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Effect of beeswax content on hydroxypropyl methylcellulose-based edible film properties and postharvest quality of coated plums (Cv. *Angeleno*)

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Running Head Title: BW effect on HPMC-BW films and coatings

Abstract

The effect of beeswax (BW) content of hydroxypropyl methylcellulose (HPMC)-BW edible coatings on stand-alone film properties and on postharvest quality of coated 'Angeleno' plums was studied. The coatings contained BW at 4 lipid content levels (0, 20, 40 and 60 g/100g, dry basis). Coated and uncoated plums were stored 4 weeks at 1 ºC and transferred to 20 ºC for 1 to 3 weeks. Addition of BW to the HPMC film matrix reduced film mechanical resistance and oxygen barrier, and improved film moisture barrier. Film mechanical properties showed a good fit with an exponential and/or linear model that could provide a useful tool to predict mechanical properties with others HPMC-BW composition mixtures. Coatings with BW reduced plum weight loss compared to HPMC-based coatings with no BW. Plum weight loss decreased as BW content increased from 20 to 40 g/100g, but above 40 g/100g BW content, weight loss was not further reduced. Whereas, water vapor permeability of stand-alone films decreased significantly as BW content increased to 60 g/100g. Coatings reduced plum softening and bleeding, with those with lower BW content being more effective, which could be related to the ability of coatings to create a modified atmosphere in the fruit. Flavor was not affected by coating application. Results indicate that HPMC-BW coatings with 20 g/100g BW would provide the best compromise to extend shelf life of 'Angeleno' plums.

KEYWORDS: water vapor permeability; oxygen permeability; mechanical properties; postharvest quality; plum.

1. Introduction

Consumer interest in health, nutrition, and food safety combined with environmental concerns has renewed efforts in edible film and coating research. The main function of edible films and coatings is to offer a protective barrier to moisture, oxygen, flavor, aroma, etc., between the food and the environment. Additionally, edible films and coatings may act as carriers of food ingredients and help improving the handling characteristics of the food. Therefore, application of edible coatings to fruits is a simple technology that allows reduction in fruit moisture loss and permits regulation of respiration as a passive modified atmosphere packaging. In addition, coatings can also act as carriers for fungicides or growth regulators and improve fruit gloss (Banks, Dadzie & Cleland, 1993; Cisneros-Cevallos & Krochta, 2002).

Edible films and coatings are made with food-grade ingredients, generally recognized as safe for human consumption. Materials used in edible films and coatings include proteins, polysaccharides, and lipids (Greener-Donhowe & Fennema, 1993). Among polymeric materials, cellulose is the most abundantly occurring natural polymer on earth with excellent film forming properties (Bravin, Peressini & Sensidoni, 2004). However, native cellulose is insoluble in water due to the high level of intramolecular hydrogen bonging in the cellulose polymer. The usefulness of cellulose to form edible films and coatings can be extended by the use of different cellulose derivatives. Among them, hydroxypropyl methylcellulose (HPMC) yields films that are flexible, odorless, tasteless, water soluble, and resistant to oils and fats (Greener-Donhowe & Fennema, 1986), and present good oxygen and aroma barrier properties (Miller & Krochta, 1997). However, their hydrophilic nature makes them rather ineffective moisture barriers. Addition of lipids to the HPMC matrix, forming composite edible films, has improved film moisture barrier properties (Kamper & Fennema, 1984; Hagenmaier & Shaw, 1990).

Previous studies showed the potential of HPMC-Beeswax (BW) edible composite coatings to extend the self life of plums (Pérez-Gago, Rojas & Del Rio, 2003; Navarro-Tarazaga, Sothornvit & Pérez-Gago, 2008a) and citrus (Pérez-Gago, Rojas & Del Rio, 2002; Navarro-Tarazaga & Pérez-Gago, 2006; Navarro-Tarazaga, Pérez-Gago, Goodner & Plotto, 2007; Navarro-Tarazaga, Del Río, Krochta & Pérez-Gago, 2008b). However, the effectiveness of these coatings depends on fruit type and cultivar. In plums cv. 'Angeleno' the coatings did not reduce weight loss, but they had an important effect maintaining flesh firmness and reducing bleeding (Navarro-Tarazaga, Sothornvit & Pérez-Gago, 2008a).

The main interest in edible films and coatings has been based on their barrier properties, with most of those studies focused on improving film and coating moisture barrier. The study of the effect of coating composition on coating properties has been usually assessed by using stand-alone films as a model. In emulsion films, barrier and mechanical properties are highly dependent on lipid content, lipid particle size and viscoelasticity of the lipid (Debeaufort, Quezada-Gallo & Voilley, 1998; Pérez-Gago & Krochta, 2001). However, coating performance should be also analyzed when formulations are applied on the fruit, because additional factors, such as skin morphology and physiology of the fruit commodity, are also important controlling mass transfer of coated fruit. Not many works studying simultaneously the effect of formulation composition on stand-alone film properties and postharvest quality of a coated fruit have been done. Therefore, the objective of this work was to study the effect of BW content of HPMC-BW coatings on postharvest quality of coated

‘Angeleno’ plums and to correlate the results with the barrier and mechanical properties of stand-alone films.

2. Materials and methods

2.1. *Materials*

HPMC (Methocel E15) was supplied by Dow Chemical Co. (Midland, MI, U.S.A.). Refined BW (grade 1) was obtained by Brillocera, S.A (Valencia, Spain). Stearic acid and glycerol were purchased from Panreac Química, S.A. (Barcelona, Spain).

2.2. Emulsion film and coating formulation

HPMC at 5 g/100g (w/w) was prepared by initial dispersion of the cellulose in hot water at 90 ± 2 ºC and later hydration at 20 ºC. Next, BW was added at 0, 20, 40 and 60 g/100g (dry basis, db). Glycerol was added as plasticizer at a HPMC:glycerol ratio of 2:1 (w/w) and stearic acid was added as emulsifier at a BW:stearic acid ratio of 5:1 (w/w). These ratios were kept constant for all formulations. Water was added to bring the mixtures to a final solid content of 7 g/100g for stand-alone films and 4 g/100g for coating formulations. Mixtures with all the ingredients were heated at 90±2 ºC to melt the BW and emulsions were formed by homogenization with a high-shear probe mixer UltraTurrax® (Mod. T25 basic; IKA-Werke GmbH & Co. KG, Staufen, Germany) for 1 min at 13,000 rpm followed by 3 min at 22,000 rpm. After cooling the emulsions in an ice bath to less than 20±2 ºC, they were continuously stirred for approximately 45 min to ensure complete hydration of the HPMC. Composition of emulsion films is shown in Table 1.

2.3. Film preparation

The film-forming solutions were degassed and applied onto a 15 cm internal diameter smooth high-density polyethylene casting plate at 30 g of total solids per plate to minimize thickness variations between formulations. The plates were placed on a leveled surface and dried at room conditions until films could be removed from the casting surface. Three replications were prepared for each formulation.

2.4. Film tensile properties

Film mechanical properties were measured according to the American Society of Testing and Materials Standard Method (ASTM) DS882-97 (ASTM, 1997). Films were conditioned 24 h at 23±2 ºC and 50±1% relative humidity (RH), cut into 50 mm x 8 mm rectangular strips, and tested for tension analysis using an Instron Universal Machine (Model 3343; Instron Corp., Canton, MA, USA). Load cell and cross head speed were 0.3 kN and 5 mm/min, respectively. Testing conditions were held constant at 23±2 ºC and 50±1% RH throughout the analysis. Young´s modulus (YM), maximum tensile stress (TS) and elongation at break (%E) were calculated from the plot of stress versus strain, considering a rectangular cross-sectional area and using the average film thickness, measured at 9 random positions. Fifteen specimens from each replicate of each formulation were analyzed.

2.5. Film water vapor permeability

A modification of the ASTM E96-80 (ASTM, 1980) gravimetric method for measuring water vapor permeability (WVP) was used (McHugh, Avena-Bustillos, & Krochta, 1993). Upon drying, films were chosen on the basis of lack of physical defects such as cracks, bubbles, or pinholes. Two specimens from each replicate of each formulation were cut and mounted on polymethacrylate test cups containing 6 mL of distilled water. The specimens were analyzed with the film surface that had been exposed to air during drying facing either the low RH environment ('facing up') or the high RH environment ('facing down'), allowing detection of any phase separation within the film. The cups were placed in a pre-equilibrated desiccator cabinet fitted with a variable speed-fan. The environment within the cabinet was held constant at 23±2 ºC and 40±1% RH using anhydrous potassium carbonate. Weights taken periodically until steady state was achieved and the average film thickness measured at six random positions were used to calculate the resulting WVP. Three replicates of each film were evaluated.

2.6. Film oxygen permeability

Oxygen permeability (OP) of stand-alone films was measured at 23 ºC and 50±1% RH using a Systech Oxygen Analyzer (Mod. 8001; Systech Instruments; Oxfordshire, UK) according to the ASTM D3985-95 standard method (ASTM, 1995). Films were placed on a stainless steel mask with an open testing area of 5 cm^2. Masked films were placed into a test cell and exposed to 98kPa N_2 + 2kPa H_2 flow on one side and pure O_2 flow on the other side. OP was calculated by dividing the oxygen transmission rate by the difference in oxygen partial pressure between both sides of the film (1 atm) and multiplying by the average film thickness, measured at 4 random positions. Three replicates of each film were evaluated.

2.7. Film thickness measurement

Film thickness was measured using a Mitutoyo digital micrometer (Model Quickmike Series 293-IP-54; Mitutoyo Corp., Kanagawa, Japan) taking measurements at random positions on the film.

2.8. Fruit sample preparation and coating application

'Angeleno' plums were hand-harvested with an average maturity index of 14.8 from a local grove in Alicante (Spain) and transferred to the IVIA postharvest facilities. After 1 day of storage at 1 ºC, samples were selected for size, color, and absence of physical damage. Plums were randomly divided into 6 homogeneous groups of 270 fruits each, which corresponded to 4 coating treatments, 1 water-dipped treatment, and 1 uncoated-untreated control treatment. Plums were immersed in either water or the coating solutions for 1.5 min and drained of excess solution. Coated, water-dipped and uncoated-untreated plums were dried in a drying tunnel at 45-50 ºC for 3 min.

After drying, plums were stored 4 weeks at 1 ºC and 85±5% RH (simulating storage conditions at packinghouses), followed by 1 to 3 additional weeks at 20 ºC and 90±5% RH (simulating retail handling conditions).

2.9. Plum weight loss

Lots of 30 plums per treatment were used to measure weight loss. The same plums were weighed at the beginning of the experiment and at the end of each storage period. The results were expressed as the percentage loss of initial weight.

2.10. Plum ethanol and acetaldehyde contents

Ethanol and acetaldehyde concentrations in juice were determined by head space gas chromatography. Three replicates per treatment of 10 plums each were juiced with an industrial juicer (LOMI model 4, Bacelona, Spain), filtered through a cheesecloth and analyzed. Five mL of juice was transferred to 10-mL vials with crimp-top caps and TFE/silicone septum seals and frozen until analysis. Ethanol and acetaldehyde contents were analyzed in a gas chromatograph (Thermo Fisher Scientific, Inc., Waltham, MA, USA) with auto-sampler, flame ionization detector (FID) and a 1.2 x 0.32 cm (ID) Poropak QS 80/100 column. A 1 mL sample of the headspace was withdrawn from vials previously equilibrated in the auto-sampler incubation chamber for 10 min at 60 ºC. The injector, column and detector temperatures were set at 175, 150 and 200 ºC, respectively. Helium was used as carrier gas at 28 mL min^{-1} velocity. Ethanol and acetaldehyde contents were identified by comparison of retention times and peak areas with standard solutions of known concentration. Results were expressed as mg/100 mL juice.

2.11. Plum firmness

Plum firmness was determined as the maximum force in Newton (N) required to penetrate the fruit flesh. Lots of 20 plums per treatment were analyzed using an Instron Universal Testing Machine (Model 3343) with a plunger of 8 mm diameter. Two tests per fruit were made, one on each of the opposite cheeks. Prior to the measurement, a disk of the skin of about 2 cm in diameter was removed to measure the plum firmness in the flesh.

2.12. Plum physiological disorders.

Physiological disorders affecting plum flesh (browning, translucency, lack of juiciness due to mealiness or leatheriness, and bleeding) were evaluated as described by Crisosto, Gordon, & Zhiguo (1999). According to this method, fruits were cut in half and visually evaluated at the mesocarp and the area around the pit. The different degrees of flesh browning and translucency were rated as 1= none, 2= very slight, 3= slight, 4= moderate on less than 50%; 5= severe on 50% to 75%; 6= extreme on most of the flesh. Mealiness, leatheriness and bleeding were rated as 1= slight, 2= moderate, 3= severe. Forty fruits per treatment were inspected at the end of each storage period.

2.13. Plum sensory evaluation.

Sensory evaluation was conducted by 10 trained judges (6 females and 4 males), 25 to 50 years old, at the end of each storage period. Panelists evaluated overall flavor and firmness of plums. Flavor was rated on a 9-point scale, where 1-3 represented a range of nonacceptable quality with the presence of off-flavor, 4-5 represented a range of acceptable quality, and 7-9 represented a range of excellent quality. Plum firmness was rated on a 7-point scale: where: 1= very soft, 4= fair, and 7= very firm. Six fruit per treatment were pealed and sectioned into segments. At least 2 segments from different fruits were presented to judges in trays labeled with three-digit random codes and served at room temperature (25 ± 1 ºC). The judges had to taste several segments of each treatment to compensate, as far as possible, for biological variation of material. Mineral spring water was provided for rinsing between samples.

2.14. Statistical analysis

A completely randomized experimental design was used to study the effect of BW content on the different film properties and plum quality parameters. STATGRAPHICS Plus 4.1 (Manugistics, Inc., Rockville, MD) was utilized to calculate analysis of variance (ANOVA). Significance between means was determined by least significant difference (LSD) at $p \leq 0.05$.

3. Results and Discussion

3.1. Film tensile properties

Figure 1 shows the effect of BW content on YM, TS and %E of the HPMC-BW films. Addition of BW to the HPMC film matrix decreased TS, %E and YM, which indicates that films became weaker and less stretchable. This effect can be attributed to the poor mechanical resistance of lipids and the development of a heterogeneous film structure, featuring discontinuities in the polymer network, that decreases the mechanical resistance of the hydrocolloid polymer matrix (Shellhammer & Krochta, 1997; Pérez-Gago & Krochta, 2001).

Several studies show that the effect of lipid content on mechanical properties of edible composite films depends on the nature of the polymer matrix. In whey protein-based films, increasing the BW content above 20 g/100g (db) significantly decreased TS and YM (Shellhammer & Krochta, 1997). Furthermore, in pea starch-based films, TS and %E decreased with the addition of BW above 30 g/100g (db), with no effect at lower BW concentrations. In addition, the reduction of TS, even though significant, was very small indicating that the main material to maintain the strength was the starch matrix. However, YM of the starch film responded differently, increasing as BW content increased from 0 to 30 g/100g (db), indicating that the films became stiffer (Han, Seo, Park, Kim & Lee, 2006). In our work with HPMC-based films, a sharp decrease in TS and %E was observed as BW content increased from 0 to 40 g/100g (db), whereas YM showed a lower decrease as BW increased from 20 to 40 g/100g (db). These results indicate that BW addition had an important effect on the HPMC matrix reducing the mechanical resistance, with a lower impact on film flexibility.

Mechanical properties are important for edible films and coatings, as they reflect the durability of films and the ability of coatings to maintain a continuous layer over the coated product. Moreover, loss in film and coating mechanical integrity due to poor mechanical properties reduces their effectiveness as a barrier to gases and water vapor (Bravin, Peressini & Sensidoni, 2004). For this reason, some works in the literature show mathematical equations that allow prediction of film mechanical properties as a function of coating composition (i.e. plasticizer, lipid or emulsifier content). Sothornvit & Krochta (2001) reported an exponential model for TS and YM and a linear dependence for %E of β-lactoglobulin films as plasticizer concentration increased. This exponential model was also observed for TS and YM in sodium caseinate films, containing oleic acid (OA) and BW, as plasticizer content increased. However, for a given OA:BW and plasticizer contents a third order polynomial relationship was obtained for the variation of TS and YM parameters as the BW ratio increased (Fabra, Talens & Chiralt, 2008). In our work, the results showed a good fit of TS and %E with an exponential model in the BW range studied, whereas YM values fit well with either a linear or an exponential model. Coefficients a, b and R^2 for film mechanical properties as BW content increased are given on Table 2. The coefficients for the exponential model indicate the greater effect of BW content on film %E and TS than on YM. This model can provide a useful tool to predict mechanical properties with others HPMC-BW composition mixtures.

3.2. Film water vapor permeability

Permeability of films to water vapor is an important property to be considered when selecting film materials for specific commodities since it indicates their ability to protect the produce from desiccation. Figure 2 shows the effect of BW content and film orientation on WVP. As BW content increased from 0 to 60 g/100g (db) the moisture barrier of the films increased, following an exponential behavior that is modeled on Table 2. Shellhammer & Krochta (1997), however, found a sharp drop in WVP of whey protein-BW emulsion films at 35 g/100g BW content (db), following a sigmoidal trend. These differences in the behavior of the WVP reduction could be due to the nature of the interactions between the protein (i.e. WPI) or the polysaccharide (i.e. HPMC) and the lipid phase, that may affect the moisture transport properties through the film.

In emulsion films, lipid distribution within the polymer matrix have been shown to affect the film moisture barrier (McHugh & Krochta, 1994; Pérez-Gago & Krochta, 2001). When a film is cast from an unstable emulsion, the hydrophilic matrix and lipid may begin to separate during drying, creating a gradient of lipid concentration across the film. As a result of such gradient, the same film would give two different measured WVPs based on film orientation (up and down). In stable emulsions, films present a homogeneous lipid distribution within the hydrophilic matrix, with no orientation effect on WVP measurements. However, no orientation effect is also observed in WVP measurements of unstable emulsions if the resulting film shows a complete phase separation with a bi-layer structure, where the lipid forms a thin layer over the hydrophilic matrix. In our work, film appearance after drying indicated some phase separation, with the film side facing the plate more shiny in appearance, indicating the presence of a HPMC enriched phase, and the side facing the air more dull in appearance, indicating a lipid-enriched phase. Considering that film orientation did not significantly influence WVP (Figure 2), but film appearance showed some phase separation, the results may indicate the formation of a bi-layer film. Kamper & Fennema (1984) also described a complete phase separation of HPMC-fatty acid emulsions films, leading to an apparent bi-layer structure in the final film, which significantly reduced film WVP.

3.3. Film oxygen permeability

Table 3 shows the OP of the HPMC-based edible films. The HPMC film without BW showed the lowest OP. BW addition to the HPMC increased film OP, but no differences were found as BW content increased from 20 to 60 g/100g (db). In general, while addition of a lipid to a protein or polysaccharide film can reduce film WVP, such addition usually increases film OP due to the lower oxygen barrier of lipids compared to polar polymers. In emulsion films, several works have reported an increase in OP as the lipid content increases, giving as a possible explanation the increase in the pathway for oxygen transmission as the lipid content increased (Ayranci & Tunc, 2003; Han Seo, Park, Kim & Lee, 2006). In our work, however, such an effect was not observed as BW content increased from 20 to 60 g/100g (db), which could be related to the structure of the film. Chick & Hernandez (2002) reported no effect on OP by increasing candelilla or carnauba wax content to lactic acid casein-based films. This was explained by a phase separation of the protein and the wax forming a bi-layer with the bottom portion of the film forming solely a protein layer. As previously explained in this work, HPMC-

BW films showed an apparent phase separation that could have led to the formation of a bi-layer film, and therefore, an increase in BW content would not affect the HPMC layer structure and film OP.

3.4. Plum weight loss

Figure 3 (A) shows weight loss of coated, water dipped and uncoated plums after 4 weeks of storage at 1 ºC plus 1 to 3 weeks of storage at 20 ºC. In general, coatings containing BW significantly reduced plum weight loss, whereas there were no differences in weight loss between uncoated and HPMC-coated plums with no BW. This indicates that in order to improve moisture barrier of 'Angeleno' plums coatings must contain a hydrophobic compound. On the other hand, no differences were found for weight loss between the control and water-dipped plums, which indicates that the immersion in water was not enough to remove the natural waxes of 'Angeleno' plums. This result contrast with the results found by Pérez-Gago, Rojas & Del Rio (2003), where water-dipped 'Autumn Giant' plums presented higher weight loss than the untreated control.

Weight loss decreased as lipid content increased from 20 to 40 g/100g, but above 40 g/100g BW content weight loss was not further reduced. This results contrast with WVP of stand-alone films where an increase of BW from 40 to 60 g/100g (db) significantly reduced WVP (Figure 2). Therefore, data from stand-alone films might be used as preliminary screening, but factors affecting coating performance should be analyzed when they are applied on the fruit. Compared to performance of stand-alone films, coating performance is affected by coating distribution over the fruit surface, especially whether it forms a continuous layer or penetrates into pores (Hagenmaier & Baker, 1993). Fruit skin morphology (presence of hairs, thickness and type of cuticle, number of stomata, lenticels, and even cracks in the lenticels) and coating physical properties such as surface tension and viscosity strongly influence mass transfer of the coated fruit (Hagenmaier & Baker, 1993). In our study, an increase of BW content from 40 to 60 g/100g (db) increased coating hydrophobicity. However, coating brittleness was also increased (Figure 1), which might have featured some discontinuities, cracks or holes, reducing the water barrier of the coating.

3.5. Plum ethanol and acetaldehyde contents

Edible coatings provide a semipermeable barrier to O_2 and CO_2, slowing down fruit respiration (Hagenmaier & Baker, 1993; Baldwin, 1999) and ripening (Kader, 1986). This usually translates in an increase in the content of volatiles associated to anaerobic conditions, such as ethanol and acetaldehyde, that depends on the oxygen barrier of the coatings. In this work, plums coated with HPMC coatings containing no BW (0 g/100g, db) showed the greatest levels of ethanol, while no consistent differences were observed among the other treatments (Figure 3B). Acetaldehyde level followed a similar behavior (data not shown). This results indicate that HPMC coatings without BW provided a greater oxygen barrier than HPMC films containing BW, which correlates with the lower OP values of HPMC stand-alone films compared to the OP values of HPMC-BW films (Table 3). The lower ethanol and acetaldehyde contents in HPMC-composite coatings as lipid content increased was previously observed in other plum and citrus cultivars, and results were always attributed to the good gas barrier properties of hydrocolloid polymers such as HPMC (Pérez-Gago, Rojas & Del Río, 2002, 2003; Navarro-Tarazaga, Sothornvit & Pérez-Gago, 2008a; Navarro-Tarazaga, Del Río, Krochta & Pérez-Gago, 2008b).

3.6. Plum firmness

The coatings maintained firmness of coated samples compared to uncoated samples when they were stored 4 wk at 1 ºC followed by 2 and 3 wk at 20 ºC, reducing texture loss up to 53% with respect to the control depending on coating composition and storage time (Figure 3C). When plums were stored 4 wk at 1 ºC plus 1 wk at 20 ºC, no differences were found between coated and uncoated plums. However, differences among treatments were found when storage time at 20 ºC was extended to 2 and 3 wks. Under these storage conditions, firmness of uncoated and water dipped plums was significantly decreased. Whereas, among the different coating, those containing 0 and 20 g/100g BW reduced firmness loss compared to coatings containing 40 and 60 g/100g BW (db), that did not result effective maintaining plum firmness.

Fruit flesh softening is related to pectin and hemicellulose degradation of cell walls (Fishman, Gross, Gillespie & Sondey, 1989). Application of some postharvest techniques, such as cold storage at the optimum temperature and the use of controlled and modified atmosphere storage, has been effective in reducing firmness loss (Ke, Rodríguez-Sinobas & Kader, 1991; Ben & Gaweda 1992). Therefore, the improvement of flesh firmness of coated plums might be related with the ability of coatings to provide a semipermeable barrier to O_2 and CO_2, acting as a modified atmosphere packaging (Baldwin, 1999; Banks, Dadzie & Cleland, 1993; Cisneros-Cevallos & Krochta, 2002). In our work, the greater ability of HPMC coatings with low BW content to maintain plum firmness might be attributed to their higher capacity to modify fruit internal atmosphere, which translated in higher ethanol content. Similar results were observed in plums and mangos coated with cellulose-based coatings presenting different gas permeabilities (Baldwin, Burns, Kazokas, Brecht, Hagenmaier, Bender & Pesis, 1999; Pérez-Gago, Rojas & Del Rio, 2003; Navarro-Tarazaga, Sothornvit & Pérez-Gago, 2008a).

3.7. Plum physiological disorders

The use of low temperature during storage extends plums market life (Crisosto, Gordon & Zhiguo, 1999). However, plums from some cultivars develop a lack of juiciness with mealy or leathery texture, flesh browning, black cavity, flesh translucency, and flesh red pigment accumulation (flesh bleeding) after prolonged cold storage and/or after ripening at room temperature. These physiological disorders are known as forms of internal breakdown (IB). ‘Angeleno’ plum cultivars develop IB at storage temperatures of around 5 °C, whereas the optimum storage temperature is around 0 °C (Crisosto, Gordon & Zhiguo,1999).

In this work, flesh bleeding was the main IB symptom observed. Flesh bleeding is the result of anthocyanin diffusion from the cells surrounding the stone and the skin, where the pigments are initially located, to the overall plum flesh. This disorder may be a consequence of tissue senescence (Lurie & Crisosto, 2005) or abnormal ripening (Dong, Zhow, Sonego, Lers & Lurie, 2001) and can be prevented by controlled-atmosphere storage (Lurie, Zeidman, Zuthi & Ben-Arie, 1992). Moreover, the cell wall degradation that produces plum flesh softening may enhance the diffusion of anthocyanins and bleeding incidence. Figure 3 (D) shows bleeding of coated and uncoated ‘Angeleno’ plums after 4 weeks of cold storage and storage at 20 °C. Generally, coatings were effective in reducing plum bleeding compared to the untreated control. On plums stored 4 weeks at 1 ºC plus 1 week at 20ºC, coatings with lower BW content were more effective reducing bleeding. This might be due to the internal atmosphere modification produced by coatings, which also resulted on reduced plum softening. However, differences on plum bleeding among coating treatments were less evident when storage at 20 ºC increased, which might be a consequence of tissue senescence.

3.8. Plum sensory evaluation

Sensory evaluation was performed to see if coatings affected flavor and firmness of coated plums. Flavor decreased with storage time for all treatments. However, there were no differences in flavor among the different treatments (data not shown), which indicates that the coatings tested did not affect ‘Angeleno’ plum flavor compared to the control. Firmness was the most limiting factor of plum quality. The results showed that ‘Angeleno’ plums could maintain marketable firmness up to 4 wk at 1 ºC followed by 1 or 2 wks at 20 ºC, whereas after prolonged storage at 20 ºC the firmness of the samples was considered unacceptable (data not shown).

4. Conclusions

HPMC-BW edible coatings in combination with low-temperature storage extended marketability on ‘Angeleno’ plums by reducing water loss, flesh softening and IB, without affecting the sensory quality of the fruit. No differences on weight loss were observed between uncoated and HPMC-coated plums with no BW, which indicates that, in order to improve moisture barrier of ‘Angeleno’ plums, coatings must contain a hydrophobic compound. Weight loss decreased as lipid content increased from 20 to 40 g/100g, but above 40 g/100g BW content, weight loss was not further reduced. These results contrast with the moisture barrier properties of stand-alone films, where an increase of BW from 40 to 60 g/100g significantly reduced WVP. These differences between coating and film performance indicate that data from stand-alone films may be used as a preliminary screening, but coating performance should be analyzed on coated fruit. Decreasing BW content (i.e. increasing HPMC content) retained plum firmness and reduced bleeding, which could be related to coatings ability to provide a semipermeable barrier to O_2, acting as a modified atmosphere packaging. Therefore, HPMC-BW coatings containing 20 g/100g BW (db) would provide the best compromise to extend shelf-life of ‘Angeleno’ plums, by reducing plum weight loss, flesh softening and internal breakdown, without affecting the sensory quality of the fruit.

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Table 1. Emulsion film and coating composition (g/100g, dry basis)

Formulation [a]	HPMC	BW	G	SA
0 BW	66.7	0	33.3	0
20 BW	50.7	20	25.3	4
40 BW	34.7	40	17.3	8
60 BW	18.7	60	9.3	12

[a] Formulation name represents BW content (g/100g, dry basis)

HPMC= hydroxypropyl methylcellulose; BW= beeswax; G= glycerol; SA= stearic acid.

Solid contents were 7 and 4 g/100g for stand-alone films and coating formulations applied to plums, respectively.

Table 2. Values of a and b coefficients and R^2 in the relationship between TS, YM, E, and WVP of HPMC-based films

Tensile property	Model	a	b	R^2
YM (MPa)	Exponential [a]	0.006	275.6	0.948
	Linear [b]	-1.32	274.6	0.946
TS (MPa)	Exponential	0.033	15.2	0.995
E (%)	Exponential	0.050	70.7	0.998
WVP [c] (mm / kPa h m^2)	Exponential	0.014	7.3	0.990

[a] $y=be^{-ax}$

[b] $y=b+ax$

[c] Model for WVP was obtained with the average values for 'up' and 'down' orientations, since WVP was not significantly affected by film orientation.

EM= elastic modulus; TS= maximum tensile stress; E= elongation at break; WVP= water vapor permeability.

Table 3. Effect of beeswax content on oxygen permeability of HPMC-based films

Formulation [a]	Oxygen permeability (mL µm/m^2 day KPa)
0 BW	232 ± 43 a
20 BW	311 ± 42 b
40 BW	293 ± 39 b
60 BW	337 ± 67 b

[a] Formulation name represents beeswax content (g/100g, dry basis)

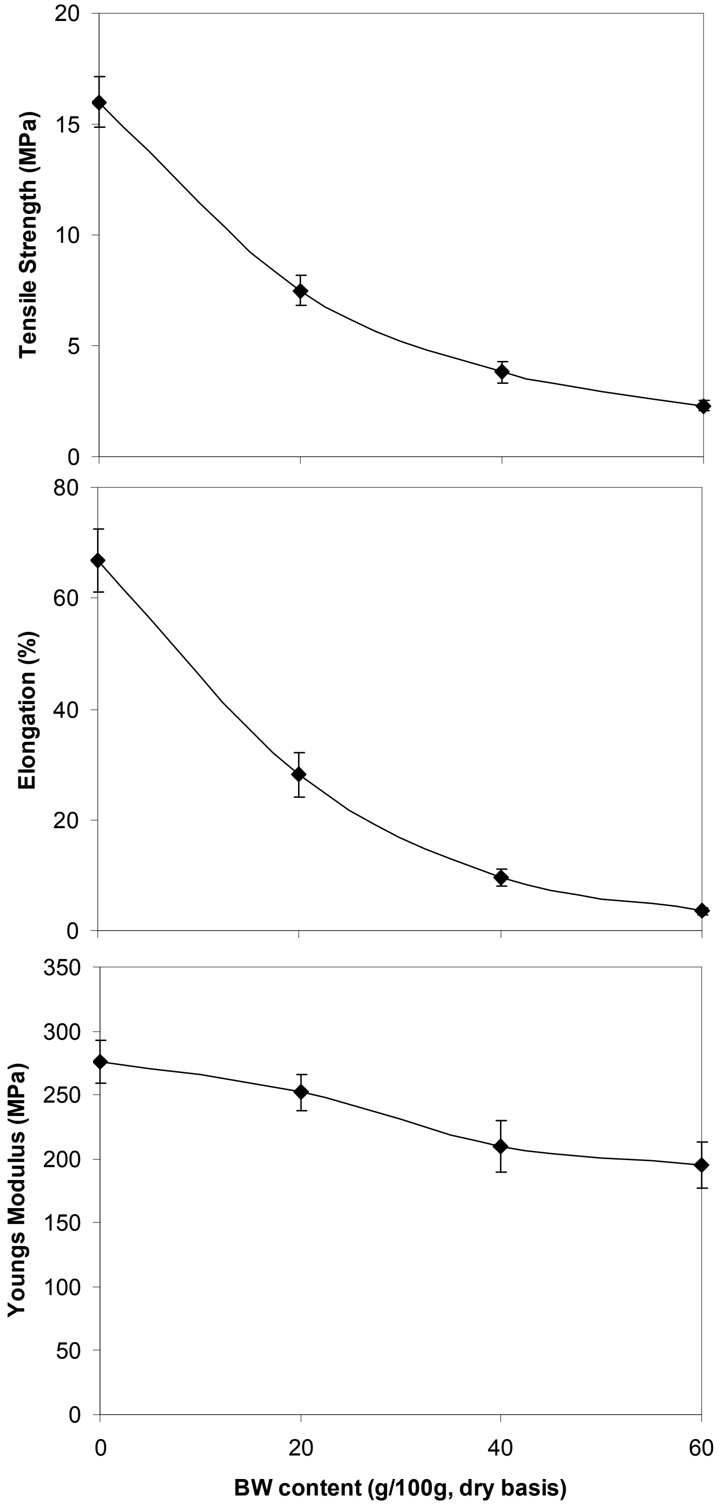
Results reported were tested at 23ºC and 50%RH.

Means with different superscripts denotes significant difference ($p < 0.05$). n= 3.

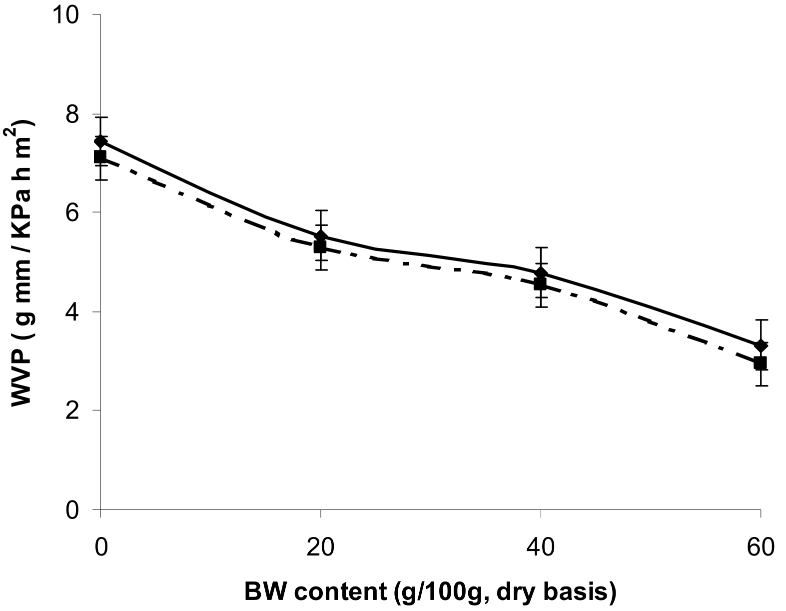
Figure 1. Effect of beeswax (BW) content on mechanical properties of HPMC-BW stand-alone edible films. Bars represent LSD values ($p<0.05$). n= 15.

Figure 2. Effect of beeswax (BW) content on water vapor permeability (WVP) of HPMC-BW stand-alone edible films. (—◆—) up (film side exposed to air during drying faced to the lower RH during analysis; (-■- -) down (film side exposed to air during drying faced to the higher RH during analysis). Bars represent LSD values ($p<0.05$). n= 3.

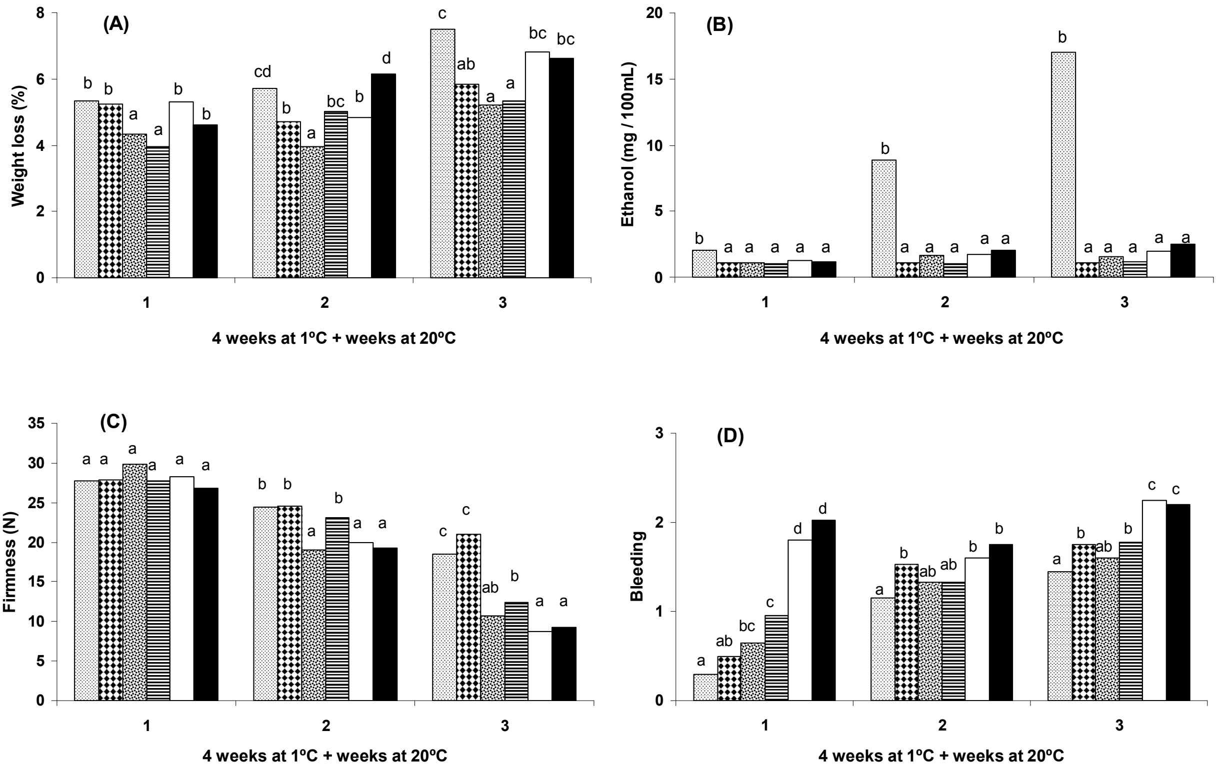
Figure 3. Weight loss (A), ethanol content in juice (B), firmness (C) and bleeding (D) of 'Angeleno' plums, uncoated (■), water dipped (□), or coated with hydroxypropyl methylcellulose (HPMC)-based coatings containing 0 (░), 20 (▦), 40 (▒) and 60 (☰) g/100g of beeswax (BW), and stored 4 weeks at 1 ºC plus 1, 2 and 3 weeks at 20 °C. Bleeding was rated as 1= slight, 2= moderate, 3= severe. Within each storage time, means with the same letter are not significantly different ($p<0.05$). Number of replications for weight loss, ethanol, firmness, and bleeding were 30, 3, 20, and 40, respectively.


Tensile Strength (MPa)
20
15
10
5
0
Elongation (%)
80
60
40
20
0
Youngs Modulus (MPa)
350
300
250
200
150
100
50
0
0
20
40
60
BW content (g/100g, dry basis)

<Figure 1>


10
8
6
4
2
0
WVP (g mm / KPa h m^2)
0
20
40
60
BW content (g/100g, dry basis)

<Figure 2>


(A)
Weight loss (%)
0
2
4
6
8
b b a a b b
cd b a bc b d
c ab a a bc bc
1
2
3
4 weeks at 1ºC + weeks at 20ºC
(B)
Ethanol (mg / 100mL)
0
5
10
15
20
b a a a a a
b a a a a a
b a a a a a
1
2
3
4 weeks at 1ºC + weeks at 20ºC
(C)
Firmness (N)
0
5
10
15
20
25
30
35
a a a a a a
b b a b a a
c c ab b a a
1
2
3
4 weeks at 1ºC + weeks at 20ºC
(D)
Bleeding
0
1
2
3
a ab bc c d d
a b ab ab b b
a b ab b c c
1
2
3
4 weeks at 1ºC + weeks at 20ºC

<Figure 3>