Color change of fresh-cut apples coated with whey protein concentrate-based edible coatings

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Received 8 March 2005; accepted 19 August 2005

Abstract

The effect of antioxidant type and content alone or in combination with edible coatings for fresh-cut apples was studied. Edible composite coatings were prepared from whey protein concentrate (WPC) and beeswax (BW). Ascorbic acid (AA) at 0.5% and 1% content, cysteine (Cys) at 0.1%, 0.3% and 0.5% content, and 4-hexylresorcinol (4-hexyl) at 0.005% and 0.02% were incorporated in the formulations as antioxidants. Apple pieces were coated with the emulsion coatings and weight loss, color (CIE L*, a*, b*, and browning index (BI)) and sensory evaluation were measured during storage. Results showed that incorporation of the antioxidant to the coating reduced browning compared to the use of the antioxidant alone. 4-Hexyl was the least effective at reducing browning, even when incorporated into the WPC-based coating. Increasing AA and Cys content decreased browning of coated samples. The most effective treatments were WPC–BW-based coatings with 1% AA or 0.5% Cys. Apple pieces treated with 0.3% and 0.1% Cys aqueous solutions showed a pinkish-red appearance, whereas this effect was not shown when similar levels of Cys were incorporated into the WPC–BW-based coating. Coating application did not reduces weight loss in fresh-cut apples, probably due to the high relative humidity of the product. A sensory panel was able to discriminate between samples coated with WPC–Cys and samples dipped in Cys aqueous solution, but not between samples coated with WPC–AA and samples dipped in AA aqueous solution.

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Keywords: Fresh-cut apple; Edible composite coatings; Whey protein concentrate; Enzymatic browning

1. Introduction

Commercialization of fresh-cut fruit, such as apples, is strongly influenced by color changes due to enzymatic reactions of phenolic compounds with released endogenous polyphenol oxidase and the diffusion of atmospheric oxygen into the tissue. The use of anti-browning agents based on citric acid or ascorbic acid, together with modified atmosphere packaging and low temperature storage increases shelf life of fresh-cut fruit. Edible films and coatings can also offer a possibility to extend the shelf life of fresh-cut produce by providing a semipermeable barrier to gases and water vapor, and therefore, reducing respiration, enzymatic browning, and water loss (Guilbert, 1986; Baldwin et al., 1995). Their protective function may be also enhanced with the addition of antimicrobials, antioxidants, flavors, nutrients, etc. Some results showing the potential of edible coatings to extend the shelf life of fresh-cut fruit and vegetables have been summarized by Wong et al. (1994), Baldwin et al. (1995), and Ahvenainen (1996).

The effectiveness of edible coatings depends on coating composition. The development of edible films and coatings has been focused upon barriers containing proteins, polysaccharides, and lipids. In previous works, we found that whey protein-based coatings without incorporation of antioxidants were more effective in reducing enzymatic browning of ‘Golden Delicious’ apples than hydroxypropyl methylcellulose-based coatings, probably due to the antioxidant effect of amino acids, such as cysteine, and/or the higher oxygen barrier that the protein exerts. However, no
differences on browning were found between the use of whey protein isolate (WPI) having 98% protein or whey protein concentrate (WPC) with 65% protein content. When different lipids were incorporated into the formulations, results indicated that lipid type had an effect on the degree of browning as measured with the colorimeter, but these differences were less evident at the end of the storage time by a sensory panel (Pérez-Gago et al., 2005). In addition, the solid content of the formulation and lipid content of whey protein-beeswax (BW) edible coatings without incorporation solid content of the formulation and lipid content of whey protein-beeswax coatings in combination with proper storage conditions could extend significantly shelf life of fresh-cut ‘Golden Delicious’ apples. Many studies to reduce enzymatic browning have been focused on the use of chemicals acting as inhibitors of enzymatic activity, others by rendering a medium inadequate for the development of the browning reaction (removing oxygen and phenolics), or by functioning as preferred substrate avoiding the formation of dark pigments. Some of the treatments that have been reported to be effective in reducing browning of fresh-cut apples include ascorbic acid and its isomers, citric acid, l-cysteine (Cys), and 4-hexylresorcinol (4-hexyl) alone or in combination (Dong et al., 2000). Ascorbic acid is probably the most widely used anti-browning agent. Its activity resides in its reducing character that reduces the o-benzoquinones back to o-diphenols. Similarly, Cys is also a reducing agent that inhibits enzymatic browning preventing brown pigment formation by reacting with quinone intermediates to form stable, colorless compounds. Acting as an enzyme inhibitor, 4-hexyl has been demonstrated to be effective at reducing browning of fresh-cut apples, pears and potatoes (Monsalve-González et al., 1993; Luo and Barbosa-Cánovas, 1995; Whitaker and Lee, 1995; Dong et al., 2000).

The objective of this work was to study the effect of antioxidant type and amount of whey protein concentrate (WPC)–BW–antioxidant-based emulsion coatings in postponing enzymatic browning of fresh-cut apples.

2. Materials and methods

2.1. Materials

Beeswax (Brillocrea, S.A., Valencia, Spain) was selected as the lipid phase of the WPC emulsion films. WPC was purchased from Bremntag Staines Logisties (Valencia, Spain) and had a protein content of 62% (dry basis). Glycerol was from Panreac Quimica, S.A. (Barcelona, Spain), l-cysteine (Cys), 4-hexylresorcinol (4-hexyl) and ascorbic acid (AA) were purchased from Sigma–Aldrich, S.A. (Barcelona, Spain).

2.2. Coating formulations

Aqueous solutions of 10% (w/w) WPC were prepared and heated for 30 min in a 90 °C water bath to denature the whey protein. The beeswax was melted in the hot protein solution and glycerol was added in the amount required to get the final film composition. The protein–plasticizer ratio selected was 3 parts WPC to 1 part glycerol (dry solid basis), and this ratio was kept constant throughout the study. The BW was added to the whey protein–glycerol mixture at 20% (dry basis). AA, Cys and 4-hexyl in aqueous solution were tested on apple slices in a preliminary experiment and the following concentrations were selected for incorporating into the emulsion coatings: (1) AA at 0.5% and 1% content, (2) Cys at 0.1%, 0.3%, and 0.5% content, and (3) 4-hexyl at 0.005% and 0.02%. Water was added to bring the emulsions to 15.6% total solid content. Samples were homogenized with a high-shear probe mixer (PolyTTron, Model PT 2100, Kinematica AG Inc., Lucerne, Switzerland) for 4 min at 30,000 rpm. After homogenization, the emulsions were placed in an ice bath to prevent further denaturation of the whey protein and to crystallize the lipid particles. The emulsions were degassed at room temperature with a vacuum pump and stored at 5 °C until application.

2.3. Fruit selection and preparation

‘Golden Delicious’ apples were purchased from a local wholesale produce distributor. Fruit were stored briefly at 4 °C until processing. The apples were cleaned, peeled, immersed in citric acid to avoid initial browning and microbial growth in the rinsing water, cut into rectangular pieces (approximately 5.5 cm × 3.5 cm × 1.5 cm), and immersed again in citric acid. Before coating application, samples were sanitized by immersion into a 100 mg/l sodium hypochlorite solution containing citric acid (pH ≈ 6) for 2 min. After draining for 3 min, the fresh-cut apple pieces were dipped into the coating solution or into aqueous solutions containing the antioxidants for 5 min. Following dipping, apple pieces were removed from the solution and excess coating was removed by draining for at least 30 min. A maximum of 15 apples were processed at the same time to minimize excessive exposure to oxygen. A sharp stainless-steel knife was used throughout the process to reduce mechanical bruising and samples were processed in a temperature controlled room at 10 ± 1 °C. Four apple pieces were placed in each polypropylene tray and 6 trays per treatment were prepared. Half of the trays were heat-sealed with micro-perforated polypropylene films, to ensure no modification of the surrounding atmosphere (35 μm thickness) (35 PA 200, Amcor Flexibles, Barcelona, Spain), and the rest remained opened. In this way, differences due to the package and/or the coating could be observed during storage. Finally, samples were stored at 5 °C.
BI following equation was used to determine BI:

\[ BI = \frac{(x - 0.31)}{0.172} \times 100 \]

where, \( x \) is the chromaticity coordinate calculated from the XYZ tristimulus values according to the following formula

\[ x = X/(X + Y + Z) \]

2.5. Colorimetric measurements

Color measurements were made periodically with a Minolta (Model CR-300, Ramsey, N.Y., U.S.A.) on 12 apple slices per treatment using the CIELAB color parameters, L*, a*, and b*. Each measurement was taken at three locations for each sample piece. A standard white calibration plate was employed to calibrate the equipment. Results were also reported as browning index (BI), defined as brown color purity, which is usually used as an indicator of browning in sugar-containing food products (Buera et al., 1986). The following equation was used to determine BI:

\[ BI = \frac{(x - 0.31)}{0.172} \times 100 \]

where, \( x \) is the chromaticity coordinate calculated from the XYZ tristimulus values according to the following formula

\[ x = X/(X + Y + Z) \]

2.6. Sensory evaluation

Two sets of experiments were performed to evaluate the effect of WPC-based coatings with antioxidant incorporated into the formulations.

In the first experiment apple slices, either coated or dipped in the antioxidant aqueous solution, were evaluated visually. Each treatment was coded, presented in random order and the judges had to rank each sample from highest to lowest degree of browning. The visual quality in each treatment based on general visual appearance (color plus effect of coating) was also determined based on the following visual scale: 9 = excellent, just sliced; 7 = very good, slightly browning; 6 = good, medium degree of browning; 5 = good, limit of marketability; 3 = fair, limit of usability; and 1 = poor, inedible (Gorny et al., 1999). A color photograph of samples rated with this scale was used by four judges to score the samples. Results for ranking based on color and visual quality were expressed as an average of the quality scores.

In the second experiment, the best treatments reducing browning (from the instrumental and sensory evaluation) were selected and a triangle test was performed to detect differences between WPC-based coated samples and samples dipped into the antioxidant aqueous solution. Coatings with no antioxidant (WPC), 1% AA (WPC–1% AA) or 0.5% Cys (WPC–0.5% Cys) were prepared. In each trial, the panelists compared samples coated with the WPC–antioxidant coating versus samples dipped in the antioxidant aqueous solution. The sensory panel consisted of 19 untrained panelists that received two sessions of instruction regarding the evaluation procedure.

Panels seated at partitioned booths were presented with three samples simultaneously, one different from the other two. To avoid discrimination due to color, panelists were provided with glasses made with red and blue transparent paper and the booth was also illuminated with appropriate lights to completely mask browning. Spring water was used for palate cleansing between samples and trials.

Panels evaluated each treatment pair replicated twice. Replicates were employed to improve test power (Dacremont and Sauvageot, 1997). To minimize adaptation, small breaks were taken between trials and after a set of three triads a stop was made to make the visual appearance as described in the first experiment (ranking and visual quality). The presentation order of treatment comparisons was counterbalanced across panelists and sample presentation was randomized within triads. The triangle test was made the first and second day after sample preparation in covered samples stored at 5 °C. Prior to the test, samples were preconditioned at room temperature.

2.7. Statistical analyses

Statistical analysis was performed using STATGRAPHICS Plus 2.1 (Manugistics Inc., Rockville, Maryland, U.S.A.). Specific differences between means were determined by least significant difference (LSD). Specific differences for color obtained by sensory evaluation were determined by the Friedman test, which is recommended with ranking (UNE 87 023, 1995). The significant effect on the triangle sensory test was obtained from tables (UNE 87 006, 1995). Significance of differences was defined at \( p \leq 0.05 \).

3. Results and discussions

3.1. Weight loss

Fig. 1 shows weight loss of coated and uncoated apple pieces measured at the end of the storage periods for samples stored uncovered 11 days at 5 °C or covered with polypropylene films as a secondary package 13 days at 5 °C. Covering the samples with polypropylene films significantly reduced weight loss of apple pieces. Analysis of variance showed that uncovered samples coated with WPC–antioxidant coatings presented significantly lower weight loss than samples treated with the antioxidant aqueous solutions, whereas, coating application had no significant effect on weight loss of covered samples compared to the antioxidant treatments alone. When compared with the untreated-control, only uncovered samples coated with WPC–Cys and WPC–0.005% hexyl coatings showed lower weight loss than the control, whereas the rest of the treatments presented similar or even higher weight loss than the control. However, when samples remained covered all the treatments, except WPC–0.1% Cys, showed lower weight loss than the control. Surprisingly, under these storage conditions apple slices dipped into the antioxidant
aqueous solution also presented lower weight loss than the control.

In general, the results based on coating application and antioxidant treatment varied with storage conditions and the lack of uniformity in the behavior of the treatments does not lead to a clear conclusion. Similar results were found by Pérez-Gago et al. (2003) when the effect of BW content and emulsion solid content was studied on weight loss of fresh-cut apples coated with WPI and BW. However, Olivas et al. (2003) found that methylcellulose–stearic acid coatings played an important role in avoiding weight loss of pear wedges, while methylcellulose-only coatings showed poor moisture barrier.

3.2. Color change

An increase in enzymatic browning in apple pieces during storage was accompanied by an increase in colorimetric $a^*$ and $b^*$ values, and a decrease in lightness ($L^*$) and hue values (data not shown). Browning index was calculated and used as a good indicator of color change during storage.

Fig. 2 shows the effect of the antioxidants incorporated into the coating solution or applied directly, on BI of fresh-cut apples stored either uncovered or covered at 5 °C. When samples remained uncovered, all the antioxidants applied in the aqueous solution reduced browning compared to the control except 0.1% Cys, which lost effectiveness after 7 days of storage. At this concentration, sliced apples presented a pinkish-red color that increased with storage time. The appearance of pinkish-red off-colored compounds, when applied at low concentration, has also been reported and attributed to phenol regeneration with deep color formation (Richard-Forget et al., 1992). When samples were packed with polypropylene films, Cys concentrations above 0.1% also induced the formation of the off-colors, which could be also due to oxidation in the package. When Cys was incorporated into the WPC-based coatings, its effectiveness was not reduced even at concentrations as low as 0.1%. This could be due to the nature of the whey protein. Whey protein is a mixture of proteins characterized by the high number of Cys residues in their molecules (Kinsella, 1984).

AA is one of the most commonly used agents to reduce browning of fresh-cut fruit surfaces. Application of AA, either in the aqueous solutions or in the WPC coating, was effective in reducing browning. However, the effectiveness depended on the storage condition. When the samples remained uncovered, no differences were found between application of the AA in the aqueous solution or incorporation...
into the WPC coating except the first and last storage period. In previous work, it was also observed that at time zero, right after coating application, the control apple pieces also had higher BI than samples coated with whey protein isolate–BW emulsion coatings (Pérez-Gago et al., 2003). The differences found in BI at the end of the storage period can probably be due to AA being consumed. AA is a highly effective inhibitor of enzymatic browning, primarily because of its ability to reduce quinones back to phenolic compounds. However, once the AA has been completely oxidized to dehydroascorbic acid by this reaction, quinones can again accumulate and undergo browning (Sarpers, 1993). In the present work when samples remained covered, incorporation of AA into the WPC coating reduced browning significantly with respect to the application of the AA in a aqueous solution. Under this storage condition, the lost of effectiveness of the aqueous solutions of AA was not appreciated, which confirms the beneficial effect of combining packaging and antioxidant application. Baldwin et al. (1996) have also reported an improvement of the efficiency of AA in delaying enzymatic browning of cut apple and potato when the antioxidant was incorporated into a carboxymethylcellulose-based coating formulation in comparison to dipping.

In recent years, much work has described the effectiveness of 4-hexyl in reducing enzymatic browning of fresh-cut fruit, such as apples and pears. In our work, application of...
4-hexyl was less effective than AA and Cys in reducing browning. When samples remained uncovered, incorporation of 4-hexyl reduced browning compared to the control and its effectiveness was slightly higher when the antioxidant was incorporated into the coating formulation compared to the application in the aqueous solution. However, when the samples remained covered, samples treated with 4-hexyl in aqueous solution showed higher browning than the control, and 4-hexyl was only effective when incorporated into the WPC coating solution, probably due to the antioxidant effect of WPC. Luo and Barbosa-Cánovas (1997) studied the effect of 4-hexyl in combination with AA on apple slices of several cultivars. They found different browning rates depending on the cultivar. Treatments with the combination 4-hexyl–AA resulted in better inhibition of enzymatic browning than AA alone for all the treatments except for ‘Golden Delicious’ fruit, where no differences were found. This was attributed to the different contribution of phenolic compounds and polyphenoloxidase activity to browning in each cultivar. That is, browning in ‘Golden Delicious’ is substrate dependent while in ‘Red Delicious’ and ‘McIntosh’ it is PPO-dependent (Coseteng and Lee, 1987). The synergistic effect of 4-hexyl combined with other antioxidants has also been reported in other fruit. In ‘d’Anjou’ pear wedges, 4-hexyl alone did not inhibit browning of cut surfaces, but suppressed core browning. However, a combination of 4-hexyl and sodium erythorbate resulted in less browning of cut surfaces, skin edges, and core than sodium erythorbate alone (Sarpers and Miller, 1998).

The effectiveness of antioxidant type and concentration also depended on storage conditions (Fig. 3). Whereas for uncovered samples dipped in the antioxidant aqueous solutions, 0.5% Cys was the most effective in reducing browning, for covered samples AA was the only antioxidant effective in reducing browning compared to the control. When the antioxidant was incorporated into the WPC emulsion coating, no differences were found between antioxidant type and concentration for uncovered samples, except at the beginning of the storage period. However, for covered samples, AA and 4-hexyl were the most and the least effective antioxidants in reducing browning, respectively. Increasing antioxidant concentration decreased browning, except for 4-hexyl-based coatings that were not influenced by antioxidant concentration.

3.3. Sensory evaluation

Browning of coated and uncoated fresh-cut apples was also assessed by a sensory panel with the objective of determining whether the color differences observed instrumentally could be observed visually. Samples were presented by groups according to storage condition and dip treatment.
Fig. 4. Effect of antioxidant type and amount alone or incorporated into whey protein concentrate (WPC)–beeswax (BW) coatings on visual browning of fresh-cut apple slices. WPC–BW emulsion coatings were prepared at 15.6% total solid content and BW content was 20% (dry basis). Judges ranked the apple pieces from 8 (highest browning) to 1 (lowest browning) and were allowed to group those treatments that were considered similar. Error bars represent differences between the sum of the rank values significantly different at the 5% level.

Samples treated with AA and 0.5% Cys, either in the aqueous solution or incorporated to the WPC edible coating were ranked with the lowest browning (Fig. 4). The apple slices dipped in 0.1% and 0.3% Cys aqueous solution had higher or similar browning to control samples. When these amounts of Cys were incorporated into the WPC edible coating, the profile of the ranking changed in such a way that these samples were evaluated with lower browning than the control. As confirmed by color measurements, 4-hexyl was not evaluated as a good treatment in reducing browning of fresh-cut ‘Golden Delicious’ apples.

When uncovered samples were evaluated for visual appearance, only coated samples with AA or 0.5% Cys incorporated into the formulations were evaluated as good (5) after 5 days of storage, whereas the rest of the treatments were below the limit of marketability (data not shown). Covered samples treated with 4-hexyl, with 0.1% Cys in aqueous solution and uncoated samples were evaluated below the limit of marketability after 5 days of storage (Fig. 5). Incorporation of 0.3% Cys to the WPC coating increased slightly the acceptability of the samples compared to the application of the antioxidant alone. It is important to notice that 0.5% AA, 1% AA and 0.5% Cys were evaluated more highly when they were applied in the aqueous solution than when they were incorporated into the WPC edible coating. Addition of these antioxidants to the WPC emulsion coating increased viscosity significantly, especially for emulsions containing Cys. This increase in viscosity made the period of drying longer and some of the judges evaluated the samples as having a slight whitish appearance. These results indicated the need to decrease the solid content of the emulsion coating and/or decrease the amount of protein in the formulation to avoid an increase in emulsion viscosity.

To avoid viscosity problems as antioxidants are incorporated into the formulations, the solid content of the emulsions was decreased to 14% and the BW content was increased to 30% (dry basis). With these coatings, a second experiment was performed to study if panelists would be able to differentiate between coated and uncoated samples by performing a triangle test. The triangle test was chosen as it allows one to distinguish between samples without having to specify the sensory characteristics that differ and it is also better at detecting small differences that are intensity ratings.
Fig. 5. Effect of antioxidant type and amount alone or incorporated into whey protein concentrate (WPC)–beeswax (BW) coatings on sensory evaluation of fresh-cut apple slices. WPC–BW emulsion coatings were prepared at 15.6% total solid content and BW content was 20% (dry basis). Visual quality (color plus effect of the coating) was based on a 9-point category scale. LSD is at the 5% level.

Table 1 shows the number of correct responses identifying the odd sample in the triangle test. The number of correct responses increased on the second day of the analysis, probably because the attributes that made the samples different were intensified. The panelists were able to differentiate between the samples containing Cys and between the samples coated with WPC and the control ($p < 0.01$). When the panelists were asked to describe any attribute that helped define the differences, the most common response was the higher smell of sulfur compounds in the Cys-coated sample and differences in texture between WPC-coated and control samples. Richard-Forget et al. (1992) have already pointed out that for complete browning control the amount of Cys required is often incompatible with product taste. Interestingly, no differences were found between coated and uncoated samples containing AA, which could make the WPC–AA coating a possible replacement for the application of AA alone.

The panelists were also asked to rank the samples according to degree of browning and to evaluate them according to the scale from 1 to 9 used by Gorny et al. (1999). The degree of browning was also followed by instrumental measurements with the colorimeter. In general, the control and the samples coated with WPC without antioxidants were ranked with the highest smell of sulfur compounds in the Cys-coated sample and differences in texture between WPC-coated and control samples. Richard-Forget et al. (1992) have already pointed out that for complete browning control the amount of Cys required is often incompatible with product taste. Interestingly, no differences were found between coated and uncoated samples containing AA, which could make the WPC–AA coating a possible replacement for the application of AA alone.

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When samples were evaluated for general visual appearance, coated apple slices with antioxidants incorporated into the formulations were evaluated as very good during the 9 days of storage, whereas samples dipped into the antioxidant aqueous solution reached the limit of marketability after 9 days of storage (Fig. 6B). The decrease in emulsion viscosity reducing solid content of the coatings improved
the sensory evaluation of the samples by the panelists (Figs. 5 and 6).

4. Conclusion

Incorporation of AA or 0.5% Cys to the WPC–BW emulsion coatings reduced enzymatic browning of ‘Golden Delicious’ apples significantly, compared to the use of the antioxidants alone. 4-Hexyl was not an effective antioxidant for ‘Golden Delicious’ fresh-cut apples in the conditions studied. Apples pieces treated with 0.3% and 0.1% Cys aqueous solution showed a pinkish-red appearance, whereas this effect was not shown when similar levels of Cys were incorporated into the WPC–BW-based coating, probably due to the Cys amino acid residues present in the whey protein. Results observed by sensory evaluation indicated the need to reduce emulsion coating viscosity by decreasing emulsion solid content to 14% and increase BW content to 30% (dry basis). The sensory panel was able to discriminate between samples coated with WPC–0.5% Cys and samples dipped in 0.5% Cys aqueous solution, but not between samples coated with WPC–1% AA and samples dipped in 1% AA aqueous solution, which could make the WPC–AA coating a possible replacement for the application of AA alone.

Acknowledgments

This work was funded by the Instituto Nacional de Investigación y Tecnología Agraria y Alimentaria through the project CAL01-021-C2-1-2. The authors also thank Dow Chemical Co. and Cat-Preymer for supplying samples of hydroxypropyl methylcellulose and technical support.

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