Research Article

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Shelf-life of fresh blueberries coated with quinoa protein/chitosan/sunflower oil edible film

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Abstract

BACKGROUND: The aim of this study was to evaluate quinoa protein (Q), chitosan (CH) and sunflower oil (SO) as edible film material as well as the influence of this coating in extending the shelf-life of fresh blueberries stored at 4 °C and 75% relative humidity. These conditions were used to simulate the storage conditions in supermarkets and represent adverse conditions for testing the effects of the coating. The mechanical, barrier, and structural properties of the film were measured. The effectiveness of the coating in fresh blueberries (CB) was evaluated by changes in weight loss, firmness, color, molds and yeast count, pH, titratable acidity, and soluble solids content.

RESULTS: The tensile strength and elongation at break of the edible film were 0.45 ± 0.29 MPa and 117.2% ± 7%, respectively. The water vapor permeability was $3.3 \times 10^{-12} \pm 4.0 \times 10^{-13}$ g s^{-1} m^{-1} Pa^{-1}. In all of the color parameters CB presented significant differences. CB had slight delayed fruit ripening as evidenced by higher titratable acidity (0.3–0.5 g citric acid 100 g^{-1}) and lower pH (3.4–3.6) than control during storage; however, it showed reduced firmness (up to 38%).

CONCLUSION: The use of Q/CH/SO as a coating in fresh blueberries was able to control the growth of molds and yeasts during 32 days of storage, whereas the control showed an increasing of molds and yeast, between 1.8 and 3.1 log cycles (between 20 and 35 days).

Keywords: quinoa protein; chitosan; edible film; fresh blueberry shelf-life; coating

INTRODUCTION

At present, there is a growing interest in the application of edible films or coatings produced from a variety of natural and biodegradable materials as an environmentally friendly technology.[1,2] Edible films and coatings are developed based on biopolymers and they have been applied to increase the shelf-life of fresh fruit.[1,2] Between 10% and 40% of postharvest fruits are inconsumable due to loss of firmness, weight loss, and/or microbial contamination, mainly due to mold,[3,4] so the use of edible coatings could help to maintain fruit freshness. Edible films are defined as a thin film, around 0.04–0.200 mm, of edible biomaterial that can be used as a coating over the food or as an external film.[5,6] Edible films and coatings can act as barriers against weight loss, O_2, and CO_2, to control postharvest microbiological growth consequently maintaining the quality and increasing the shelf-life of fresh foods.[1,2] Edible films made from hydrocolloids and their blends have been widely studied.[7,8] However, there is still a need for basic studies that determine the functional properties of edible films for specific applications as packaging materials in the food industry such as fresh fruit and ready-to-eat foods.[9–14]

Several authors have reported that berries have the potential to act as a functional food ingredient since they are rich in anthocyanin and in compounds that provide pigmentation to fruits, which is in addition to serving as natural antioxidants and possessing a broad spectrum of therapeutic and anticarcinogenic properties.[15,16] However, postharvest respiration, transpiration, and microbial attack cause quality deterioration in fresh fruits, thus limiting shelf-life. For example, fresh blueberries have a shelf-life of approximately 10–40 days depending on the cultivar type, fruit maturity, method of harvest, and storage conditions.[17,18] To this end, edible coatings have already been studied in the context of improving the shelf-life of fresh berries such as strawberries.[19–24] Moreover, some studies have reported on the use of edible film in ready-to-eat blueberries, with results showing a shelf-life of 15 days when kept between 0 and 20 °C.[18,25,26]

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In forming composite and edible films, quinoa protein has been shown to be a good biopolymer, and when blended with chitosan (at pH 3, because it is positively charged and soluble), it yields mechanically resistant films without the use of a plasticizer.[27] Additionally, when low levels of high oleic sunflower oil are added to quinoa protein/chitosan films, water vapor permeability (WVP) improves as a result of hydrophobic interactions.[6] Thus quinoa/chitosan/sunflower oil edible (Q/CH/SO) composite may lead to new applications in the food industry. The fields of application of Q/CH/SO coating blend are low-pH fresh fruits, to increase shelf-life and consumer acceptability.

The aims of this study were to characterize the mechanical, barrier, and structural properties of the edible film formed by quinoa protein (Q) at pH 11 (at this pH it has negative charge and high solubility), chitosan (CH), and high-oleic sunflower oil (SO), and also to evaluate the effectiveness of this edible film in improving the storage life of blueberries.

MATERIALS AND METHODS

Materials

Quinoa flour (*Chenopodium quinoa* Willd.) was supplied by Cooperativa las Nieves, located in the VI Region of Chile. Chitosan was obtained from giant squid (*Dosidicus gigas*) provided by the Pontificia Universidad Católica, Peru. Sunflower oil was purchased from Camilo Ferrón SA, Chile (≥70 g 100 g^{-1} oleic acid). Finally, Southern Highbush blueberries (*Vaccinium corymbosum* L. cv. O'Neal) were provided by the Chilean agricultural society Vitroplanta Ltda, and these were transported to the laboratory in ice coolers filled with ice packs.

Preparation and characterization of edible film

Preparation of Q

The quinoa flour was suspended in distilled water (18 g 100 mL^{-1}), and the pH was adjusted to pH 11 with 1 mol L^{-1} NaOH. This suspension was stirred for 60 min at room temperature and centrifuged at 21 000 × *g* for 30 min at 15 °C. The soluble protein content was measured according to the Bradford method[28] and expressed as milligrams of protein per milliliter.

Preparation of CH solution

A solution containing 2 g 100 mL^{-1} of CH in 1 g 100 mL^{-1} of lactic acid was prepared. The solution was sonicated for 30 min (Fisher Scientific FS30H, Germany) and left overnight at 4 °C to eliminate bubbles.

Preparation of edible film composed of Q/CH/SO

Blends of Q, SO, and CH were prepared according to Abugoch *et al.*[27] and Valenzuela *et al.*[6] by mixing solutions of Q (0.62% ± 0.11% w/v), SO (3.8% w/v), and CH (2% w/v) in a ratio of 1:1 (v/v). The blends were prepared by mixing Q and SO at room temperature for 10 min before homogenization with a high-speed Ultra-Turrax (Silverson L4R Machines, UK) for 10 min at 10 000 rpm. Then, CH (2% g 100 mL^{-1}) was incorporated into the blend by mixing with a blade homogenizer (Bosch MSM6A3R 750w, China) for 10 min at 1000 rpm. The pH was then adjusted to 3.0 with lactic acid. The film-forming CH/Q/SO blend was sonicated for 30 min (Fisher Scientific FS30H, Germany) to eliminate bubbles. This blend was cast on to a horizontal surface in low-density polyethylene boxes (14 cm diameter). Films were dried in a conventional oven (Heraus, Type TU 60/60 Germany) oven without controlled conditions of relative humidity (RH), under environmental conditions of relative humidity (75%) and temperature (50 °C). The films were dried to a constant weight at 50 °C before being carefully removed and conditioned in an environmental chamber (Model LTH-0150E, Labtech Co., Korea) for 72 h at 23 °C and 60% RH before use.

Mechanical, barrier, and structural properties of edible film

Thickness

The thickness (mm) of five coated samples was determined and averaged together. The measurements were taken at nine points on each film using a digital micrometer (Mitutoyo 293340, Japan).

Mechanical properties

The tensile properties of the film, tensile strength (TS) and percentage elongation at break (%*E*) were determined using the Official Chilean Standard Method,[29] equivalent to the ISO R1184-1970 standard method, on a universal tensile testing machine (model LR5K, Lloyd, UK), which was operated with a 5 kN load cell and controlled by DAPMAT Version 3.0 software. Five film samples were cut into 10 mm × 50 mm strips and tested using a double clamp with a separation of 30 mm and a test speed of 20 mm min^{-1}. The reported TS and %*E* values were the averages of at least five measurements. The TS was expressed in MPa and was calculated as follows:

$$TS = F/CSA^2 \quad (1)$$

where TS is the tensile strength (MPa) *F* is the maximum force (N) when the film ruptures, and CSA (mm^2) is the initial cross-sectional area of the film.

E% was calculated as follows:

$$E\% = \frac{(D_f - D_i)}{D_i} \times 100 \quad (2)$$

where *E*% is the percentage elongation at break, D_f is the distance elongation at break (mm), and D_i is the initial distance between the baselines (mm).

Water vapor permeability (WVP)

Measurements for WVP were performed according to the Official Chilean Standard Method,[30] equivalent to the ASTM D1653-93 and DIN 52615 standard methods, using the wet cup method. A cup was filled with distilled water to 6 mm from the top edge. The film was adhered to the cup with silicone gel (Dow Corning high-vacuum grease), and the cup was placed in an environmental chamber (model LTH-0150E, Labtech, Co., Korea) at 23 °C and 60% RH. The weight of the cup was measured daily over 21 days. The reported WVP values were the averages of six measurements. WVP was estimated as follows:

$$WVP = \frac{\Delta m}{tA\Delta P} \times \varepsilon \quad (3)$$

where WVP is the water vapor permeability (g m^{-1} s^{-1} Pa^{-1}, Δm is the change in mass over time (g); *t* is time (s), *A* is the film area (m^2), ΔP is the partial difference in vapor pressure between the atmosphere and pure water (112.353 kPa at 23 °C), and ε is the thickness (m).

Film microstructure

Characterization of the film's microstructure was determined by scanning electron microscopy (SEM) (model JSC 6400 scanning electron microscope, JEOL, Tokyo, Japan). Prior to examination, the film was mounted on a cylindrical die 10 mm in diameter using double-sided adhesive tape. This was then sputter coated in gold for 3 min at 20 kV in an argon atmosphere (PELCO 91000) to render it electrically conductive. Images were registered on black and white photographic film (Kodak TMAX 100 TMX-120).

Application and evaluation of the coating on physicochemical and microbiological properties affecting blueberry quality

Coating application on blueberries

Blueberries were selected for study based on uniformity of size and color and on having no visible mechanical damage or fungal infection. Fresh blueberries were randomly assigned to the uncoated, control group, which did not receive any manipulation. The remaining blueberries were placed in the coating group and subsequently immersed in the coating solution for 1.5 min. The excess film-forming solutions were drained, and the coated blueberries were dried in an oven with a forced-air dryer at room temperature (±20 °C) for 45 min. Approximately 140 g blueberries were weighed and placed inside commercial PET (polyethylene terephthalate), vented clamshell containers and stored in a refrigerated chamber at 4.0 ± 0.5 °C and 75% RH. Two lots with three replicates per lot were analyzed on days 0, 4, 7, 11, 13, 18, 20, 25, 28, 32, and 35 of storage.**

Physicochemical properties

Weight loss (WL). Just after air-drying, blueberries were separated into lots of 140 g before being stored. The weight of these lots was taken again during the experiment, and the results were expressed as the percentage of loss compared to the initial weight.

Firmness. Firmness was determined from 20 blueberries of each lot with a universal tensile testing machine (Model LR5K, Lloyd) controlled by DAPMAT Version 3.0 software. Firmness was reported as the peak force and was expressed in newtons ($N\,mm^{-1}$). Blueberries were individually set on their side, and firmness was determined by the maximum penetration force (N) with a 1 mm diameter metal probe. The penetration depth was 2 mm.

Color. The surface color from 90 g blueberries was measured with a colorimeter (Hunter Lab system, model Miniscan 2.0/45, USA) using the Hunter Lab color scale. Luminosity (L), a^*, and b were determined.[31] The chroma and the hue angle were calculated according to the following equations:

$$\text{Chroma} = \left(a^{*2} + b^{*2}\right)^{1/2} \quad (4)$$

$$\text{Hue} = (\text{atan}\,(b^*/a^*)/6.2832 \times 360) + 180 \quad (5)$$

where a^* and b^* are Hunter Lab parameters.

pH, soluble solids content (SSC), and titratable acidity (TA). The samples were ground and filtered. Measurements of pH were carried out using a pH meter (pH-537; KFW Microprocessors, USA). Total SSC was measured using a digital refractometer (PR1; Atago Co. Ltd, Japan) at 25 ± 2 °C. The TA was determined according to AOAC 942.15 (AOAC, 1995)[32] and expressed as grams of citric acid per 100 of fruit.

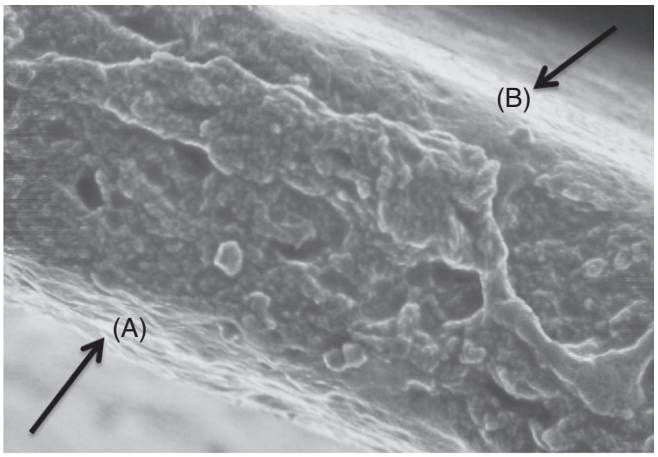

Figure 1. SEM micrographs of the cross-section (300×) and surfaces of quinoa protein/chitosan/sunflower oil blend film: (a) film exposed to air; (b) film in contact with the casting support.

Mold and yeast counts

Microbiological analyses were performed in triplicate according to the Official Standard method.[33] For the yeast and mold count, 10 g blueberries were ground and suspended in 90 mL peptone water (0.1% v/v). The suspension was mixed in a blender (Seward Stomacher 400) for 5 min. Serial dilutions (10^{-1}, 10^{-2} and 10^{-3}) of the blueberry homogenates were plated on the surface of selective media (potato dextrose agar, Oxoid, Basingstoke, UK), and the uninverted plates were incubated at 25 °C for 5 days. Mold and yeast counts were expressed as log CFU (colony-forming units) per gram of blueberries.

Statistical analysis

StatGraphics Plus 5 software was used for all statistical analyses. Analysis of variance (ANOVA) and multiple Tukey range tests were used to determine significant differences ($P < 0.05$).

RESULTS AND DISCUSSION

Edible film formed by Q extracted at pH 11 and blended with CH and SO at pH 4 (Q/CH/SO) was characterized. The effectiveness of this edible film was then evaluated in blueberries in the context of increasing postharvest life.

Film characterization

The film thickness of Q/CH/SO was 0.0581 ± 0.0235 mm. This value was similar to Q/CH film when the Q was extracted at pH 8, but lower than when the Q was extracted at pH 8 in Q/CH/SO film.[6] The TS value of the Q/CH/SO film was 0.45 ± 0.29 MPa, which was lower than values found for CH or Q/CH films.[6,27] The incorporation of SO into the polymeric matrix decreases TS values,[6] and this property is also affected by the pH at which Q extraction takes place.[27,34] On the other hand, the %E value was 117.2 ± 7.6, which was higher than values from CH.[6,27] Given the same concentrations of SO in the polymeric matrix of Q/CH, a lower %E value is observed when the protein is extracted at pH 11.[6]

Structural characterization, as performed through SEM, is shown in Fig. 1. It was possible to observe a rough surface, heterogeneous and with the presence of pores. Valenzuela *et al.*[6] showed a superficial structure, homogeneous, continuous, and without the

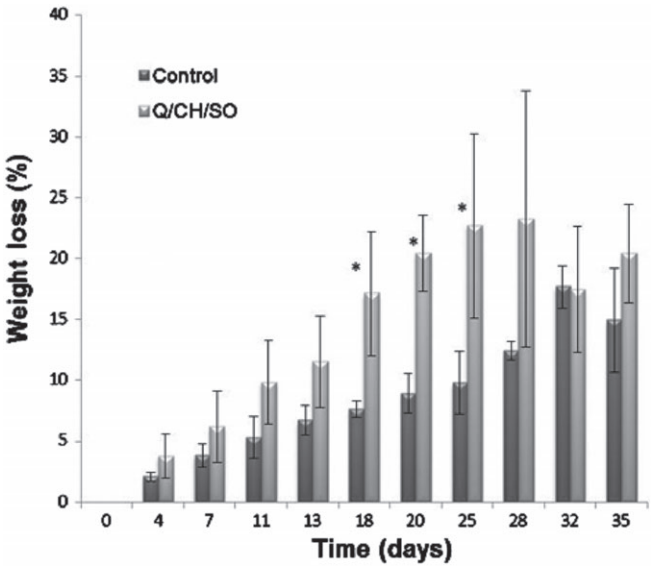

Figure 2. Weight loss (%) of control and blueberries coated with quinoa protein/chitosan/sunflower oil film (Q/CH/SO), throughout storage. *Significant differences on each day ($P < 0.05$).

presence of pores for Q/CH or CH SEM films.[6,27] Thus the addition of SO in a continuous polymeric phase composed of CH and Q can provide the film's heterogeneity and give the material a rougher surface, with the presence of pores. This observation is in accordance with other authors who have used lipidic material in polymeric edible films.[35,36] Finally, the WVP obtained for Q/CH/SO was $3.27574 \times 10^{-12} \pm 3.98879 \times 10^{-13}$ (g s^{-1} m^{-1} Pa^{-1}). This value was lower than in edible films formed by Q at pH 9/CH and Q at pH 8/CH/SO.[6,27] It was for this reason that in the present study quinoa protein was extracted at pH 11 in order to obtain the Q/CH/SO edible film applied to fresh blueberries.

Application and evaluation of the coating on physicochemical and microbiological properties affecting blueberry quality

Weight loss

It is possible to observe (Fig. 2) that WL increased throughout the storage period for control and CB. WL values observed between control and CB were not significantly different ($P > 0.05$) after 13 days of refrigerated storage, but the WL of CB was significantly higher than control samples from day 18 until day 25 of refrigerated storage. After this period, no significant differences ($P > 0.05$) were observed between the WL of coated and control blueberries. WL of between approximately 6.7% (control) and 11% (CB) was observed after 13 days at 5 °C; Sanford *et al.*[37] reported 7.6% weight loss when blueberries were stored for 14 days at 5 °C. The decrease in moisture content is related to WL and to loss of firmness since blueberries are harvested fully ripe.[38,39]

Firmness

Fruit firmness was affected by the coating after its application and during storage at 4.0 ± 0.5 °C (Fig. 3). After application, the firmness of coated blueberries was 32% lower compared to the uncoated blueberries. Control and coated berries presented significant differences ($P < 0.05$) in firmness, and until day 28 of storage the firmness values for coated blueberries were lower than values for the uncoated group (Fig. 4). From day 32 until the end of the storage period the firmness of coated berries showed no significant differences ($P > 0.05$) from the control samples. A loss in firmness of around 26% was observed for uncoated blueberries, and 32% for coated fruits. The decrease in water content observed in CB is related to loss of firmness blueberries;[38] according to Paniagua *et al.*[40] moisture loss is the major cause of firmness change during postharvest storage of blueberry.

Color

Change in color is one of the factors that determine the quality of fresh blueberries.[37,40] The surface color of coated and

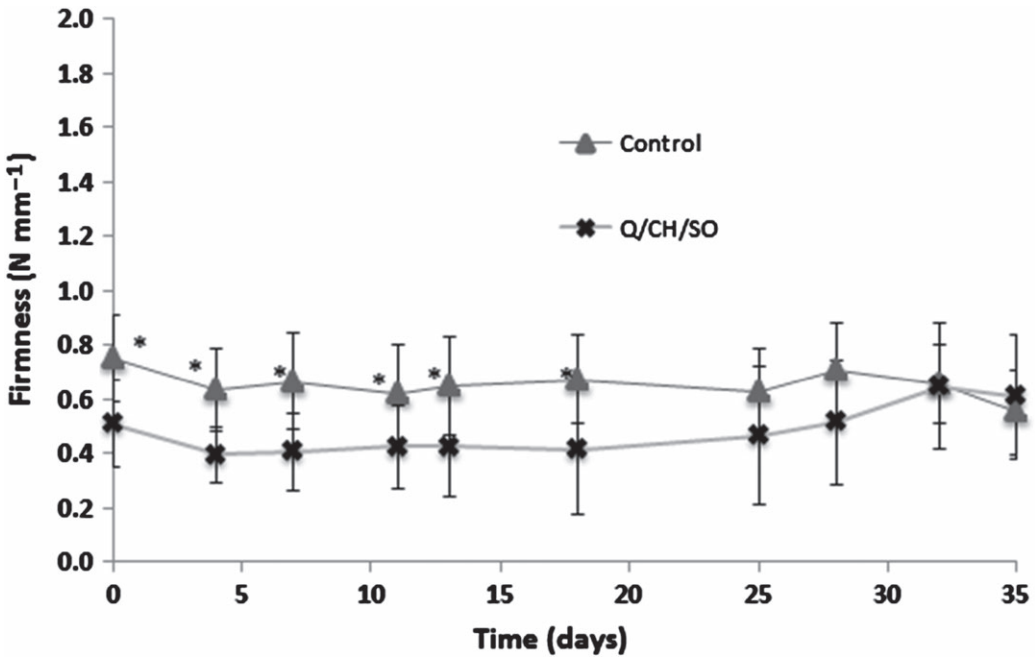

Figure 3. Firmness of control and coated blueberries with quinoa protein/chitosan/sunflower oil film (Q/CH/SO), throughout storage. Error bars represent SD. *Significant differences between coated and control blueberries ($P < 0.05$).

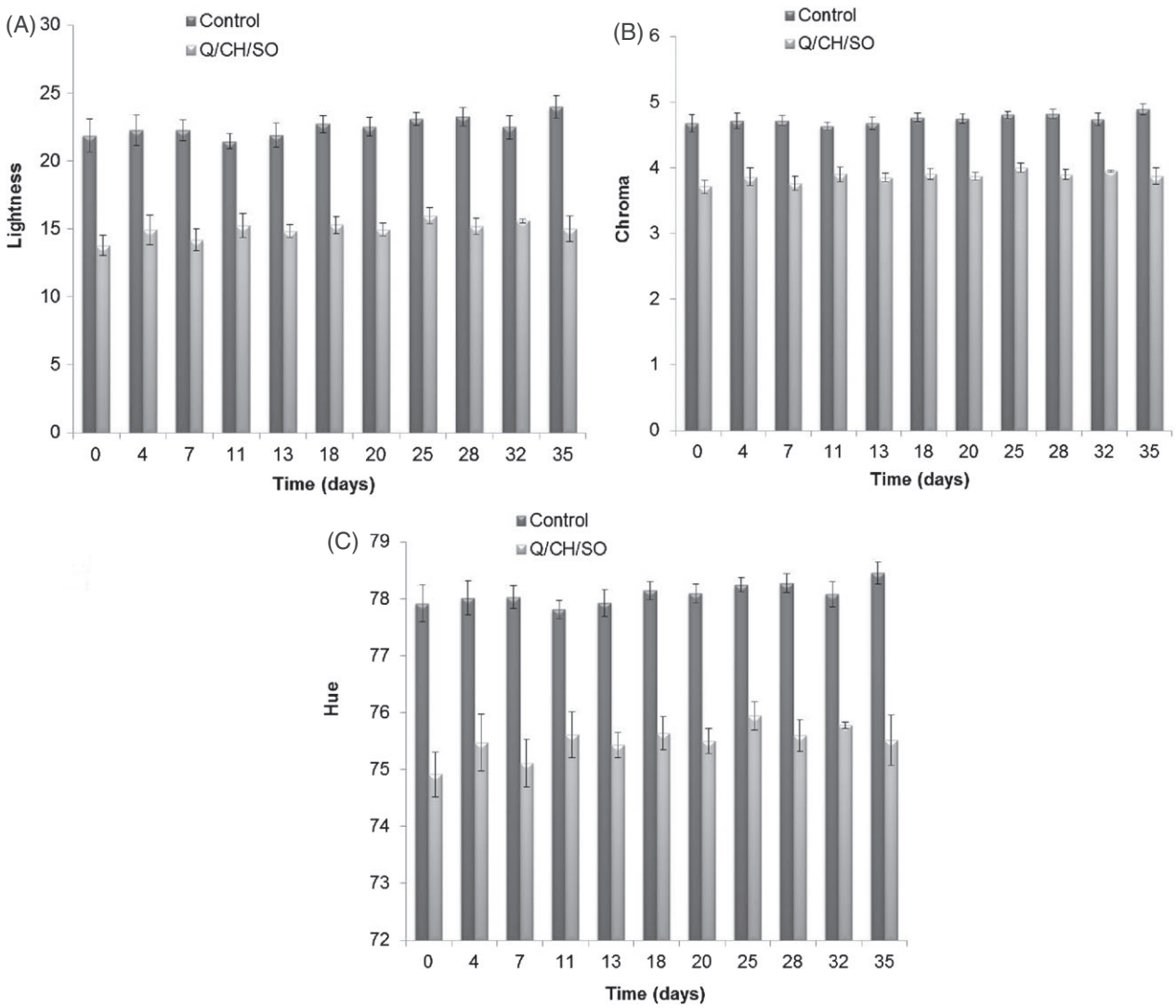

Figure 4. Lightness (A), chroma (B), and hue angle (C) of control and coated blueberries with quinoa protein/chitosan/sunflower oil (Q/CH/SO) film throughout storage.

control blueberries did not significantly change ($P > 0.05$) during storage (Fig. 4), and previous studies on fresh blueberries also found color parameter values similar to control blueberries in the present study.[17] However, there were significant differences ($P < 0.05$) between coated and control blueberries in all of the color parameters. The color in fresh blueberries is determined by the waxy bloom rather than by the anthocyanin content,[41,42] which is why this parameter was affected when fruits were coated.

Soluble solids content, pH, and titratable acidity

The effects of Q/CH/SO coating on SSC, pH, and TA during refrigerated storage are shown in Fig. 5. SSC increased during the storage period (Fig. 3). This increase was significantly higher ($P < 0.05$) in coated than in uncoated samples. SSC values ranged from 12.9% to 14.1% for control samples and from 13.3% to 15.7% for coated samples. Only at the end of storage (day 35) were no significant differences ($P > 0.05$) in SSC found between the control and CB. This increase of SSC is possibly related to moisture loss, since blueberries are harvested fully ripe.[39,40]

The pH values for control samples were increased slightly from 3.5 to 3.9 for control samples, and from 3.5 to 3.6 for coated samples during the study period (Fig. 5). While significant differences ($P < 0.05$) were found between coated and uncoated samples, this change was significantly ($P < 0.05$) lower in coated than in uncoated blueberries. The control samples showed a significant increase in pH values at 25 days of storage, reaching pH 3.9; this pH value was maintained until the end of storage. Perkins-Veazie *et al.*[38] reported that the pH of the O'Neal blueberry cultivar significantly increased after 25 days of storage pH remains without significant changes. In contrast, the highest pH value obtained by the coated blueberries in the present study was 3.6 ± 0.13 at 28 days of storage, and after that pH remained without significant modifications. Films could present a good-quality protection regarding pH, maintaining pH values between 3.4 and 3.6 until day 32, similar to fresh blueberries.

In regard to TA (uncoated and coated fruits), citric acid levels were found to be around 0.4–0.5 g 100 g^{-1} (Fig. 4). The TA for each blueberry cultivar is different, with Skupień[43] reporting TA content values between 0.51 and 1.77 g 100 g^{-1} of citric acid. In the present study, significant differences ($P < 0.05$) were found between uncoated and coated blueberries, with coated blueberries having the overall highest mean total acid content. During

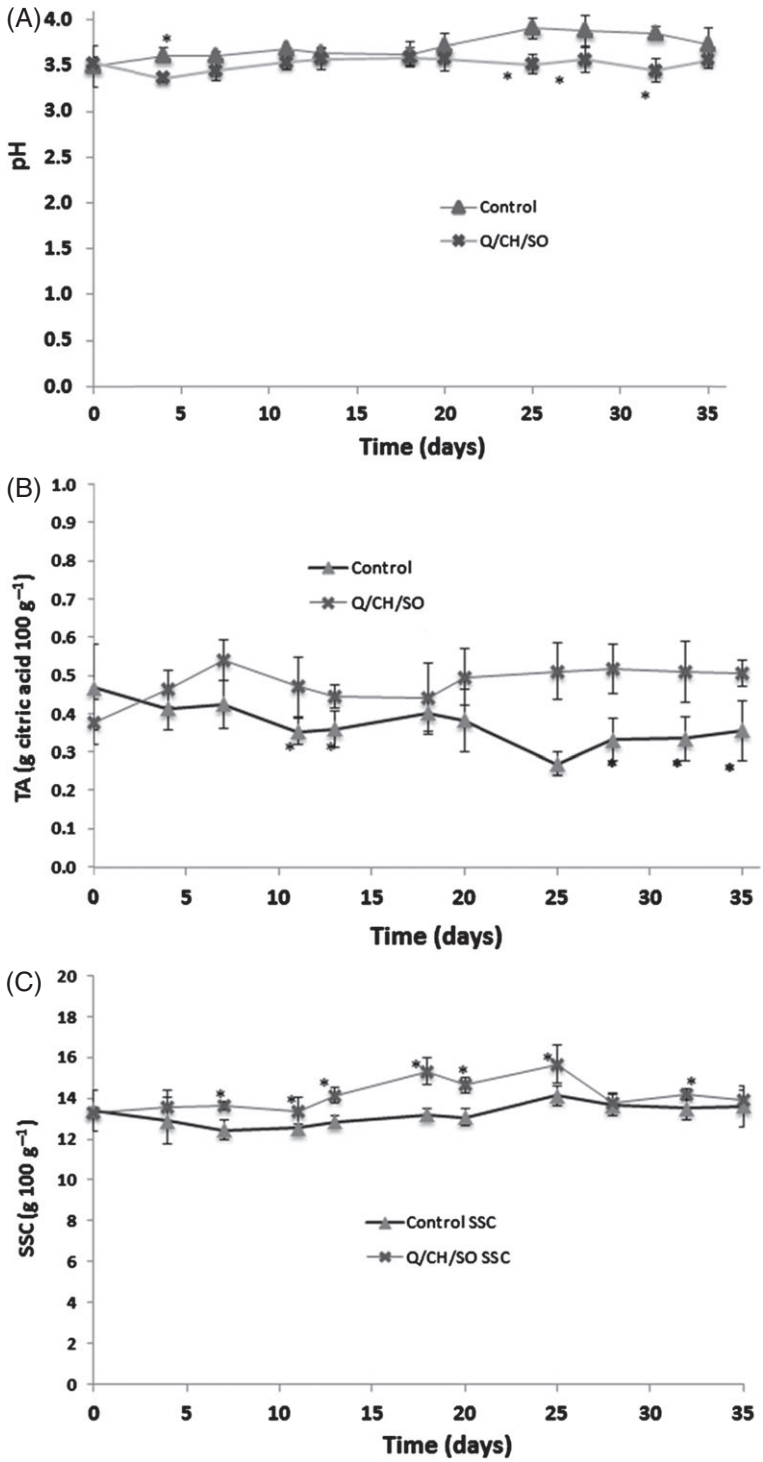

Figure 5. pH, titrable acidity (TA), and **s**oluble solids content (SSC) of control and coated blueberries with quinoa protein/chitosan/sunflower oil film (Q/CH/SO), throughout storage. Error bars represent SD. *Significant differences between control and Q/CH/SO ($P < 0.05$).

storage, significant differences were found from day 25 until the end of storage in both the coated and control samples. Citric acid is the main organic acid present in the juice of Highbush blueberries, and concentration markedly decreases as the fruit ripens.[44] The TA values of the control samples showed a significant decrease ($P < 0.05$) from 0.5 ± 0.0 at the beginning of the experiment to 0.41 g citric acid 100 g^{-1} after 35 days of storage. In contrast, coated blueberries were more stable during storage, with values ranging from 0.4 ± 0.1 to 0.5 ± 0.0 (Fig. 2). This result would indicate that, according to this index, the Q/CH/SO coating allowed for a slower maturation.

Microbiological analysis

The postharvest shelf-life of blueberries is determined by fungal spoilage. The effects that coating treatments had on the growth of molds and yeast are shown in Fig. 6. The Q/CH/SO coating was

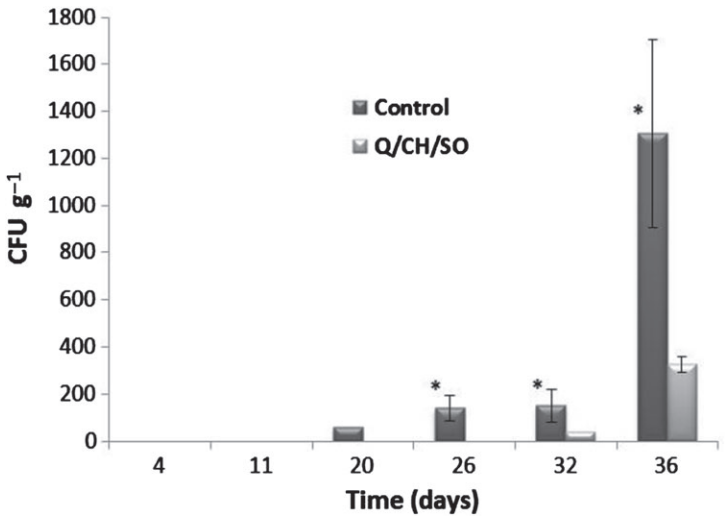

Figure 6. Evolution of mold and yeast counts during the refrigerated storage of control and coated blueberries with quinoa protein/chitosan/sunflower oil film (Q/CH/SO). *Significant differences between control and Q/CH/SO ($P < 0.05$).

effective in controlling fungal growth until 35 days of storage. After 20 days of storage, there was a significant lack of mold and yeast counts on coated blueberries as compared to the control group, which showed fungal growth at 20 days of storage. The antifungal activity of edible film with chitosan has been reported by many authors.[43,45] Sebti *et al.*[46] suggest that chitosan could have antimicrobial activity due to its positive charge, which in turn could interfere with the negatively charged residues of macromolecules on the cell surface, thus causing leaks through the cell membrane. Because of this, the antimicrobial activity of this edible chitosan film could be limited by ionic interactions between chitosan and other components. However, as can be seen in Fig. 6, the edible film demonstrated antifungal activity, which would suggest that the chitosan present in the Q/CH/SO film had free positive charges that protected against the growth of fungi.

CONCLUSIONS

A composite edible film of Q/CH/SO has been obtained with improved mechanical and water barrier properties compared with CH only. The results showed that the use of Q/CH/SO as an edible coating in fresh blueberries extended their shelf-life by controlling the growth of molds during storage. Firmness and color in fresh blueberries were affected in coated fruits. Nevertheless, coated blueberries had a slightly delayed fruit ripening during storage.

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