International Food Research Journal 24(4): 1553-1561 (August 2017)

Journal homepage: *http://www.ifrj.upm.edu.my*

Quality evaluation of blueberries coated with chitosan and sodium alginate during postharvest storage

*Chiabrando, V. and Giacalone, G.

DISAFA, Department of Agriculture, Forest and Food Science, Via L. da Vinci 44, Grugliasco (TO), Italy

Article history

Received: 27 April 2015
Received in revised form: 22 June 2016
Accepted: 19 August 2016

Keywords

Berries
Duke
Modified atmospher
Packaging
Polylactic acid

Abstract

Edible coatings are used to improve fruits appearance and storage and to extend their shelf-life. The effects of chitosan and sodium alginate as edible coatings on the quality of fresh blueberries during storage were studied. Berries were treated with coatings, packed in polylactic acid punnets and then stored at 0°C for 6 weeks. Fruit quality was evaluated for weight loss, firmness, surface color, titratable acidity, total soluble solids content, total anthocyanin content, total phenolic content, total antioxidant capacity and yeasts and molds count. Sodium alginate coated samples showed higher values of firmness and lightness during storage, but lower values of total soluble solids content and titratable acidity compared to the other samples. Furthermore, sodium alginate coated blueberries showed higher total phenolic content. Unfortunately, the results showed that alginate coating promoted the growth of yeasts and molds at the end of storage period. On the contrary, chitosan coating delayed ripening as indicated by lower respiration rate, higher total soluble solids content and titratable acidity values compared to other treatments. Moreover chitosan coating inhibited the growth of yeasts and molds. For these reasons, chitosan coating could be considered for commercial application in extending shelf life and maintaining quality of blueberry during storage and marketing.

Introduction

Blueberry is an appreciated fruit due to its organoleptic and nutritional properties, but it is a highly perishable product during postharvest storage and shelf-life. The long-term goals of postharvest research for blueberries are to improve product availability, to maintain product quality and to improve economics for producers (Giacalone and Chiabrando, 2012). Adequate postharvest technologies to be combined with cold storage are fundamental. Several pre and postharvest technologies have been used to control blueberry decay (Connor *et al*., 2002; Chiabrando *et al*., 2006; Trigo *et al*., 2006; Chiabrando and Giacalone, 2011; Giacalone and Chiabrando, 2012). In this sense, the use of edible coatings could be a new technological alternative to improve fruit quality during postharvest storage and shelf-life (Campos *et al*., 2011, Vieira *et al*., 2016).

Edible coatings may contribute to extend the shelf life of fruit and vegetables producing a semipermeable barrier to external elements that can reduce moisture loss, solutes migration, respiration and oxidative reactions and retard the natural physiological ripening process (Vargas *et al*. 2008). Maintenance of fruit quality has been reached using different coatings such as chitosan in peach and mandarins (Li and Yu, 2001; Contreras-Oliva *et al*., 2012), pectin coating in melon (Ferrari and Sarantoulos, 2013), alginate in apple (Olivas *et al*., 2007; Rojas-Grau *et al*., 2007), hydroxypropylmethylcellulose and whey protein in plum (Navarro-Tarazaga *et al*., 2008; Reinoso *et al*., 2008). In blueberry, some effects on fruit quality have been obtained with edible coating based on chitosan (Duan *et al*., 2011), aloe vera (Vieira *et al*., 2016) and quinoa protein/chitosan/sunflower oil edible film (Abugoch *et al*., 2016)

Alginate is a hydrophilic biopolymer that has a coating function because of its unique colloidal properties, which include its use for thickening, suspension forming, gel forming and emulsion stabilising (Acevedo *et al*., 2012). Sodium alginate has been effective on maintaining postharvest quality in tomato (Zapata *et al*., 2008) and peach (Maftoonazad *et al*., 2008). Chitosan, a high molecular weight polysaccharide, is soluble in organic acids, and could be used as a preservative coating material for fruits, moreover chitosan shows antifungal activity (Outtara *et al*., 2000; Devlieghere *et al*., 2004; Han *et al*., 2005; Chien *et al*., 2007).

Therefore, the aim of this work was to evaluate the effectiveness of chitosan and sodium

*Corresponding author.
Email: *: valentina.chiabrando@unito.it*

alginate edible coatings, alone or associated, in improving the postharvest quality of cold-stored highbush blueberries.

Material and Methods

Fruit material

Blueberries (*Vaccinium corymbosum L.*, cv Duke) were used in this study. The berries were hand-harvested at full maturity (100% blue), in Peveragno (CN, Italy), into 250 g punnets and transported to the laboratory. Fruits were selected, based on their uniformity of size and color. Rotten and damaged fruits were discarded.

Preparation of coating solutions

Three different coatings were prepared.

A 2% (w/v) acid-soluble chitosan (Sigma Italiana SRL, Ozzano Emilia, Italy) coating solution was prepared by dissolving the chitosan in 1% aqueous acetic acid (1.0% v/v) under agitation, with 50% glycerol as plasticizer and 0.15% Tween 20 (w/v) as a surfactant. The coating was homogenized with a high-speed Ultra-Turrax (Silverson L4RMachines, UK) for 90 s at 10000 rpm and then stored overnight at room temperature (±20°C). Blueberries were immersed in this solution for 2 min, the excess of film-forming solution was drained and then the blueberries were air dried at room temperature (±20°C) for 30 min to ensure coating dryness.

A 1.5% (w/v) sodium alginate (Sigma Italiana SRL, Ozzano Emilia, Italy) solution was prepared dissolving the powder in distilled water upon stirring at 70 °C for 2 h. Then the solution was cooled to room temperature (±20°C) according to Poverenov *et al*. (2014). Blueberries were immersed in this solution for 2 min and then in 5% aqueous solution of CaCl2 for 2 min (Sigma Italiana SRL, Ozzano Emilia, Italy) to perform gelation of alginate molecules by cross-linking. The samples were then air dried at room temperature (±20°C) for 30 min to ensure coating dryness.

A 1.5% (w/v) chitosan and 1% (w/v) sodium alginate coating solution was prepared by mixing 3% chitosan solution and 2% sodium alginate solution at a 1:1 ratio with 25% glycerol and 0.15% Tween 20 (w/v). Blueberries were immersed in the solution for 2 min, the excess of the film-forming solution was drained and then blueberries were air dried at room temperature (±20°C) for 30 min to ensure coating dryness. Samples immersed in distilled water was used as control.

Packaging and storage conditions

Approximately 120 g of blueberries were weighed and placed inside commercial PLA (Polylactic acid, biodegradable film) punnets and wrapped automatically with PLA film (Compac, Italy). The PLA film had an O2 transmission rate of 40 cm3/m2/24h at 23°C, a CO2 transmission rate of 200 cm3/m2/24h at 23°C and a moisture vapor transmission rate of 18 g/m2/24h at 23°C and 85% RH. For each treatment nine punnets were prepared. The experimental unit was one punnet. At each sampling time, three individual punnets were selected per treatment to measure the quality attributes. The packages were then stored in a refrigerated chamber at 0±0.5°C and 75% RH for 45 days.

Weight loss

Weight loss was determined by weighing the packages at the start of the experiment (0 time) and at 15 days intervals during storage. Values are reported as percent of weight loss per initial fruit weight.

Atmosphere composition

The concentrations of oxygen and carbon dioxide inside the packages were monitored daily by sampling (0.5 mL) the headspace using a CANAL 121 (Vizag, Gas Analysis, France). A syringe was inserted into the package through a rubber seal placed on the film. Gases were analysed with an electrochemical sensor for O2 level and an infrared sensor for CO2 level. The instrument was calibrated towards air. Results were expressed as KPa of O2 and CO2 inside the packs.

Quality evaluations

Berry physicochemical quality attributes were measured before coating treatments (time 0) and then after 15, 30 and 45 days of storage.

Total soluble solids content (TSSC), pH, and titratable acidity were measured using juice extracted from blueberries sample blended in a tissue homogeniser. Soluble solids concentration was determined by a digital refractometer (Atago refractometer model PR-32, Co., Ltd, Japan) and the results expressed as °Brix. Titratable acidity and pH were measured by titrating 1:10 diluted juice, using 0.1 N NaOH and an automatic titrator (Compact 44–00, Crison Instruments SA, Barcelona, Spain).

Flesh color measurements were determined on berries surface with a Minolta Chroma Meter CR-400 (Konica Minolta, Japan). The instrument was setting with the illumining D65 and an observation angle of 2° and calibrated with a standard white plate. Ten measurements (15 berries) per treatment

and sampling time were made. The results were expressed as CIELAB *(L^*,a^*,b^*)* color space. The L^* value describes the lightness.

Textural measurements were carried out at harvest and after 15, 30 and 45 days of storage and 15 berries were randomly selected according to Doving *et al*. (2005) for each coating treatment. Before analysis, samples were cooled to room temperature (20°C) for 3 hr, because most fruits and vegetables showed decreasing firmness with increasing temperature (Bourne, 1980). Fruit firmness was determined by penetration using a Texture Analyzer TaxT2i® (Stable Micro System, UK). Measurements were performed at a crosshead speed of 3 mm/s with a 3 mm diameter punch in the equatorial part of the blueberry (Chiabrando *et al*., 2009). A 5-Kg load cell was used for firmness determination and the probe was programmed to penetrate the berry for 3 mm after the contact with the flesh. Fruit firmness was tested individually on berry samples for each coating treatment. The maximum penetration force (N), which is related to the firmness of the samples, was the parameter selected for further statistical analysis.

Determination of total anthocyanin, total phenolic and total antioxidant capacity

To determine the total anthocyanin, phenolic and antioxidant capacity, extracts were prepared by weighing 10 g of berries into a centrifuge tube, adding methanol (25 ml) and homogenising the sample for 1 min. Extractions were performed under reduced light conditions. Tubes were centrifuged (3000 rpm for 15 min) and the clear supernatant fluid collected and stored at -26°C.

Total anthocyanin content was quantified according to the pH differential method of Cheng and Breen (1991). Anthocyanins were estimated by their difference of absorbance at 515 and at 700 nm in buffer at pH 1.0 and at pH 4.5, where A = (A515 - A700)pH1.0 -(A515 - A700)pH4.5. Results were expressed as mg of cyanidin-3- glucoside (C3G) per 100 g of fresh berries.

Total phenolic content was determined with the Folin–Ciocalteu reagent by the method of Slinkard and Singleton (1977), using gallic acid as a standard. Absorption was measured at 765 nm. Results were expressed as mg gallic acid equivalents (GAE) per 100 g of fresh berries.

Total antioxidant activity was determined using ferric reducing antioxidant power (FRAP) assay, following the methods of Pellegrini *et al*. (2003) with some modifications. Results were expressed as mmol Fe^{2+}/kg of fresh berries.

Microbiological analysis

The analyses of yeasts and molds count were carried out at the end of storage period, according to the methodology described by the Compendium of Methods for the Microbiological Examination of Foods (Vanderzant and Splittstoesser, 1992). Yeast and mould count were performed using a chloramphenicol glucose agar (CGA) (ISO 21527, 2008). All the plates were incubated at room temperature (± 20°C) for 3-5 d. At the end of the incubation period, the obtained microbial colonies were counted and reported per log CFU (colony-forming units) per grams of blueberries.

Statistical analysis

Data were elaborated by analysis of variance, using statistical procedures of the STATISTICA ver. 6.0 (Statsoft Inc., Tulsa, OK, USA), the sources of variance being coating treatments and storage time. Tukey's HSP test (honest significant differences) was used to determine significant differences among treatment means. A variation of a given factor was considered significant when the probability, p, for this factor was less than 0.05.

Results and Discussion

Weight loss

Biodegradable films with appropriate oxygen transmission rate play an important role in the development of modified atmosphere and quality maintenance of fresh fruits during storage. Water vapor permeability of packaging materials is essential to limit weight losses of product during storage.

During storage, significant ($p \leq 0.05$) increasing in weight losses was observed, in particular after 45 days of storage. Berries weight losses were about 4-5% during 45 days of storage (Figure 1). These values are low if compared to traditional storage condition, where the average weight loss was about 9-10% after 45 days of storage (Chiabrando *et al*., 2006).

Among samples, significantly higher weight loss values were obtained in alginate + chitosan samples at the end of storage (Figure 1) compared to other treatments. A factor that has caused the lack of a better performance of the used coatings to reduce weight and moisture loss in the present study could be the non-uniform coverage of the blueberries and the adhesion degree of the coating to the surface of the fruit, which is influenced by the surface moisture (Baldwin 1994).

Differences in the ability to reduce weight loss are attributed to the different water vapour permeability

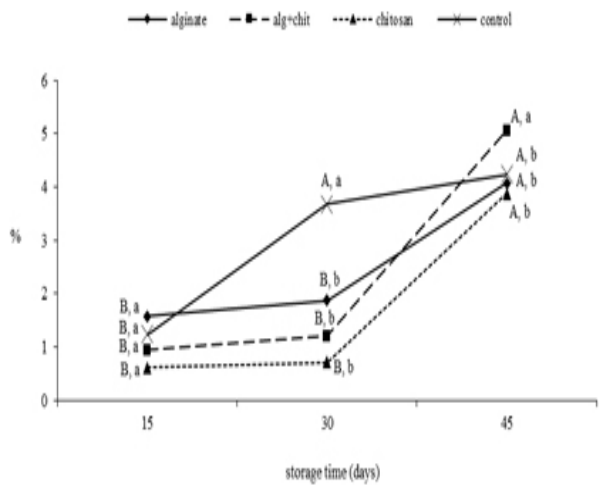

Figure 1. Weight loss (%) in 'Duke' blueberries during postharvest storage in PLA packages and coated with sodium alginate (1.5%), sodium alginate + chitosan (1.5% + 1%) or chitosan (2%). Means sharing the same letters during storage time (A, B, C) and in the same storage time (a, b, c) are not significantly different from each other (Tukey's HSD test, p≤ 0.05). Data is average of 3 replicates ± SD.

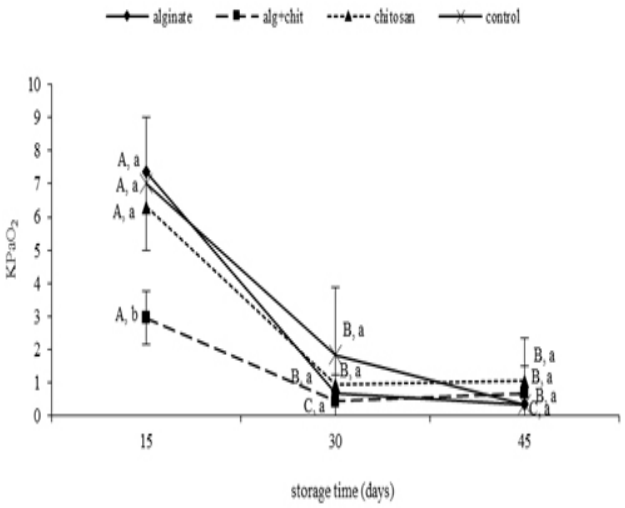

Figure 2. Dynamics of Oxygen ($KPaO_2$) content in the headspace of 'Duke' blueberries during postharvest storage in PLA packages and coated with sodium alginate (1.5%), sodium alginate + chitosan (1.5% + 1%) or chitosan (2%). Means sharing the same letters during storage time (A, B, C) and in the same storage time (a, b, c) are not significantly different from each other (Tukey's HSD test, p ≤ 0.05). Data is average of 3 replicates ± SD.

of the polysaccharides used in the formulation of the edible coatings (Vargas *et al*., 2008). In these sense, the addition of glycerol (50%) as plasticiser in the chitosan coating gave significant results in terms of reducing blueberry weight loss. According to previous research on strawberry, fruit with coatings without plasticise showed higher weight losses than fruits with more than 40% of glycerol coatings (García *et al*., 1998). The same result was obtained in apples (Moldão-Martins *et al*., 2003). In this sense, weight loss results indicated that chitosan coating provides a better water barrier property than other coating, thus reducing weight loss of coated berries and delaying dehydration of fruits during storage.

Atmosphere composition

The O_2 content in all blueberries samples decreased as a result of natural fruit respiration. The dynamics of oxygen concentration is showed in Figure 2. The higher decrease of O_2 content and the higher increase of CO_2 (Figure 3) content during the first 30 days of storage was observed in alginate + chitosan samples. After 30 and 45 days of storage, no significant differences between treatments were observed. In general, no detectable influences of coating treatments was found.

Quality evaluations

Physicochemical properties of blueberry before processing (time 0) and the evolution during

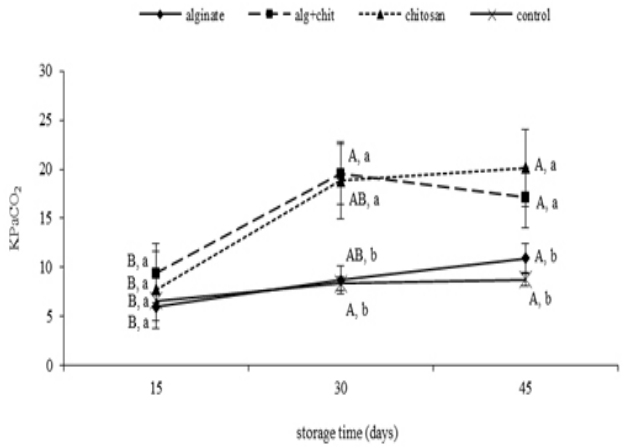

Figure 3. Dynamics of Carbon Dioxide (KPaCO2) content in the headspace of 'Duke' blueberries during postharvest storage in PLA packages and coated with sodium alginate (1.5%), sodium alginate + chitosan (1.5% + 1%) or chitosan (2%). Means sharing the same letters during storage time (A, B, C) and in the same storage time (a, b, c) are not significantly different from each other (Tukey's HSD test, p ≤0.05). Data is average of 3 replicates ± SD.

postharvest storage period are presented in Table 1. Firmness of berries before coating treatments (day 0) was 2.69 N. During storage firmness values showed an increase after 15 days and then a significant (p≤0.05) decrease in all samples. This mechanical behavior is in agreement with previous works (Chiabrando *et al*., 2009) and could be due to increased moisture loss and enhanced shrivelling during storage period. In this sense, a higher firmness during storage could be

Table 1. Effect of different coatings on firmness (N), total soluble solids content (T.S.S.C.; °Brix), titratable acidity (T.A.; meq/l), lightness (L*), total anthocyanin content (mg cyanidin 3-gluc/100g FW), total polyphenol content (mg gallic acid/100g FW) and total antioxidant activity (mmol Fe2+/kg FW) of 'Duke' blueberries during postharvest storage in PLA packages. Means sharing the same letters in row (A, B, C) and in column (a, b, c) are not significantly different from each other (Tukey's HSD test, $p \leq 0.05$). For firmness and lightness, data is average of 15 replicates ± SD. For T.S.S.C., titratable acidity, anthocyanin, polyphenol and antioxidant activity, data is average of 3 replicates ± SD

		storage time (days)			
Quality parameter	treatments	0	15	30	45
Firmness (N)	alginate	2.69 ± 0.36 ^{A,a}	2.81 ± 0.38 ^{A,a}	2.66 ± 0.21 ^{A,a}	2.50 ± 0.34 ^{a,a}
	alg+chit	2.69 ± 0.36 ^{A,a}	2.84 ± 0.35 ^{A,a}	2.44 ± 0.49 ^{a,b}	2.49 ± 0.45 ^{a,a}
	chitosan	2.69 ± 0.36 ^{A,a}	2.80 ± 0.31 ^{A,a}	2.48 ± 0.47 ^{a,b}	1.95 ± 0.55 ^{b,b}
	control	2.69 ± 0.36 ^{A,a}	2.80 ± 0.30 ^{A,a}	2.33 ± 0.50 ^{a,b}	2.33 ± 0.35 ^{a,ab}
T.S.S.C. (°Brix)					
	alginate	11.77 ± 1.21 ^{A,a}	11.00 ± 1.53 ^{AB,b}	10.20 ± 1.36 ^{B,a}	10.20 ± 1.32 ^{B,a}
	alg+chit	11.77 ± 1.21 ^{A,a}	10.80 ± 2.15 ^{B,a}	10.60 ± 1.52 ^{B,a}	10.10 ± 1.42 ^{BC,b}
	chitosan	11.77 ± 1.21 ^{A,a}	11.9 ± 1.64 ^{A,a}	11.10 ± 1.61 ^{AB,ab}	11.00 ± 1.34 ^{AB,a}
	control	11.77 ± 1.21 ^{A,a}	10.40 ± 1.82 ^{B,a}	11.90 ± 1.26 ^{A,a}	10.10 ± 2.38 ^{B,a}
T.A. (meq/l)					
	alginate	72.57 ± 2.12 ^{A,a}	59.48 ± 2.52 ^{C,b}	62.28 ± 4.12 ^{B,a}	51.54 ± 1.91 ^{C,b}
	alg+chit	72.57 ± 2.12 ^{A,a}	75.08 ± 2.44 ^{A,a}	56.98 ± 3.27 ^{C,b}	69.32 ± 2.30 ^{B,a}
	chitosan	72.57 ± 2.12 ^{A,a}	52.30 ± 3.61 ^{C,b}	43.28 ± 3.23 ^{C,c}	62.04 ± 2.22 ^{B,a}
	control	72.57 ± 2.12 ^{A,a}	60.32 ± 3.53 ^{B,a}	42.98 ± 2.18 ^{C,c}	61.08 ± 2.22 ^{B,ab}
Lightness (L*)					
	alginate	33.75 ± 2.35 ^{A,a}	23.82 ± 2.15 ^{B,a}	28.24 ± 2.63 ^{B,a}	28.78 ± 2.32 ^{B,a}
	alg+chit	33.75 ± 2.35 ^{A,a}	20.86 ± 2.44 ^{B,a}	18.01 ± 1.75 ^{B,a}	22.09 ±2.04 ^{B,b}
	chitosan	33.75 ± 2.35 ^{A,a}	18.73 ± 1.98 ^{B,a}	15.22 ± 1.55 ^{B,a}	17.51 ± 1.85 ^{B,a}
	control	33.75 ± 2.35 ^{A,a}	27.23 ± 2.74 ^{B,a}	28.46 ±2.81 ^{B,a}	29.09 ± 2.46 ^{B,a}
Anthocyanin (mg cyanidin 3-gluc/100 g FW)					
	alginate	22.82 ± 1.81 ^{B,a}	24.35 ± 1.61 ^{AB,a}	16.36 ± 1.12 ^{C,c}	27.77 ± 1.67 ^{A,a}
	alg+chit	22.82 ± 1.81 ^{AB,a}	13.31 ± 1.54 ^{C,c}	18.26 ± 1.12 ^{B,a}	23.59 ± 1.80 ^{A,c}
	chitosan	22.82 ± 1.81 ^{AB,a}	17.99 ± 1.30 ^{C,b}	19.40 ± 1.85 ^{B,a}	26.90 ± 1.81 ^{A,a}
	control	22.82 ± 1.81 ^{B,a}	15.22 ± 1.21 ^{C,c}	23.97 ± 1.77 ^{B,b}	29.94 ± 2.21 ^{A,a}
Polyphenol (mg gallic acid/100 g FW)					
	alginate	307 ± 9.32 ^{AB,b}	284.32 ± 10.12 ^{B,b}	263.94 ± 9.97 ^{C,a}	330.61 ± 10.32 ^{A,a}
	alg+chit	307 ± 9.32 ^{A,a}	283.32 ± 11.20 ^{B,b}	253.14 ± 9.55 ^{C,c}	300.52 ± 11.20 ^{A,b}
	chitosan	307 ± 9.32 ^{A,a}	227.87 ± 12.12 ^{C,c}	265.23 ± 10.22 ^{B,b}	259.59 ± 9.64 ^{B,c}
	control	307 ± 9.32 ^{A,a}	314.50 ± 10.97 ^{A,a}	290.04 ± 9.62 ^{B,a}	300.25 ± 11.67 ^{AB,b}
Antioxidant activity (mmol Fe^{2+}/kg FW)					
	alginate	15.26 ± 0.21 ^{A,a}	14.96 ± 0.22 ^{A,a}	15.57 ± 0.25 ^{A,a}	15.18 ± 0.22 ^{A,a}
	alg+chit	15.26 ± 0.21 ^{A,a}	14.87 ± 0.25 ^{A,a}	14.55 ± 0.21 ^{A,a}	14.56 ± 0.23 ^{A,a}
	chitosan	15.26 ± 0.21 ^{A,a}	15.58 ± 0.24 ^{A,a}	14.69 ± 0.22 ^{A,a}	15.29 ± 0.31 ^{A,a}
	control	15.26 ± 0.21 ^{A,a}	15.27 ± 0.25 ^{A,a}	15.15 ± 0.22 ^{A,a}	14.25 ± 0.28 ^{A,a}

due to the formation of a resistant superficial tissue consequent of a higher moisture loss, according to Souza et al. (2005) in papaya. However, the difference among edible coatings samples firmness after 15 days storage was not substantial ($p \leq 0.05$) (Table 1). Contrarily, at the end of storage period (45 days) alginate and alginate + chitosan coatings helped maintain the firmness of Duke over the control and chitosan coated samples. This behavior could be explained with the immersion in CaCl2 solution of the blueberries treated with sodium alginate. The higher firmness of berries treated with $CaCl_2$ confirms the role of calcium in maintaining cell wall structure and membranes (Soliva-Fortuny and Martin-Belloso, 2003). However, chitosan coating did not significantly reduce softening in blueberries as reported previously for other coatings in strawberries (Tezotto-Uliana *et al*., 2014). The authors attributed this trend to the fact that coating immersion leaves the fruit wet, leading to faster softening. Moreover, the changes in fruit firmness during ripening results from alterations in cell wall structure and depolymerisation of pectic substances and hemicelluloses.

Total soluble solids content significantly decreased during blueberries storage and was likely due to the continued ripening process during storage (Table 1). Berries treated with chitosan coating had more stable TSSC values than the control and the other treatments according to the results of Chien *et al*. (2007) and Chiabrando and Giacalone (2011) (Table 1). It was interpreted that during postharvest storage, acid metabolism converted acid to sugar,

Table 2. Effect of different coatings on microbiological changes of 'Duke' blueberries after 45 days of storage in PLA packages. Values with different letters in the same column are significantly different by Tukey test ($p \leq 0.05$) (n = 3).

	yeast (log CFU/g)	mould (log CFU/g)
alginate	4.56 a	1.81 a
alg+chit	4.68 a	2.00 a
chitosan	<1 b	<1 b
control	2.81 b	1.26 b

thus resulting in the decrease of TA and the increase of TSSC (Duan *et al*., 2011).

In general, titratable acidity values decreased significantly over time, but without a defined trend (Table 1). A decrease in total acidity is typical during postharvest storage and has been attributed to the use of organic acids (such as citric acid) as substrates for the respiratory metabolism (Gol *et al*., 2013). Between treatments, after 15 and 30 days of storage, blueberries treated with alginate and alginate + chitosan showed higher values compared with the other treatments. In this case, coatings reduce the respiration rate, therefore delay the utilization of organic acids, according to Yaman and Bayoindirli (2002). At the end of storage period, blueberries treated with chitosan and in association (alg+chit) showed the highest values, thus, in this case coating treatment helped retain titratable acidity of the samples ($p \leq 0.05$). On the contrary, alginate samples showed significant lower titratable acidity values compared to control and other coatings. A decline in acidity demonstrates maturation development, thus, in this case, coating improved blueberries maturation. This results were also obtained by Abugoch *et al*. (2015) in blueberries coated with quinoa protein/ chitosan/sunflower oil edible film.

The results of changes in the color of berry skin in term of lightness values during storage (Table 1) showed a general decreasing trend, more evident in all coated samples compared to control, due to the post-harvest ripening process. The coating treatments led to a decrease in luminosity of samples, and, after 30 and 45 days of storage, the blueberries treated with coating containing chitosan showed significantly lower lightness values. This is probably due to the changes in the surface reflection properties when the blueberries are coated, that can provoke this luminosity decrease. In this sense, Hoagland and Parris (1996) reported that chitosan film turned opaque during film formation at the final stage of drying, resulting in decreased of L* values.

On the contrary, control and sodium alginate coated berries showed significant higher L* values compared to other samples, with significant differences after 30 and 45 days of storage. The length of storage reduced lightness values significantly already after 15 days of storage (Table 1), since then the L* values resulted quite constant.

Determination of Total Anthocyanin, Total phenolic and Total Antioxidant Capacity

The total anthocyanin content of fruit subjected to different coating treatments are reported in Table 1. The storage time as well as the coating treatments influenced total anthocyanins content ($p \leq 0.05$). The obtained results showed that with progressing storage duration, total anthocyanins content declined throughout 30 days of storage in all the treatments and then increased at the end (45 days) (Table 1). The same results were obtained also in pomegranate (Miguel *et al*., 2004), in strawberry and in raspberry (El Ghaouth *et al*., 1991; Han *et al*., 2004). The authors concluded that the increased in anthocyanins is related to the activity of the anthocyanin biosynthetic pathway enzymes, because storage conditions and edible coatings can produce abiotic stress on produce, modifying its metabolism and affecting the production of such secondary metabolites. Between treatments, significantly higher values were obtained in control samples and could be related to water loss and an increase in anthocyanin concentration. Moreover, the stability of anthocyanins probably depends on biological as well as environmental and stress conditions (Alighourchi *et al*., 2008).

Total phenolic content was significantly affected by coating treatments (Table 1). In general, polyphenol contents decreased and then increased during storage in according with anthocyanin behavior, with the exception of chitosan samples. The increase in phenolic content during storage is affected by several causes of physiological stress, which can promote enzymatic oxidation of these compounds according to the results reported by Connor *et al*. (2002) in blueberry, cv Elliott.

Antioxidant capacity has been used to evaluate the antioxidant potential status of tissue, which is a function of the type and amount of bioactive compounds present. In this work, total antioxidant activity was not significantly affected by cold storage as well as coating treatments during storage ($p \leq 0.05$).

Microbiological analysis

The quality of blueberries during storage has been limited by its highly perishable nature including susceptibility to postharvest decay associated, in particular, with yeasts and fungal infections. Results on total yeast and mold counts of different edible

coatings treated berries are presented in Table 2. Blueberries treated with chitosan edible coating minimized the growth of yeasts and molds to values <1 log CFU g−1 compared to the control and the others coatings. The chitosan coating on the blueberries effectively inhibited the growth of microorganisms during post-harvest period according to Chien *et al*. (2007) in mango, Hernandez-Munoz *et al*. (2006) in strawberry and Gonzalez-Aguilar *et al*. (2009) in papaya. On the contrary, sodium alginate coating promoted the growth of yeasts and molds compared to chitosan samples and control, and showed significant higher values in yeast and mold counts at the end of storage period.

Conclusion

Results indicate the possibility of using alginate and chitosan edible coating in blueberry with no reduction in shelf-life. In this study different coatings showed different effects on post-harvest quality. In general, alginate coating showed higher values of firmness and lightness during storage, but lower values of total soluble solids content and titratable acidity. Regarding yeast and mold growth, the treatment with sodium alginate coating induces an undesired increase of the proliferation during postharvest storage period. Chitosan showed lower weight losses, higher total soluble solids content and titratable acidity values compared to other treatments. In addition, chitosan-based coatings had the added advantage of an antifungal property. In conclusion, chitosan coating could be considered for commercial application in extending shelf life and maintaining quality of blueberries during storage and marketing.

References

Abugoch, L., Tapia, C., Plasencia, D., Pastor, A., Castro-Mandujano, O., López, L. and Escalona V. 2016. Shelf-life of fresh blueberries coated with quinoa protein/chitosan/sunflower oil edible film. Journal of the Science of Food and Agriculture 96: 619–626.

Acevedo, C. A., López, D. A., Tapia, M. J., Enrione, J., Skurtys, O., Pedreschi, F., Brown, D. I., Creixell, W. and Osorio, F. 2012. Using RGB image processing for designating an alginate edible film. Food and Bioprocess Technology 5: 1511-1520.

Alighourchi, H. Barzegar, M. and Abbasi, S. 2008. Anthocyanins characterization of 15 Iranian pomegranate (*Punica granatum* L.) varieties and their variation after cold storage and pasteurization. European Food Research and Technology 227: 881–887.

Baldwin, E. A. 1994. Edible coatings for fruits and vegetables, past, present and future. In Krochta, J. M., Baldwin E. A. and Nisperos-Carriedo M. O. (Eds). Edible Coatings and Films to Improve Food Quality, p. 25–64. Lancaster: Technomic Pub. Co.

Bourne, M. C. 1980. Textural evaluation of horticultural crops. HortScience 15: 51–56.

Campos, C. A., Gerschenson, L. N. and Flores, S. K. 2011. Development of edible films and coatings with antimicrobial activity. Food and Bioprocess Technology 4: 849-875.

Chiabrando, V., Peano, C., Beccaro, G., Bounous, G. and Rolle, L. 2006. Postharvest quality of highbush blueberry (*Vaccinium corymbosum* L.) cultivars in relation to storage methods. Acta Horticulture 715: 545–551.

Chiabrando, V., Giacalone, G. and Rolle, L. 2009. Mechanical behaviour and quality traits of highbush blueberry during postharvest storage. Journal of the Science of Food and Agriculture 89: 989–992.

Chiabrando, V. and Giacalone, G. 2011. Shelf-life extension of highbush blueberry using 1-methylcyclopropene stored under air and controlled atmosphere. Food Chemistry 126: 1812-1816.

Cheng, G. W. and Breen, P.J. 1991. Activity of Phenylalanine Ammonia-lyase (PAL) and Concentration of Anthocyanins and Phenolics in Developing Strawberry Fruit. Journal of the American Society for Horticultural Science 116: 856-867.

Chien, P. J., Sheu, F. and Yang, F. H. 2007. Effects of edible chitosan coating on quality and shelf life of sliced mango fruit. Journal of Food Engineering 78: 225– 229.

Contreras-Oliva, A., Pérez-Gago, M. B. and Rojas-Argudo, C. 2012. Effects of chitosan coatings on physicochemical and nutritional quality of clementine mandarins cv. 'Oronules'. Food of Science and Technology International 4: 303-315.

Connor, M. A., Luby, J. J., Hancock, F. J., Berkheimer, S. and Hanson, J. E. 2002. Changes in fruit antioxidant activity among blueberry cultivars during cold-temperature storage. Journal of Agriculture and Food Chemistry 50: 893–898.

Devlieghere, F., Vermeulen, A. and Debevere, J. 2004. Chitosan: Antimicrobial activity, interactions with food components and applicability as a coating on fruit and vegetables. Food Microbiology 21: 703–714.

Doving, A., Mage, F. and Vestrheim, S. 2005. Methods for testing strawberry fruit firmness: a review. Small Fruit Review 4: 11–34.

Duan, J., Wu, R., Strik, B. C. and Zhao, Y. 2011. Effect of edible coatings on the quality of fresh blueberries (Duke and Elliott) under commercial storage conditions. Postharvest Biology and Technology 59: 71–79.

El Ghaouth, A., Ponnampalam, R. and Boulet, M. 1991. Chitosan coating effect on storability and quality of fresh strawberries. Journal of Food Science 56: 1618–1621.

Ferrari, C. C. and Sarantoulos, C. I. G. L. 2013. Effect of Osmotic Dehydration and Pectin Edible Coatings on Quality and Shelf Life of Fresh-Cut Melon. Food and

Bioprocess Technology 6: 80-91.

García, M. A., Martino, M. N. and Zaritzky, N. E. 1998. Starch-based coatings: effect on refrigerated strawberry *(Fragaria ananassa)* quality. Journal of the Science of Food and Agriculture 76: 411–420.

Giacalone, G. and Chiabrando, V. 2012. Problems and methods to improve the market-life of berry fruit. In Tuberoso, C. (Ed). Berries: Properties, Consumption and Nutrition, p. 179-196. New York: Nova Science Publishers.

Gol, N. B., Patel, P. R. and Rao, T. V. R. 2013. Improvement of quality and shelf-life of strawberries with edible coatings enriched with chitosan. Postharvest Biology and Technology 85: 185–195.

Gonzalez-Aguilar, G. A., Valenzuela-Soto, E., Lizardi-Mendoza, J., Goycoole, F., Martinez-Tellez, M. A., Villegas-Ocho, M. A., Monroy-Garcia, I. N. and Ayala-Zavala, J. F. 2009. Effect of chitosan coating in preventing deterioration and preserving the quality of fresh-cut papaya 'Maradol'. Journal of the Science of Food and Agriculture 89: 15–23.

Han, C., Zhao, Y., Leonard, S. W. and Traber M. G. 2004. Edible coating to improve storability and enhance nutritional value of fresh and frozen strawberries *(Fragaria ananassa)* and raspberries *(Rubus ideaus)*. Postharvest Biology and Technology 33: 67–78.

Han, C., Lederer C., McDaniel M. and Zhao Y. 2005. Sensory evaluation of fresh strawberries *(Fragaria ananassa)* coated with chitosan-based edible coatings. Journal of Food Science 70: 172–180.

Hernandez-Munoz, P., Almenar, E., Ocio, M. J. and Gavara, R. 2006. Effect of calcium dips and chitosan coatings on postharvest life of strawberries (Fragaria x ananassa). Postharvest Biology and Technology 39: 247–253.

Hoagland, P. D. and Parris, N. 1996. Chitosan/pectin laminated films. Journal of Agriculture and Food Chemistry 44: 1915–1919.

Kittur F. S., Saroja N., Haibibunnisa M. S. and Tharanathan R. N. 2001. Polysaccharide-based composite coating formulations for shelf-life extension of fresh banana and mango. European Food Research and Technology 213: 306–311.

ISO 21527. 2008. Microbiology of food and animal feeding stuffs. Horizontal method for the enumeration of yeasts and moulds. Switzerland: International Standards Organization.

Li, H. and Yu, T. 2001. Effect of chitosan coating on incidence or brown rot, quality and physiological attributes for postharvest peach fruit. Journal of the Science of Food and Agriculture 81: 269–274.

Maftoonazad, N., Ramaswamy, H. S. and Marcotte, M. 2008. Shelf-life extension of peaches through sodium alginate and methyl cellulose edible coatings. International Journal of Food Science and Technology 43: 951–957.

Miguel, G., Fontes, C., Antunes, D., Neves, A. and Martins, D. 2004. Anthocyanin concentration of 'Assaria' pomegranate fruits during different cold storage conditions. Journal of Biomedicine and Biotechnology 5: 338–342.

Moldão Martins, M., Beirão da Costa, S. M. and Beirão da Costa, M. L. 2003. The effects of edible coatings on postharvest quality of the "Bravo de Esmolfe" apple. European Food Research and Technology 217: 325–328.

Navarro-Tarazaga, M. L. L., Sothornvit, R. and Pérez Gago, M. B. 2008. Effect of plasticizer type and amount on hydroxypropyl methylcellulose-beeswax edible film properties and postharvest quality of coated plums (cv. Angeleno). Journal of Agriculture and Food Chemistry 56: 9502–9509.

Olivas, G. I., Mattinson, D. S. and Barbosa Cánovas, G. V. 2007. Alginate coatings for preservation of minimally processed Gala apples. Postharvest Biology and Technology 45: 89-96.

Outtara, B., Simard, R. E., Piette, G., Begin, A. and Holley, R. A. 2000. Diffusion of acetic and propionic acids from chitosan-based antimicrobial packaging films. Journal of Food Science 65: 768–773.

Pellegrini, N., Serafini, M., Colombi, B., Del Rio, D., Salvatore, S., Bianchi, M. and Brighenti, F. 2003. Total Antioxidant Capacity of Plant Foods, Beverages and Oils Consumed in Italy Assessed by Three Different *in Vitro* Assays. Journal of Nutrition 133: 2812-2819.

Poverenov, E., Danino, S., Horev, B., Granit, R., Vinokur, Y. and Rodov, V. 2014. Layer-by-layer electrostatic depositino of edile coating on fresh-cut melon model: anticipated and unexpected effect of arginate-chitosan combination. Food and Bioprocess Technology 7: 1424-1432.

Reinoso, E., Mittal, G. S. and Lim, L. T. 2008. Influence of whey protein composite coatings on plum (*Prunus domestica* L.) fruit quality. Food and Bioprocess Technology 1: 314–325.

Rojas Graü, M. A., Tapia, M. S., Rodríguez, F. J., Carmona, A. and Martin Belloso, O. 2007. Alginate and gellan-based edible coatings as carriers of antibrowning agents applied on fresh-cut Fuji apples. Food Hydrocolloid 21: 118-127.

Slinkard, K. and Singleton, V. L. 1977. Total Phenol Analysis: Automation and Comparison with Manual Methods. American Journal of Enology and Viticulture 28: 49-53.

Soliva-Fortuny, R. C. and Martin-Belloso, O. 2003. New advances in extending the shelf-life of fresh-cut fruits: a review. Trends in Food Science and Technology 14: 341–53.

Souza, B. S., Durigan, J. F., Donadon, J .R. and Lima, M. A. 2005. Quality and physiological behavior of fresh cut "Formosa" papaya. Braz. Journal of Food Technology 8: 243–247.

Tezotto-Uliana, J. V., Fargoni, G. P., Geerdink, G. M. and Kluge, R. A. 2014. Chitosan applications pre- or post-harvest prolong raspberry shelf-life quality. Postharvest Biology and Technology 91: 72–77.

Trigo, M. J., Sousa, M. B., Botelho, M. L. and Veloso, G. 2006. Quality of gamma irradiated blueberries. Acta Horticulture 715: 573–577.

Vargas, M., Pastor, C., Chiralt, A., McClements, J. and González Martínez, C. 2008. Recent advances in edible coatings for fresh and minimally processed fruits. Critical Review Food Science and Nutrition 48: 496–511.

Vanderzant, C. and Splittstoesser, D. F. 1992. Compendium of methods for the microbiological examination of foods. 3rd ed. Washington: American Public Health Association.

Vieira, J. M., Flores-Lopez, M. L., Jasso de Rodríguez, D., Soussa, M. C., Vicente, A. A. and Martins J. T. 2016. Effect of chitosan–Aloe vera coating on postharvest quality of blueberry *(Vaccinium corymbosum)* fruit. Postharvest Biology and Technology 116: 88–97.

Yaman, O. and Bayoindirli, L. 2002. Effects of an Edible Coating and Cold Storage on Shelf-life and Quality of Cherries. Food Science and Technology 35: 146–150.

Zapata, P. J., Guillén, F., Martínez Romero, D., Castillo, S., Valerom D. and Serrano, M. 2008. Use of alginate or zein as edible coatings to delay postharvest ripening process and to maintain tomato (*Solanum lycopersicon* Mill.) quality. Journal of the Science of Food and Agriculture 88: 1287–1292.