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This is the author's final version of the contribution published as:

Chiabrando, Valentina; Giacalone, Giovanna. Anthocyanins, phenolics and antioxidant capacity after fresh storage of blueberry treated with edible coatings. *INTERNATIONAL JOURNAL OF FOOD SCIENCES AND NUTRITION*. 33 (3) pp: 248-253.

DOI: 10.3109/09637486.2014.986075

The publisher's version is available at:

<http://www.tandfonline.com/doi/full/10.3109/09637486.2014.986075>

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Anthocyanins, phenolics and antioxidant capacity after fresh storage of blueberry treated with edible coatings

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Abstract

The influence of different edible coatings on total phenolic content, total anthocyanin and antioxidant capacity in highbush blueberry (*Vaccinium corymbosum* L. cv Berkeley and O'Neal) was investigated, mainly for industrial applications. Also titratable acidity, soluble solids content, firmness and weight loss of berries were determined at harvest and at 15-day intervals during 45 storage days at 0 °C, in order to optimize coating composition. Application of chitosan coating delayed the decrease in anthocyanin content, phenolic content and antioxidant capacity. Coating samples showed no significant reduction in the weight loss during storage period. In cv Berkeley, the use of alginate coating showed a positive effect on firmness, titratable acidity and maintained surface lightness of treated berries. In cv O'Neal, no significant differences in total soluble solids content were found, and the chitosan-coated berries showed the minimum firmness losses. In both cultivars, the addition of chitosan to coatings decreases the microbial growth rate.

Keywords: Berkeley, chitosan, O'Neal, sodium alginate

Introduction

Fruits and vegetables are rich in phenolic compounds, such as anthocyanins, flavonols, isoflavones and catechins which have strong antioxidant capacity. Anthocyanins, natural pigments that are responsible of the blue, violet and red colors of fruits, are one of the major flavonoid classes.

The well known nutritional value and antioxidant capacity of blueberries allow these species to be considered as an excellent “functional food” and has contributed in recent years to the growth of a profitable market for these commodities. Blueberries (*Vaccinium corymbosum* L.) contain essential nutrients and a variety of phytochemicals, such as polyphenols and flavonoids, which have been suggested to provide important health benefits (Gil et al., 1997; Kalt et al., 2001; Wu et al., 2004). Statistical data suggests that regular consumption of fruits and vegetables, including berries, is associated with reduced risk of chronic diseases, such as cancer and cardiovascular disease (Chu et al., 2002; Duthie, 2007; Giampieri et al., 2012). These compounds are not completely stable, and, after harvest, undergo changes during processing and storage, which may alter their biological activity (Alvarez-Suarez et al., 2014). Factors that may affect antioxidant activity include maturity, genetic differences, pre-harvest conditions, post-harvest storage and processing. Post-harvest storage can affect phenolic compound levels and antioxidant capacity in berry fruits (Connor et al., 2002). In blueberry, phenolic compounds are highly unstable and may be lost during processing (Hakkinen et al., 2000; Srivastava et al., 2007). Processing also has marked effects on phenolic content and antioxidant capacity in fruits.

Strawberries, blueberry and raspberries stored at temperatures of 0°C resulted in an increase in antioxidant capacity (Kalt et al., 1999). Controlled atmosphere storage of strawberry fruit decreased anthocyanin content in internal tissues (Gil et al., 1997).

In recent years, edible coatings are used to improve fruits appearance and conservation. Edible coatings have been studied for extending shelf life of some fresh berry fruits and blueberries (Duan et al., 2011; Ribeiro et al., 2007; Vargas et al., 2008, Zhang & Quantick, 1998). Edible coatings may control the internal gas atmosphere of the fruit, may serve as a barrier to water vapor, reducing

moisture loss and delaying fruit quality losses (Baldwin et al., 1995). The interaction between the antioxidant capacity, anthocyanin and phenolic content and the use of edible coatings during storage of highbush blueberry has still not been investigated much.

Therefore, the objective of this work was to determine the effects of different coatings, alone or in conjunction, on antioxidant potential, anthocyanin and phenolic content and overall quality of highbush blueberry under commercial storage conditions.

Materials and methods

Fruit

Two early season cultivars of commercial importance, Berkeley and O'Neal, were chosen. 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 eliminated.

Preparation of coating solutions

Three different coatings were prepared. A 2% (w/v) acid-soluble chitosan (Sigma-Aldrich Co., Steinheim, Germany) coating solution was prepared by dissolving chitosan in 1% aqueous acetic acid with 50% glycerol and 0.15% Tween 20 (w/v) in according with Duan et al. (2011). The coating was homogenizing for 90 s and then storing overnight at room temperature. Blueberries samples were immersed in the chitosan solution for 2 min and air dried at room temperature for 30 min.

A 1.5% (w/v) sodium alginate (Sigma-Aldrich Co., Steinheim, Germany) solution was prepared dissolving the sodium alginate powder in distilled water upon stirring at 70°C during 2 h. Then the solution was cooled to 25°C according to Poverenov et al. (2013). Blueberries samples were immersed in the alginate solution for 2 min and then immersed in 5% aqueous solution of CaCl₂ for 2 min (Sigma-Aldrich Co., Steinheim, Germany) to perform gelation of alginate molecules by cross-linking. The samples were air dried at room temperature for 30 min.

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) according to Duan et al. (2011). Blueberries samples were immersed in the chitosan + sodium alginate solution for 2 min and air dried at room temperature for 30 min. A sample washed in water was used as control.

Packaging and storage conditions

A total of 100–130 g of blueberries were placed in polylactic acid (PLA, biodegradable film) punnets and wrapped automatically with PLA film (Compac, Reggio Emilia, Italy). The PLA film has an O₂ transmission rate of 40 cm³/m²/24 h at 23°C, a CO₂ transmission rate of 200 cm³/m²/24 h at 23°C and a moisture vapor transmission rate of 18 g/m²/24 h at 23°C and 85% R.H. For each treatment nine punnets were prepared. The packages were then stored at 0°C in dark for 45 days.

Anthocyanin contents, phenolic contents, total antioxidant capacity

To determine the anthocyanin contents, phenolic contents and total antioxidant capacity, extracts were prepared by weighing 10 g of berries into a centrifuge tube, adding methanol (25 ml) and homogenizing 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. Three extracts for each treatments at each evaluation point was made.

Anthocyanin content was quantified according to the pH differential method of Cheng & Breen (1991). Anthocyanins were estimated by their difference of absorbance at 510 and at 700 nm in buffer at pH 1.0 and at pH 4.5, where $A = (A_{515} - A_{700})_{pH1.0} - (A_{515} - A_{700})_{pH4.5}$. Results were expressed

as mg of cyanidin- 3-glucoside (C3G) per 100 g of fresh berries. The reported values are the mean±SD of nine replicates (Tables 1 and 2). Total phenolic contents were determined with the Folin– Ciocalteu reagent by the method of Slinkard & 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. The reported values are the mean±SD of nine replicates (Tables 1 and 2).

Antioxidant activity was determined using a modification (Deighton et al., 2000) of the ferric-reducing antioxidant power (FRAP) assay (Benzie & Strain, 1996). The antioxidant capacity of berry extract is determined by its ability to reduce ferric iron to ferrous iron in a solution of TPTZ prepared in sodium acetate at pH 3.6. The reduction of iron in the TPTZ-ferric chloride solution (FRAP reagent) results in the formation of a blue-colored product (ferrous tripyridyltriazine complex) and the absorbance of which is read spectrophotometrically at 593 nm, 4 min after the addition of berry extract to the FRAP reagent. Results were expressed as mmol Fe²⁺/kg of fresh berries. The reported values are the mean±SD of nine replicates (Tables 1 and 2).

Weight loss

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

Quality evaluations

Blueberries' physicochemical quality attributes were measured before cold storage (time 0) and then after 15, 30 and 45 days of storage. Total soluble solids content, pH and titratable acidity were measured using juice extracted from a 50 g (replicate) berries sample blended at high speed in a homogenizer. Three replicate were used for each treatment at each evaluation point. Soluble solids concentration was determined by a digital refractometer (Atago refractometer model PR-32, Tokyo, Japan) and the results were expressed as °Brix. Titratable acidity and pH were measured by titrating 1:10 diluted juice, using 0.1N NaOH and an automatic titrator (Compact 44–00, Crison, Barcelona, Spain). The reported values are the mean ±SD of three replicates (Tables 1 and 2).

Textural measurements were carried out before cold storage (time 0) and after 15, 30 and 45 days of storage. Berries samples for analysis were randomly selected and sample size was of 30 berries each testing, according to Doving et al. (2005). Each berry was considered a replicate. Before the analysis, samples were warmed to room temperature (20°C) for 3 h, 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, Godalming, UK). Measurements were performed in the equatorial part of the blueberry, at a cross-head speed of 3 mm/s and with a 3mm diameter punch (Chiabrando et al., 2009). A 5- kg load cell was used for firmness determination and the probe was programmed to penetrate 3mm into the blueberry. Fruit firmness was tested individually on 30 berries 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.

Color of coated berries was measured at day 0, 15, 30 and 45 of storage individually in each berry (30 for each treatments, each berry was considered a replicate). Surface color was analyzed with a tri-stimulus CR-400 Chroma Meter (Konica Minolta Sensing, Tokyo, Japan) with the illuminating D75 and observation angle of 10° calibrated with a standard white plate (Y=94.00, x=0.3158, y=0.3322). One readings of L* (lightness), b* (yellow chromaticity) and a* (green chromaticity) coordinates were recorded. The reported values are the mean±SD of 30 replicates (Tables 1 and 2).

Microbiological analysis

To evaluate the microbiological efficiency of the coatings, microbiological analyses of yeasts and molds 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 & Splittstoesser, 1992). Yeast and molds counts were performed using a chloramphenicol glucose agar (CGA) (ISO 21527, 2008). All the plates were incubated at room temperature for 3–5 d. Three replicates were analyzed and microbiological counts were expressed as colony forming units (CFU) of the sample (Table 3).

Statistical analysis

Data were analyzed by the analysis of variance, using statistical procedures of the STATISTICA ver. 6.0 (Statsoft Inc., Tulsa, OK). The source of variance is coating treatments. Tukey's HSP test (honest significant differences) was used to determine significant differences amongst treatment means. Mean values were considered significantly different at $p \leq 0.05$.

Results

Anthocyanin contents, phenolic contents, total antioxidant capacity

Mean values for antioxidant activity, total phenolic content and anthocyanin content for each cultivar and storage interval are shown in Tables 1 and 2. In both cultivars, anthocyanin content was higher in the first post-harvest time point compared to harvest with significant differences between coatings. In particular, in cv Berkeley, anthocyanin content of the alginate-coated berries and control berries was 42.23 (mg cyanidin 3-gluc/100 g FW) (highest) and 34.63 (mg cyanidin 3-gluc/100 g FW) (lowest), respectively (Table 1). At the end of storage period (45 days), all the coated blueberries showed significantly higher values compared with control berries. Changes in berry weight that occurred during the post-harvest period may have influenced the values for antioxidant activity, when expressed on a fresh weight basis. For instance, it is possible that a portion of the increase in these variables during storage was also due to water loss.

Total phenolic content showed different trend between samples during post-harvest storage period (Table 1). In alginate-coated samples, total phenolic content showed a slight increase during the first 15 days, exhibited a small peak at day 30, and then declined at the end of storage. In alginate + chitosan samples and chitosan-coated samples, total phenolic content decreased during the first 15 storage days, then increased to its maximum on day 30, after which decreased slightly during the last storage days. The same trend was also found by Pen & Jiang (2003) in chitosan coating Chinese water chestnut and Meng et al. (2008) in grape fruit. In control blueberries, total phenolic content remaining relatively unchanged during storage. Between treatments, chitosan-coated samples showed the highest total phenolic content values (Table 1).

Antioxidant activity values decreased continuously during storage period (Table 1). After 45 days of storage alginate-coated samples showed significantly lower antioxidant activity values compared with other samples. The application of chitosan coating delayed changes of contents of anthocyanin, total phenolics and antioxidant activity compared with other treatment in accordance with previous work (Zhang & Quantick, 1997).

| Quality parameter | treatments | days of storage | | | |
|---|------------|-----------------|-----------------|-----------------|-----------------|
| | | 0 | 15 | 30 | 45 |
| Firmness (N) | alginate | 2.2 ± 0.23 | 3.19 ± 0.63 a | 2.60 ± 0.61 a | 2.69 ± 0.63 a |
| | alg+chit | 2.2 ± 0.23 | 2.30 ± 0.68 bc | 2.34 ± 0.78 ab | 2.11 ± 0.68 b |
| | chitosan | 2.2 ± 0.23 | 1.95 ± 0.44 c | 1.79 ± 0.42 b | 1.73 ± 0.71 c |
| | control | 2.2 ± 0.23 | 2.75 ± 0.33 b | 2.71 ± 0.85 a | 2.51 ± 0.45 ab |
| T.S.S. (°Brix) | alginate | 10.5 ± 1.63 | 12 ± 1.53 b | 12.10 ± 1.25 a | 11.90 ± 1.88 ab |
| | alg+chit | 10.5 ± 1.63 | 11.20 ± 1.03 c | 12.00 ± 1.68 a | 11.40 ± 1.63 b |
| | chitosan | 10.5 ± 1.63 | 13.1 ± 1.22 a | 12.20 ± 1.32 a | 12.10 ± 1.05 a |
| | control | 10.5 ± 1.63 | 10.60 ± 1.35 d | 11.20 ± 1.47 b | 11.50 ± 1.21 ab |
| Tit. acidity (meq/l) | alginate | 81.46 ± 3.21 | 96.08 ± 3.33 b | 73.98 ± 3.13 b | 85.74 ± 3.15 a |
| | alg+chit | 81.46 ± 3.21 | 130.48 ± 3.22 a | 60.44 ± 3.21 c | 83.68 ± 2.63 a |
| | chitosan | 81.46 ± 3.21 | 81.16 ± 2.63 c | 72.54 ± 2.63 b | 76.98 ± 3.14 b |
| | control | 81.46 ± 3.21 | 101.54 ± 2.99 b | 80.76 ± 2.77 a | 76.66 ± 3.21 b |
| Lightness (L*) | alginate | 32.1 ± 1.23 | 30.28 ± 0.63 a | 31.42 ± 1.23 a | 31.66 ± 2.15 a |
| | alg+chit | 32.1 ± 1.23 | 21.50 ± 2.15 b | 24.05 ± 1.87 b | 22.99 ± 1.99 b |
| | chitosan | 32.1 ± 1.23 | 16.51 ± 1.90 c | 16.54 ± 1.22 c | 16.64 ± 1.43 c |
| | control | 32.1 ± 1.23 | 29.40 ± 1.88 a | 28.28 ± 1.33 a | 30.98 ± 2.15 a |
| Anthocyanin (mg cyanidin 3-gluc/100 g FW) | alginate | 21.3 ± 0.93 | 42.23 ± 2.13 a | 28.53 ± 1.43 a | 38.42 ± 2.19 a |
| | alg+chit | 21.3 ± 0.93 | 37.66 ± 1.99 b | 25.49 ± 1.63 b | 38.04 ± 2.14 a |
| | chitosan | 21.3 ± 0.93 | 38.80 ± 2.10 b | 12.55 ± 1.75 c | 39.56 ± 2.11 a |
| | control | 21.3 ± 0.93 | 34.62 ± .93 c | 24.35 ± 1.98 b | 30.05 ± 2.31 b |
| Polyphenol (mg gallic acid/100 g FW) | alginate | 297.00 ± 9.13 | 320.90 ± 9.22 a | 325.40 ± 8.98 a | 289.80 ± 7.33 b |
| | alg+chit | 297.00 ± 9.13 | 269.80 ± 8.63 c | 304.20 ± 8.53 b | 290.30 ± 7.34 b |
| | chitosan | 297.00 ± 9.13 | 258.20 ± 8.33 c | 307.00 ± 8.31 b | 300.50 ± 8.10 a |
| | control | 297.00 ± 9.13 | 289.70 ± 8.25 b | 272.30 ± 7.77 c | 282.50 ± 7.44 b |
| Antioxidant activity (mmol Fe ²⁺ /kg FW) | alginate | 18.41 ± 0.21 | 14.87 ± 0.33 b | 14.25 ± 0.24 | 13.58 ± 0.32 b |
| | alg+chit | 18.41 ± 0.21 | 14.36 ± 0.13 b | 15.04 ± 0.33 | 15.19 ± 0.42a |
| | chitosan | 18.41 ± 0.21 | 16.01 ± 0.22 a | 15.04 ± 0.36 | 15.19 ± 0.42 a |
| | control | 18.41 ± 0.21 | 14.59 ± 0.24 b | 14.84 ± 0.41 | 15.42 ± 0.40 a |

Tab. 1. Cv. Berkeley. Quality parameters of blueberry treated with different coatings during 45 storage days at 0°C.

*alginate= 1.5% sodium alginate coating, alg+chit= 1.5% chitosan and 1% sodium alginate coating, chitosan= 2% chitosan coating; control= without coating.

Values are the mean ± SD. Different letters in the same storage time means significantly different ($p \leq 0.05$). Storage time without letters means no significant differences.

In the cv O'Neal, as storage advanced, the levels of anthocyanin increased in both control and treated blueberries. This increase being significantly higher in the chitosan-treated berries compared to the other treatments. Chitosan-coated berries showed the highest values and control samples the lowest, in accordance with cv Berkeley. After 45 days of storage, chitosan coated samples also showed the highest values of total phenolic and antioxidant activity compared with other treatments. At the end

of storage period, total phenolic and antioxidant activity values exhibited a slight decrease in all the treatments, compared to the values found at time 0 (Table 2). According to Wang & Gao (2013) in strawberry, chitosan treatments retarded the decrease of phenolic content and antioxidant activity during cold storage period.

| Quality parameter | treatments | days of storage | | | |
|---|------------|-----------------|------------------|------------------|------------------|
| | | 0 | 15 | 30 | 45 |
| Firmness (N) | alginate | 1.73 ± 0.36 | 1.77 ± 0.38 b | 1.65 ± 0.21 b | 1.46 ± 0.34 a |
| | alg+chit | 1.73 ± 0.36 | 0.96 ± 0.35 c | 0.99 ± 0.49 c | 0.80 ± 0.45 b |
| | chitosan | 1.73 ± 0.36 | 2.43 ± 0.31 a | 1.58 ± 0.47 b | 1.27 ± 0.55 b |
| | control | 1.73 ± 0.36 | 1.69 ± 0.30 b | 1.90 ± 0.50 a | 1.48 ± 0.35 a |
| T.S.S. (°Brix) | alginate | 9.80 ± 0.98 | 9.50 ± 1.03 b | 9.10 ± 1.09 b | 9.40 ± 1.21 |
| | alg+chit | 9.80 ± 0.98 | 10.70 ± 1.12 b | 10.10 ± 1.11 a | 10.00 ± 1.11 |
| | chitosan | 9.80 ± 0.98 | 11.50 ± 1.10 a | 10.80 ± 1.21 a | 10.10 ± 1.11 |
| | control | 9.80 ± 0.98 | 9.80 ± 1.08 b | 9.30 ± 1.21 b | 9.50 ± 1.13 |
| Tit. acidity (meq/l) | alginate | 161.87 ± 1.35 | 118.86 ± 1.77 a | 148.90 ± 1.98 a | 102.62 ± 1.98 b |
| | alg+chit | 161.87 ± 1.35 | 71.00 ± 1.85 c | 101.08 ± 1.77 c | 66.16 ± 1.47 c |
| | chitosan | 161.87 ± 1.35 | 119.32 ± 1.89 a | 134.44 ± 1.75 b | 116.42 ± 1.96 b |
| | control | 161.87 ± 1.35 | 92.64 ± 1.85 b | 156.58 ± 1.85 a | 135.72 ± 1.97 a |
| Lightness (L*) | alginate | 34.35 ± 1.93 | 27.86 ± 1.33 a | 32.24 ± 1.44 a | 29.06 ± 1.98 a |
| | alg+chit | 34.35 ± 1.93 | 20.79 ± 1.05 b | 22.59 ± 1.54 b | 21.15 ± 1.97 b |
| | chitosan | 34.35 ± 1.93 | 13.55 ± 1.11 c | 16.71 ± 1.49 b | 16.79 ± 1.78 c |
| | control | 34.35 ± 1.93 | 26.77 ± 1.23 a | 31.78 ± 1.38 a | 28.08 ± 1.77 a |
| Anthocyanin (mg cyanidin 3-gluc/100 g FW) | alginate | 21.3 ± 0.91 | 39.93 ± 0.89 a | 38.04 ± 1.22 a | 36.52 ± 1.15 b |
| | alg+chit | 21.3 ± 0.91 | 36.90 ± 1.23 b | 29.67 ± 0.93 b | 31.57 ± 1.02 c |
| | chitosan | 21.3 ± 0.91 | 42.23 ± 1.78 a | 48.69 ± 2.15 a | 48.69 ± 0.83 a |
| | control | 21.3 ± 0.91 | 39.53 ± 1.23 a | 38.80 ± 1.88 a | 30.05 ± 0.89 c |
| Polyphenol (mg gallic acid/100 g FW) | alginate | 469.59 ± 10.81 | 301.06 ± 11.21 b | 300.79 ± 12.35 b | 317.99 ± 16.47 b |
| | alg+chit | 469.59 ± 10.81 | 290.31 ± 10.81 b | 311.01 ± 13.24 a | 355.35 ± 10.35 a |
| | chitosan | 469.59 ± 10.81 | 318.26 ± 13.81 a | 298.91 ± 13.54 b | 367.18 ± 10.98 a |
| | control | 469.59 ± 10.81 | 328.21 ± 10.65 a | 300.79 ± 15.16 b | 319.07 ± 12.25 b |
| Antioxidant activity (mmol Fe ²⁺ /kg FW) | alginate | 15.88 ± 0.18 | 15.11 ± 0.21 a | 16.17 ± 0.28 a | 14.66 ± 0.17 b |
| | alg+chit | 15.88 ± 0.18 | 15.18 ± 0.28 a | 14.35 ± 0.23 b | 14.93 ± 0.20 b |
| | chitosan | 15.88 ± 0.18 | 15.15 ± 0.17 a | 14.83 ± 0.17 b | 15.22 ± 0.20 a |
| | control | 15.88 ± 0.18 | 13.68 ± 0.25 b | 16.22 ± 0.19 a | 13.57 ± 0.23 b |

Tab. 2. Cv. O'Neal. Quality parameters of blueberry treated with different coatings during 45 storage days at 0°C.

*alginate= 1.5% sodium alginate coating, alg+chit= 1.5% chitosan and 1% sodium alginate coating, chitosan= 2% chitosan coating; control= without coating.

Values are the mean ± SD. Different letters in the same storage time means significantly different ($p \leq 0.05$). Storage time without letters means no significant differences.

Weight loss

All samples showed a progressive loss of weight during storage period. Loss of weight in fresh blueberries is mainly due to the loss of water caused by transpiration and respiration processes, which is determined by the gradient of water vapor pressure between the fruit and the surrounding air. In this work, observed weight losses of blueberries were about 3–5% during 45 storage days. These values are very low if compared with traditional storage condition, where the weight loss was about 15% after 9 weeks of storage (Chiabrande et al., 2006).

In cv Berkeley, after 45 days of storage, the weight losses of the alginate-coated berries and control berries were 3.72% (highest) and 3.09% (lowest), respectively. On the contrary, in cv O'Neal the highest weight loss was in alginate + chitosan (4.95%), the lowest was in alginate-coated berries (3.84%). Coating samples showed no significantly retarded in the weight loss of blueberry fruits during storage period.

Quality evaluations

In cv Berkeley, the total soluble solids content and the titratable acidity did not vary significantly among treated samples (Table 1). During storage, total soluble solids content showed a slight increase after 15 and 30 days of storage, and then declined at the end of storage period. Titratable acidity showed a peak after 15 days of storage, except for chitosan samples, and then remaining relatively unchanged. According to Valero et al. (2013), alginate edible coating delayed acidity losses.

In cv O'Neal, the total soluble solids content of blueberry fruits did not vary significantly during storage (Table 2). No significant differences were also detected among treatments. Berries samples showed high level of titratable acidity at harvest (161.87 meq/l) and then values decreased during storage period. In particular, in alginate + chitosan samples, the acidity changed from 161.87 meq/l to 66.26 meq/l. Titratable acidity values exhibited a high decrease, in particular, after 15 days of storage. Decrease in total acidity is typical during post-harvest storage of fruit and has been attributed to the use of organic acids as substrates for the respiratory metabolism (Kader & Ben-Yehoshua, 2000).

Changes in external colour of blueberries were monitored by measuring lightness (L^*). Table 1 reveals that in cv Berkeley, chitosan and chitosan + alginate-coated berries darkened as evidenced by decreasing values of L^* , which became significantly different after 15 days of storage. L^* value of chitosan samples decreased quickly during the first 15 days of storage and then remained relatively unchanged. Control and alginate-treated blueberries showed a very little decline in L^* values.

In cv O'Neal (Table 2), L^* values tended to decrease in all coated samples after 15 days of storage, and then increased during storage. Alginate-coated samples and control showed the lowest changes in L^* values during storage. In chitosan-treated blueberries, L^* values showed a significant decrease already after 15 days. Chitosan coatings led to a decrease in luminosity of samples. Changes in the surface reflection properties when the blueberries are coated can provoke this luminosity decrease. Hoagland & Parris (1996) reported that chitosan coating turned opaque during film formation at the final stage of drying, resulting in decreased L^* values.

Texture loss is the most noticeable change occurring in fruits during prolonged storage and it is related to metabolic changes and water content. Cv Berkeley was firmer compared with cv O'Neal, at harvest and throughout the post-harvest period (Tables 1 and 2). Between coatings, the use of alginate edible coating applied on blueberries showed a significant ($p \leq 0.05$) effect on keeping texture. Both alginate and alginate + chitosan treated blueberries showed beneficial results on firmness retention during the entire storage period. This result of alginate application is in accordance with Rojas-Grau et al. (2008) on fresh-cut apple and with Fan et al. (2009) in strawberry. Moreover, alginate edible coatings also slowed down the softening process in different plum cultivars during storage (Valero et al., 2013). Retention of firmness can be explained by retarded degradation of components responsible for

structural rigidity of the fruit, primarily the insoluble pectin and proto-pectin. Cv O’Neal showed low firmness values during storage (Table 2). Blueberries samples consistently maintained firmness measurements below 1.70 N, in particular alginate + chitosan coated samples. In alginate and control samples, firmness values remained relatively unchanged during the first 15 days of storage, and then declined slightly.

Microbiological analysis

The use of edible coating was effective in reducing microbial colony forming units during refrigerated storage period (Table 3). Chitosan edible coating showed significantly lower counts compared with the other treatments, in particular in controlling yeasts. Table 3 showed in cv Berkeley all the edible coatings applied on blueberries had a marked effect in reducing yeasts and molds counts as compared to the control. On the contrary, in cv O’Neal, alginate coating controlled better molds colony and chitosan coating yeasts proliferation. As stated by Olivas & Barbosa-Canovas (2005), coatings create a modified atmosphere that may change the growth rate of microorganisms. This modified atmosphere inhibits the growth of molds and yeast during post-harvest storage conditions.

| cv | treatments | | |
|----------|------------|----------------|---------------|
| | | yeasts (ufc/g) | molds (ufc/g) |
| Berckley | alginate | 55 | 82 |
| | alg+chit | 750 | 64 |
| | chitosan | <10 | 27 |
| | control | 470 | 600 |
| O'Neal | alginate | 170000 | <10 |
| | alg+chit | 15000 | 460 |
| | chitosan | <10 | 410 |
| | control | 8300 | 550 |

Tab. 3. Microbial counts (cfu g⁻¹) of blueberries treated with different coatings and stored for 45 days at 0°C

*alginate= 1.5% sodium alginate coating, alg+chit= 1.5% chitosan and 1% sodium alginate coating, chitosan= 2% chitosan coating; control= without coating.

Different letters in the same storage time means significantly different ($p \leq 0.05$). Storage time without letters means no significant differences.

Conclusion

Sodium alginate and chitosan edible coatings influenced the rate of changes in some chemical and physiological properties of the blueberries during storage. Coatings of berries slowed down the rate of deterioration and improved the post-harvest quality. The minimum loss of firmness was obtained in blueberries coated with sodium alginate. Sodium alginate-coated blueberries were also found to be less opaque, in term of L*, than those coated with chitosan. While it has been confirmed that the addition of chitosan to coatings decreases the microbial growth rate of the fruit and retarded the

decrease of phenolic content, anthocyanin and antioxidant activity. The maintenance of quality of blueberries by alginate coating revealed that such a coating can be considered for commercial application during storage and marketing. Alginate edible coatings can help maintain desirable quality characteristics of blueberries during storage. But, we recommend the application of chitosan coating to control microbial decay.

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