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**Postharvest  
Biology and  
Technology**

Postharvest Biology and Technology 36 (2005) 77–85

[www.elsevier.com/locate/postharvbio](http://www.elsevier.com/locate/postharvbio)

## Effect of whey protein- and hydroxypropyl methylcellulose-based edible composite coatings on color change of fresh-cut apples

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Received 12 April 2004; accepted 27 October 2004

### Abstract

Edible composite coatings were prepared from whey protein isolate (WPI), whey protein concentrate (WPC) or hydroxypropyl methylcellulose (HPMC) as the hydrophilic phase, and beeswax (BW) or carnauba wax (CarW) as the lipid phase. Apple pieces were coated with the emulsion coatings and weight loss and color (CIELAB color parameters, L\*, a\*, b\*, and browning index (BI)) were measured during storage. Results show that apple pieces coated with whey protein-based coatings had higher L\*-, and lower b\*-, a\*-, and BI-values than HPMC-based coated and uncoated apple pieces, which indicate that whey proteins exert an antibrowning effect. Coatings containing BW were more effective in decreasing enzymatic browning than coatings containing CarW. The sensory panel differentiated samples coated with whey protein-based coatings from samples coated with HPMC-based coatings. However, differences due to lipid type were less evident at the end of the storage time. Coating application did not reduce weight loss in fresh-cut apples, probably due to the high relative humidity of the product.

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**Keywords:** Fresh-cut apple; Edible composite coatings; Whey protein; Hydroxypropyl methylcellulose; Enzymatic browning

### 1. Introduction

Enzymatic browning is a major problem reducing shelf-life of fresh-cut fruit and vegetables due to the reaction of phenolic compounds with atmospheric oxygen diffusing into the tissue. This reaction is catalyzed by endogenous polyphenol oxidase, which is

released through cutting injury of the fresh-cut products. The main approach to inhibit browning is the use of antibrowning agents based on citric acid or ascorbic acid. In addition, the use of modified atmosphere packaging further increases the shelf-life of fresh-cut products. Edible films and coatings can offer a possibility to extend the shelf-life of fresh-cut products by providing a semipermeable barrier to gases and water vapor, and thereby, reducing respiration, enzymatic browning, and water loss (Baldwin et al., 1995a). Their protective function may also be enhanced with the addition of antimicrobials, antioxidants, flavors, nutrients,

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etc. Some of the 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. (1995b), Ahvenainen (1996) and Park (1999). Most of the work in the literature consists of coating formulations, which include some preservatives and/or antioxidants. However, little is known about the effect of coating composition without the incorporation of additives, such as antioxidants and/or antimicrobials.

The development of edible films and coatings has been focused upon barriers containing proteins, polysaccharides, and lipids. Among the film-forming compounds studied, hydroxypropyl methylcellulose (HPMC), whey protein isolate (WPI) and whey protein concentrate (WPC) have shown good film-forming properties and the capability of yielding tough and flexible transparent films with good oxygen barrier properties at low relative humidity. However, due to the hydrophilic nature of these compounds, they provide a poor moisture barrier. Incorporation of lipids reduces film water vapor permeability and the moisture barrier of these composite films has been shown to depend, among other factors, on the lipid type and amount (Kamper and Fennema, 1984; Kamper and Fennema, 1985; Kester and Fennema, 1986; McHugh and Krochta, 1994; Shellhammer and Krochta, 1997; Pérez-Gago and Krochta, 2000).

Few reports of application to fresh-cut fruit of HPMC-based and whey protein-based coatings without antioxidants are found in the literature. In a previous paper, we showed that the solid content of the formulation and lipid content of WPI-beeswax (BW)-edible coatings without incorporation of antioxidants had an affect on the degree of browning of fresh-cut apples. The optimum solid content of the emulsion and BW-content, in order to reduce browning, were 16 and 20%, respectively (Pérez-Gago et al., 2003). In a similar study with HPMC-BW, the solid content of the emulsion and the BW content did not affect color of coated fresh-cut apples (unpublished data). However, emulsion coatings with high viscosity due to an increase in the solid content and/or low BW content of the emulsion (i.e., coatings with high HPMC content) were considered unacceptable from the commercial point of view, since drying time was considerably increased and the appearance was poor. Therefore, the optimum solid content of the emulsion and BW con-

tent of HPMC-BW coatings selected was 8 and 40%, respectively.

In the present work, the objective was to compare the effectiveness of HPMC-, WPI- and WPC-based coatings without the incorporation of antibrowning agents, and to study the effectiveness of lipid type in postponing enzymatic browning of fresh-cut apples.

## 2. Materials and methods

### 2.1. Materials

Beeswax (BW) and carnauba wax (CarW) (Brillo-cera S.A., Valencia, Spain) were selected as the lipid phase of the whey protein and HPMC emulsion films. WPI was purchased from Davisco Foods International (Le Sueur, MN, USA) and WPC from Brenntag Stinnes Logisties (Valencia, Spain). The protein content in the WPC was 62% (dry basis). Hydroxypropyl methylcellulose (HPMC) (Methocel E15) was supplied by Dow Chemical Co. (Midland, MI, USA). Glycerol and stearic acid were from Panreac Quimica, S.A. (Barcelona, Spain).

### 2.2. Coating formulations

Emulsion coatings consisted HPMC, WPI or WPC as the hydrophilic phase and BW or CarW as the lipid phase, suspended in water.

To make the HPMC-lipid emulsion coatings, 5% HPMC (w/w) solution was prepared by first dispersing the powder in a small amount (about one-third of the total water added) of hot water at 80 °C, and next adding the rest of the cold water. Hydration of the HPMC was completed by cooling and mixing the 5% solution to less than 30 °C for 30 min. Stearic acid and glycerol were added as emulsifier and plasticizer, respectively. The HPMC-plasticizer phase consisted of three parts HPMC to one part glycerol (dry basis), and this ratio was kept constant throughout the study. Lipid (BW or CarW)-stearic acid ratio was also kept constant and consisted of two parts lipid to one part fatty acid (dry basis). Either the BW or CarW were added to the HPMC-stearic acid-glycerol mixture at 40% (dry basis). To help melting of the BW and CarW, solutions were heated to 10–20 °C above the melting point of the lipids, so they melted immediately

prior to homogenization. Once the lipids were melted, samples were homogenized with a high-shear probe mixer (PolyTron, Model PT 2100; Kinematica AG Inc., Lucerne, Switzerland) for 4 min at 30,000 rpm. Before homogenization, cold water was added to bring the emulsions to 8% total solids content. Further cooling was achieved by placing the emulsions in an ice-bath to bring them to room temperature. Agitation was continued for approximately 20 min after reaching this temperature to ensure complete hydration of the HPMC. The emulsions were degassed at room temperature with a vacuum pump and stored at 5 °C until application.

To prepare the WPC and WPI-lipid emulsion coatings, aqueous solutions of 10% (w/w) whey protein were prepared and heated for 30 min in a 90 °C water bath to denature the whey protein. The lipid was melted in the hot protein solution and glycerol was added in the amount required to obtain the final film composition. The protein-plasticizer ratio selected was three parts WPI to one part glycerol (dry basis), and this ratio was kept constant throughout the study. Either the BW or CarW were added to the whey protein-glycerol mixture at 20% (dry basis). Water was added to bring the emulsions to 16% total solid content. Samples were homogenized with a high-shear probe mixer (Poly-Tron, 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 also degassed at room temperature with a vacuum pump and stored at 5 °C until application. Composition of the emulsion coatings on a dry basis is shown in Table 1.

Table 1  
Coating composition on a dry basis (%)

Components	HPMC-based coatings	Whey protein-based coatings
HPMC/WPC/WPI	30.0	60.0
Lipid	40.0	20.0
Stearic acid	20.0	—
Glycerol	10.0	20.0
Solid content (%)	8	16

HPMC: hydroxypropyl methylcellulose; WPC: whey protein concentrate; and WPI: whey protein isolate.

### 2.3. Fruit selection and preparation

‘Golden Delicious’ apples were purchased from a local wholesale produce distributor. The fruit were stored briefly at 4 °C until processing. The apples were cleaned, peeled, immersed in citric acid to avoid initial browning, cut into rectangular pieces (approximately 5.5 cm × 3.5 cm × 1.5 cm), and sanitized by immersion into a 100 mg L<sup>-1</sup> sodium hypochlorite solution containing citric acid (pH ≈ 6) for 2 min. After draining, the fresh-cut apple pieces were dipped in the coating solution for 5 min. Following dipping, apple pieces were removed from the solution and excess coating was removed by draining for 20 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 six trays per treatment and storage temperature were prepared. Half of the trays were heat-sealed with microperforated polypropylene films to ensure no modification of the surrounding atmosphere (35 µm thickness) (35 PA 200, Amcor Flexibles, Barcelona, Spain). The rest of the trays remained opened. Finally, samples were stored 1 day at 20 °C, or 7 days at 5 °C.

### 2.4. Weight loss determination

Weight loss was measured at the end of the storage period by weighing three trays containing four apple pieces, which represented three different replicates. The results were expressed as the percentage loss of initial weight.

### 2.5. Colorimetric measurements

Color measurements were made periodically with a Minolta (Model CR-300, Ramsey, NY, USA) 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 spectrophotometer. Results were also reported as a browning index (BI), defined as brown-color purity, which is usually used as an indicator of the extent of browning in sugar-containing food products (Buera et al., 1986).

The following equation was used to determine BI:

$$BI = \frac{(x - 0.31)}{0.172} \times 100 \quad (1)$$

where  $x$  is the chromaticity coordinate calculated from the  $X\ Y\ Z$  tristimulus values according to the following formula  $x = X/(X + Y + Z)$ .

### 2.6. Sensory evaluation

At the beginning and at the end of each experiment, apple fruit slices 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 (hedonic) scale: 9 = excellent, just sliced; 7 = very good; 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 three judges to score the samples. Results for ranking based on color and visual quality, performed by the same panel members, were expressed as an average of the scores.

### 2.7. Statistical analyses

Statistical analysis was performed using STATGRAPHICS Plus 2.1 (Manugistics Inc., Rockville, MD, USA). Specific differences between means were determined by least significant difference (LSD). Specific differences for color obtained by sensory eval-

uation were determined by the Friedman test, which is recommended with ranking (UNE 87 023, 1995). Significance of differences was defined at  $p \leq 0.05$ .

## 3. Results and discussions

### 3.1. Weight loss

**Table 2** shows weight loss of coated and uncoated apple pieces (control) measured at the end of the storage periods for samples stored 1 day at 20 °C, or 7 days at 5 °C. Samples remained either uncovered or covered with polypropylene films as a secondary package. Covering the samples with polypropylene films significantly reduced weight loss of apple pieces. However, coating application did not significantly improve moisture loss compared to the control. Few differences were observed between coating treatments, in spite of differences in lipid type, lipid content and the solid content of the emulsion between the whey protein-based and the HPMC-based coatings (**Table 1**). In general, the lack of uniformity in the behavior of the treatments at the different storage conditions leads to no conclusions based on the effect of coating composition on weight loss. Similar results were found by Pérez-Gago et al. (2003) who studied the effect of BW content and solid content of the emulsion on weight loss of fresh-cut apples coated with WPI and BW. However, Olivas et al. (2003) found that incorporation of stearic acid to methylcellulose coatings played an important role in avoiding weight loss of pear wedges compared to methylcellulose coatings without stearic acid.

Table 2  
Percentage weight loss of fresh-cut coated and uncoated apples

Treatments	Storage conditions			
	One day at 20 °C		Seven days at 5 °C	
	Open <sup>a</sup>	Closed <sup>b</sup>	Open <sup>a</sup>	Closed <sup>b</sup>
WPI-BW	9.18 ± 0.30 ab	0.58 ± 0.07 a	25.03 ± 3.15 a	1.16 ± 0.17 cd
WPI-CarW	9.14 ± 0.88 ab	0.68 ± 0.04 a	20.69 ± 3.09 a	0.80 ± 0.15 ab
WPC-BW	6.85 ± 1.35 a	0.75 ± 0.07 a	21.48 ± 0.70 a	1.40 ± 0.12 d
WPC-CarW	8.02 ± 1.72 ab	1.07 ± 0.79 a	25.07 ± 2.62 a	1.03 ± 0.19 bc
HPMC-BW	13.33 ± 1.05 c	0.51 ± 0.01 a	21.04 ± 2.63 a	0.73 ± 0.07 a
HPMC-CarW	10.33 ± 2.13 b	0.60 ± 0.11 a	20.89 ± 4.44 a	0.79 ± 0.12 ab
Control	9.14 ± 1.58 ab	0.75 ± 0.07 a	21.32 ± 2.31 a	0.83 ± 0.21 ab

Means within each storage time with different letters are significantly different at  $p = 0.05$ .

<sup>a</sup> Samples were stored uncovered.

<sup>b</sup> Samples were stored covered with microperforated polypropylene films.

Table 3

Analysis of variance on the color index ( $L^*$ ,  $a^*$ ,  $b^*$ , hue and chroma) of coated and uncoated fresh-cut apple slices

Open, 20 °C		Closed, 20 °C		Open, 5 °C		Closed, 5 °C	
Lipid type	Hydrocolloid type	Lipid type	Hydrocolloid type	Lipid type	Hydrocolloid type	Lipid type	Hydrocolloid type
$L^*$	3.01 <sup>a</sup>	15.83 <sup>**</sup>	4.72 <sup>*</sup>	7.82 <sup>**</sup>	9.89 <sup>**</sup>	29.32 <sup>**</sup>	10.43 <sup>**</sup>
$a^*$	5.38 <sup>**</sup>	7.68 <sup>**</sup>	4.72 <sup>*</sup>	19.95 <sup>**</sup>	19.89 <sup>**</sup>	48.18 <sup>**</sup>	1.95 <sup>a</sup>
$b^*$	5.63 <sup>**</sup>	5.59 <sup>**</sup>	23.52 <sup>**</sup>	11.47 <sup>**</sup>	19.00 <sup>**</sup>	14.49 <sup>**</sup>	42.02 <sup>**</sup>
Hue	3.38 <sup>*</sup>	5.07 <sup>**</sup>	0.01 <sup>a</sup>	3.72 <sup>*</sup>	19.57 <sup>**</sup>	37.27 <sup>**</sup>	5.11 <sup>**</sup>
Chroma	5.78 <sup>**</sup>	5.67 <sup>**</sup>	25.55 <sup>**</sup>	12.63 <sup>**</sup>	18.97 <sup>**</sup>	13.60 <sup>**</sup>	43.12 <sup>**</sup>

*F*-ratios are shown for the sources of variations.<sup>a</sup> Not significant.<sup>\*</sup> Significant at  $p = 0.05$ .<sup>\*\*</sup> Significant at  $p = 0.01$ .

The results also contrast with the behavior of stand-alone films (without coating the fruit surface). Issues of lipid type and content have been studied as affecting the barrier properties of protein and polysaccharide-based emulsion films. Many studies have shown that the barrier properties of stand-alone films depend on lipid type and content (Martin-Polo et al., 1992a,b; Gontard et al., 1994). At similar lipid content, the moisture barrier depends on the degree of saturation and lipid polarity. Thus, waxes are the most effective as moisture barriers because of high hydrophobicity due to their high content of long-chain fatty alcohols and alkanes (Morillon et al., 2002). Shellhammer and Krochta (1997) found that viscoelastic waxes, such as BW are more effective in reducing water vapor permeability of stand-alone films made with WPI and glycerol plasticizer than non-viscoelastic waxes, such as CarW. However, in our work both waxes seemed to perform similarly in reducing weight loss of fresh-cut apples.

Coatings containing protein or polysaccharide alone do not provide a good moisture barrier (Krochta, 1997). Even though the incorporation of hydrophobic components improved this barrier property, the water vapor permeability of these composite films increased as relative humidity increased. The surface of fresh-cut fruit has high relative humidity. For this reason, the performance of the coatings in reducing weight loss is not as good as when the coatings are applied to whole fruit.

### 3.2. Color change

Increased enzymatic browning in apple pieces during storage was accompanied by an increase in col-

orimetric  $a^*$ - and  $b^*$ -values, and a decrease in lightness ( $L^*$ ) and hue values. The calculated browning index (BI) was a good indicator of color change during storage. Table 3 shows the analysis of variance (ANOVA) results on the color index for the coated and uncoated fresh-cut apples stored under different conditions. The lipid- and hydrocolloid-type (protein or polysaccharide) factors were a significant source of variation for almost all of the color index, indicating that coating composition significantly affected the color of the fresh-cut apples.

Figs. 1 and 2 show the effect of coating type on BI and  $L^*$ , respectively, of fresh-cut apples stored either unpacked or packed 7 days at 5 °C, or 1 day at 20 °C. Application of all the emulsion coatings significantly reduced BI compared to control apples dipped in sodium hypochlorite and citric acid solutions, except for samples that remained uncovered and stored at 5 °C where HPMC–CarW coatings did not control browning compared to uncoated samples ( $p \leq 0.01$ ) (Fig. 1). HPMC–lipid based coatings were, in general, less effective than whey protein–lipid-based coatings. No differences were found between samples coated with WPI and WPC ( $p \leq 0.05$ ).

Lightness of fresh-cut apples was only improved in samples coated with whey protein-based coatings (Fig. 2). Fresh-cut apples coated with whey protein–lipid-based coatings had significantly higher  $L^*$ -values than control apples dipped in sodium hypochlorite and citric acid solutions. HPMC–lipid-based coatings did not improve the lightness of coated apples compared to the control.

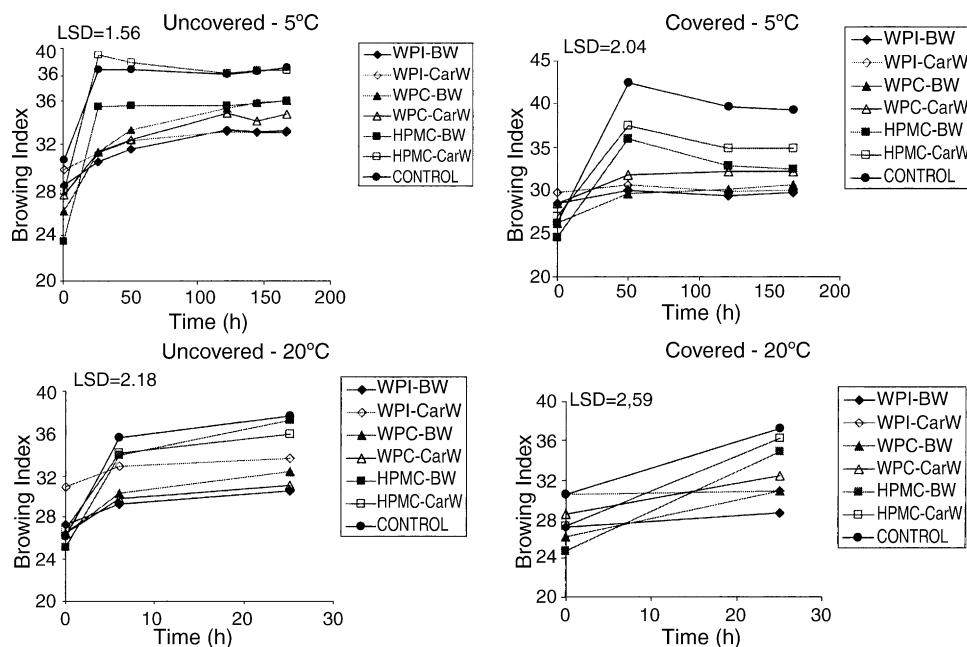


Fig. 1. Browning index of coated and uncoated fresh-cut apple slices. Samples were stored at 5 and 20 °C, either uncovered or covered with polypropylene films. LSD are given at the 5% level.

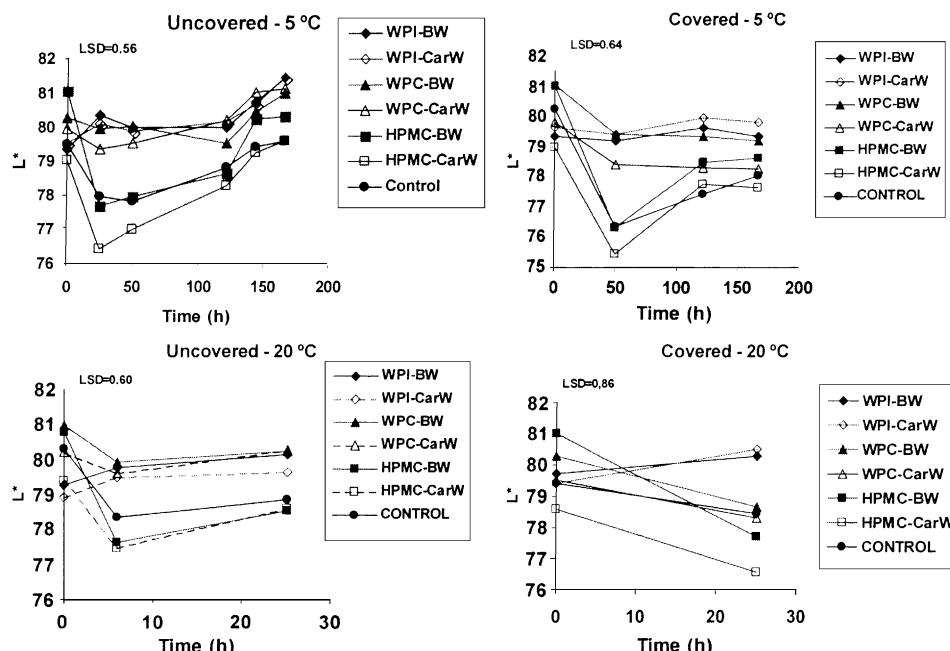


Fig. 2. Luminosity ( $L^*$ ) of coated and uncoated fresh-cut apple slices. Samples were stored at 5 and 20 °C, either uncovered or covered with polypropylene films. LSD are given at the 5% level.

In previous work, Pérez-Gago et al. (2003) observed that WPI–BW emulsion coatings without antioxidants were effective in reducing enzymatic browning of fresh-cut apples. Le Tien et al. (2001) also studied the antioxidant capacity for film formulations based on calcium caseinate or WPC, with or without carboxymethyl cellulose sodium salt, and observed that whey proteins exhibit higher antioxidative power than calcium caseinate. In both studies, the results were attributed to the coatings acting as an oxygen barrier and/or having antioxidant properties, since the presence of several cysteine side residue amino acids might provide them with the ability to reduce enzymatic browning. Lee et al. (2003) studied the effect of carrageenan and WPC-based edible coatings on the initial respiration rate of fresh-cut apples and showed that the rate at 25 °C decreased by about 5 and 20% in carrageenan- and WPC-coated apple slices, respectively. The higher efficiency in reducing the respiration rate of WPC coatings was attributed to their water-insolubility and good oxygen barrier properties (McHugh and Krochta, 1994), as well as to the possible inhibitory effect of calcium ions in respiration (Poovaiah et al., 1988). In their experiment, color change of apple slices was only studied for the coatings in combination with antibrowning agents when stored at 3 °C, so the effect of carrageenan and WPC alone was not determined.

Olivas et al. (2003) studied the ability of methylcellulose and methylcellulose–stearic acid coatings to preserve the quality of fresh-cut ‘Anjou’ pears. In this work, some additives (ascorbic acid, calcium chloride and sorbic acid) were incorporated into the coating formulations. Since no differences in BI were found between the use of coatings containing additives and the use of the additives alone, it was concluded that there was no effect from the methylcellulose and methylcellulose–stearic acid coatings on the BI, and so the extension of shelf-life was only due to the presence of ascorbic acid and sodium chloride.

Lipid type also had a significant effect on browning. BW-based coatings were more effective in reducing browning than CarW-based coatings, except for uncovered samples stored 1 day at 20 °C ( $p \leq 0.05$ ). Nevertheless, both BW- and CarW-based coatings were effective in reducing browning compared to the uncoated control. CarW-based emulsions were more yellow than BW-based emulsions, which could be the reason for the color differences in coated samples.

Pérez-Gago et al. (2003) reported that an increase in BW content reduced the BI of coated fresh-cut apples; however, the increase in BW content imparted a whitish appearance to coated samples that was considered unacceptable from the commercial point of view by the sensory panel. This could indicate that the color of the emulsion coatings might affect the final color of the coated sample.

As expected, storage at 5 °C and covering the samples improved quality of the fresh-cut apples by reducing enzymatic browning (Figs. 1 and 2). Covered samples had a significantly lower BI than uncovered samples stored at 5 °C ( $p \leq 0.05$ ) due to the reduced exposure to atmospheric oxygen. Whereas, at 20 °C, there were no differences between covered and uncovered samples, probably due to the faster rate of browning observed at this storage temperature. The level of the BI after 7 days of storage at 5 °C was not significantly different to that of samples stored 1 day at 20 °C, which shows the effectiveness of reducing temperature in prolonging the shelf-life of fresh-cut fruit.

### 3.3. Sensory analysis

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. At the end of the storage period, the judges were asked to rank the apple slices from lowest (1) to highest (7) degree of browning, and were allowed to group those samples that were considered to have similar color. In addition, the judges also evaluated the effect of coating application on the visual appearance of the apple slices based on color and general appearance.

Whey protein-based treatments resulted in higher sensory scores than uncoated apples. Even though HPMC-coated samples scored slightly higher than the control, no significant differences were found between them (Fig. 3A). Apple slices coated with whey protein-based coatings and covered with polypropylene films were evaluated as being within the limits of commercialization after 7 days of storage at 5 °C, even though the formulations did not carry any antioxidant, whereas, the control was considered inedible.

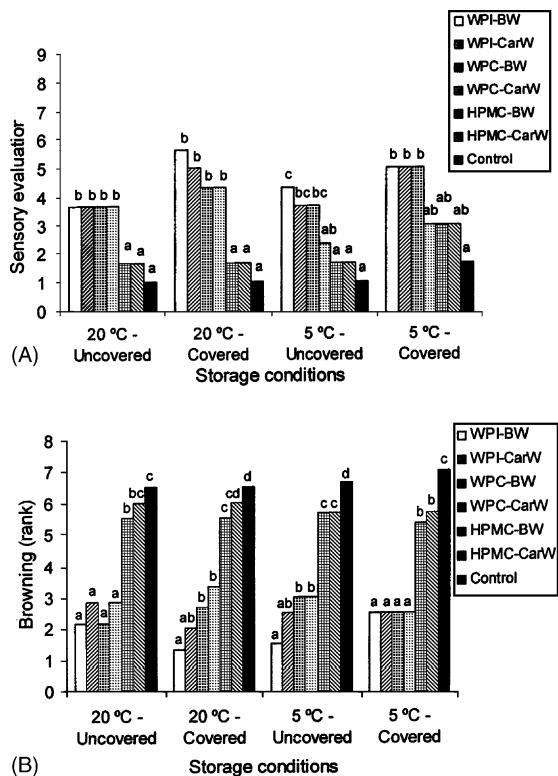


Fig. 3. Effect of coating type on visual appearance (A) and browning ranking (B) of fresh-cut apple slices. Visual appearance (color plus effect of the coating) was based on a visual (hedonic) scale (9 = excellent, just sliced; 7 = very good; 5 = good, limit of marketability; 3 = fair, limit of usability; and 1 = poor, inedible). Judges ranked the apple slices from 7 (highest browning) to 1 (lowest browning) and were allowed to group those treatments that were considered similar. Means within each storage time with the same letter are not different ( $p < 0.05$ ).

When comparing the degree of browning, the judges separated coated samples into two groups: (1) whey protein-coated apples and (2) HPMC-coated samples (Fig. 3B). Apple slices coated with whey protein-based coatings were ranked with lower browning values than apples coated with HPMC-based coatings. The latter did not differ significantly from uncoated apples except for covered samples stored at 5 °C. These results are in agreement with the results obtained by the colorimeter regarding the effect of protein and polysaccharide on browning of fresh-cut apples. Differences among samples due to lipid type were not observed by the judges whereas these differences were detected by the colorimeter.

#### 4. Conclusion

Whey protein-based coatings were more effective in reducing enzymatic browning of 'Golden Delicious' apple slices than HPMC-based coatings, probably due to the antioxidant effect of amino acids, such as cysteine and/or the higher oxygen barrier that the protein exerts. No differences in browning were found between the use of WPI or WPC. Lipid type affected the degree of browning as measured with the colorimeter, but these differences were less evident at the end of the storage time by the sensory panel. The results suggest that the addition of antibrowning agents to whey protein coatings in combination with proper storage conditions could significantly extend the shelf-life of fresh-cut apples.

#### 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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