. Ascorbic acid contents and color parameters of coated and uncoated peppers.[z]

Treatment[y]	Ascorbic acid content (mg/100 g edible portion)	Color parameter L	a	b
A, B, D (day 0)	115 a[x]	52.6 a	–2.3 a	17.6 a
A (day 14)	117 a	51.1 a	0.85 a	22.1 b
B (day 14)	110 a	50.3 a	–0.1 a	20.9 b
D (day 14)	126 a	49.1 b	4.05 b	24.3 b

[z]Each value is the mean of four independent analyses of two independent composites of 5 to 6 peppers for treatments A, B, D on day 14. For treatments A, B, and D on day 0, the values were from six independents analyses of 6 to 12 peppers.
[y]See Table 1 for descriptions of treatments.
[x]Numbers in the same column followed by the same letter are not significantly different according to Duncan's multiple range test ($\alpha = 0.05$).

Fig. 1. Appearance of coated and control peppers after an extended storage of 21 d at 25 °C and 75% to 80% RH.

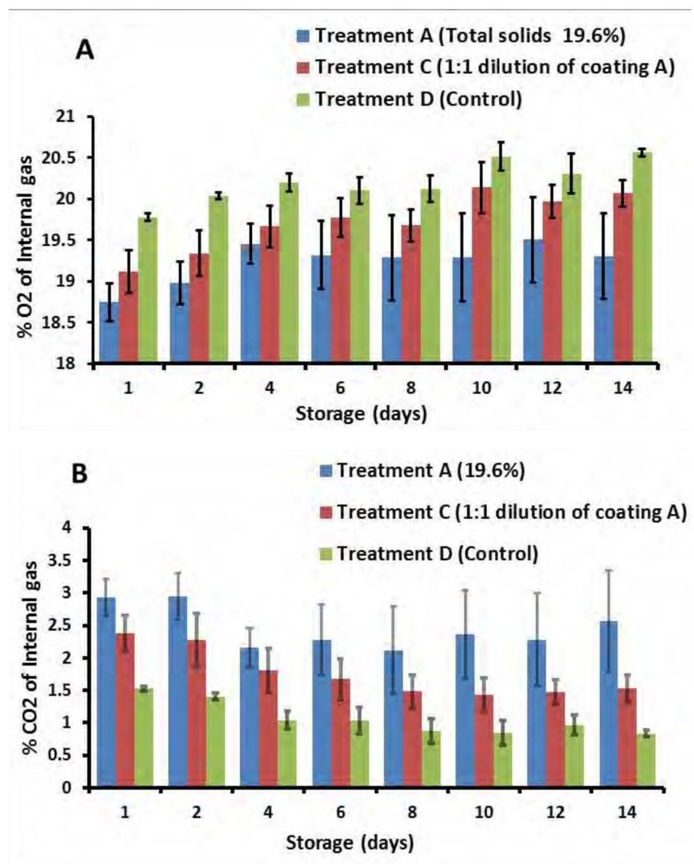

Fig. 2. (**A**) Internal O_2 and (**B**) CO_2 concentrations of coated (Treatments A and C) and uncoated (Treatment D) peppers during storage for 14 d at 25 °C and 75% to 80% RH. See Table 1 for descriptions of treatments. Vertical bars represent the SE of 5 replications per measurement.

Table 3. Effect of coatings on water loss and sensory scores of bell peppers.[z]

Treatment	Water loss (%) (objective)	Firmness (sensory)[y]	Overall preference (sensory)	Color (sensory)	Glossiness (sensory)
Control	9.8a[x]	1.6b	1.3b	3.6c	2.9b
Coating C (8% wax)	3.4b	4.2a	3.6a	3.9bc	3.1b
Coating B (15.2% wax)	3.4b	4.3a	4.0a	4.5a	4.6a
Coating A (16% wax)	2.9b	4.1a	3.6a	4.2ab	3.0b

[z]See Table 1 for descriptions of treatments.
[y]The sensory scores are the overall average of four independent sessions after 3, 6, 9 and 12 d of storage.
[x]Numbers in the same column followed by the same letter are not significantly different according to Duncan's multiple range test ($\alpha = 0.05$).

about 19% and 2.2% in coated fruit (Fig. 2). These concentrations are far from the reported limits for peppers at refrigerated storage temperatures (Saltveit, 1997). Although maintenance of higher O_2 concentrations would be necessary to avoid anaerobiosis in peppers at 25 °C than at their optimum storage temperatures of 7 °C to 13 °C (Hardenburg et al., 1986), the O_2 levels measured in coated peppers in this study are unlikely to limit aerobic respiration. Carbon dioxide levels greater than 5% have been reported to cause injury to peppers, but specifically at storage temperatures lower than 10 °C (Wang, 1977). The sensitivity of plant tissues to elevated CO_2 levels decreases at higher temperatures (Mitz, 1979). Thus, injurious CO_2 concentrations would be higher than 5% at 25 °C, indicating that the CO_2 levels recorded during ambient temperature storage in this study are tolerable for peppers.

The ADH activities for coated peppers (treatment A) and uncoated peppers (treatment D) were 0.23 ± 0.04 and 0.28 ± 0.06 unit/mg protein, respectively. The PD activities for coated and uncoated peppers were 0.09 ± 0.07 and 0.20 ± 0.02 unit/mg protein, respectively. Slight increases ($P > 0.05$) of both enzyme activities observed in uncoated peppers during storage suggest symptom of water stress in the pepper fruit, resulting from the >10 % water loss due to transpiration. The coating formulations seemed to be adequate as determined by the activities of ADH and PD, thus further indicating that adequate gas exchange occurred.

After 20 d of storage at 25 °C and 75% to 80% RH, which was an accelerated storage condition, there were some spots of white mold on the pedicels of some coated and uncoated peppers. Since this storage condition is quite extreme for peppers, we believe that if the same fruit were stored at the recommended storage temperature of 7 °C to 13 °C (Hardenburg et al., 1986), these peppers could be expected to remain in good condition for a much longer time. Peppers without coating that are stored at the recommended temperatures with optimum relative humidity have a potential shelf life of up to 4 weeks (Kader, 1992).

Panelists rated the coated peppers as firmer than the uncoated peppers (Table 3). Coating formulation B received the highest scores for glossiness and color, and the uncoated control received the lowest. For overall acceptability, peppers in the uncoated control were rated lowest and the differences in overall acceptability among the coated pepper treatments were not significant.

Conclusions

Selected lipid-based edible films improved the shelf life of bell peppers and delayed color change, loss of firmness, and loss of weight during storage compared with uncoated peppers. Coated peppers were well accepted by expert panelists and were rated higher than uncoated peppers. Although differences among the tested coatings were small, coating A appeared to be best as a water barrier and coating B appeared to be best for glossiness. No evidence of anaerobic respiration was observed in coated peppers with the present levels of 10% to 20% solids in the coatings. Under aerobic conditions, without deprivation of oxygen, the ascorbic acid content remained unchanged. This study shows that lipid-based edible coatings have potential for commercial application for bell peppers and for use in situations in which refrigerated storage is not available.

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