Effects of chitosan based coatings enriched with procyanidin by-product on quality of fresh blueberries during storage

Mannozzi C.[a*], Tylewicz, U.[a], Chinnici F.[a,b], Siroli L.[a], Rocculi P.[a,b], Dalla Rosa M.[a,b] and Romani S.[a,b]

[a] *Department of Agricultural and Food Sciences, University of Bologna, Cesena, Italy.*

[b] *Interdepartmental Centre for Agri-Food Industrial Research, University of Bologna, Cesena, Italy.*

*Corresponding author:

Cinzia Mannozzi, University of Bologna, Department of Agricultural and Food Sciences, p.zza Goidanich 60, 47521 Cesena (FC), Italy, e-mail: (cinzia.mannozzi2@unibo.it)

Abstract

The aim of this work was to evaluate the efficacy of an innovative edible coating, based on chitosan from mushrooms enriched with procyanidins extracted from grape seeds, on fresh blueberry quality maintenance, (weight loss, pH, dry matter, colour, firmness and antioxidant activity) and microbial growth, during 14 days of storage at 4° C.

For weight loss, pH and dry matter no relevant differences were detected among the control and the differently coated samples at each considered storage time. Chitosan and chitosan + procyanidins coatings promoted a slight decrease of luminosity and an increase of blue hue colour of blueberry samples during the whole storage period. The use of coating promoted an increase in the antiradical activity that was the highest in blueberries coated with chitosan + procyanidins. Microbiological analysis results indicated that the chitosan-based coated samples had a significantly higher yeast and mould growth inhibition compared to the uncoated sample.

Keywords Edible coating, chitosan, procyanidins, blueberries, antioxidant activity

1. Introduction

Blueberries are increasingly appreciated for their rich composition in flavonoids, phenolic acids, tannins and anthocyanins giving them a great nutritional value. Anthocyanins are natural pigments, largely distributed in nature and generally present in many fruit and vegetables. In particular, berries demonstrated to have a great antioxidant activity, due to their high content in phenolic acids and flavonoids, which can cause a strong antioxidant capacity in different products (Pellegrini et al., 2003). In addition, phenolic compounds may exert beneficial effects on human health associated with the consumption of fruit and vegetables (Cheynier, 2012).

However, fresh fruits deteriorate rapidly due to loss of water and cellular juice (product of superficial lesions), senescence, mould growth and/or putrefaction phenomena (Yang et al., 2014). Moreover, bioactive compounds are prone to alterative oxidative reactions, which can negatively affect phenolic levels and antioxidant capacity in berry fruits during post-harvest storage (Connor, Luby, Hancock, Berkheimer, & Hanson, 2002). Physical deteriorations that occur during postharvest storage of blueberries are mainly due to loss of firmness and microbial decay (Li, Luo, & MacLean, 2011).

Different technologies have been used in order to delay the fruit deterioration and to extend their shelf-life such as refrigeration, modified atmosphere packaging and UV irradiation (Chiabrando & Giacalone, 2011; Yang, et al., 2014).

The use of edible films or coatings represents an alternative and/or additional way for fruit preservation, because of their ability to reduce moisture, solute migration, respiration and transpiration rate, to maintain firmness and generally delay senescence (Tezotto-Uliana, Fargoni, Geerdink & Kluge, 2014).

In order to improve the efficiency and stability of edible coatings it is essential to find adequate composition of their formulations. The basic coating ingredients are polysaccharides, proteins and lipids, either as pure substances or in combination. Edible coatings have high potential to carry active and functional ingredients such as antimicrobial, antioxidant and antibrowning agents, colorants, nutrients that can enhance the nutritional values and the stability of products during their shelf-life (Rojas-Graü, Tapia, & Martín-Belloso, 2008).

Chitosan (poly β-(1,4)N-acetyl-D-glucosamine) polymer is industrially produced from chemical deacetylation of the chitin found in exoskeletons of crustaceans. This biopolymer can also be extracted from the cell wall of mushrooms, being biodegradable, non-toxic and non-allergenic, which contribute to its use in many fields, including food, biomedicine, agriculture and environmental protection (Shahidi, Arachchi, & Jeon, 1999; Kim & Rajapakse, 2005). Moreover, it has been shown to have mechanical and antimicrobial properties, no toxicity, biodegradability and to inhibit the growth of fungi on the surface of different fruits (Rojas-Graü et al., 2008; Treviño□Garza, García, del Socorro Flores□González, & Arévalo□Niño, 2015).

Procyanidins are one of the most abundant flavonoids present in grape seeds and skin. They are mainly proanthocyanidins (condensed tannins) mostly constituted of oligomeric flavonoids as catechin, epicatechin, epicatechin gallate and epigallocatechin (Souquet, Cheynier, Brossaud, & Moutounet, 1996). During food processing and storage, plant phenolic compounds are converted to a variety of reaction products that could contribute to the quality of plant-based foods, along with the genuine plant components (Cheynier, 2012). Moreover, these bioactive compounds can be used to add value and to improve the nutritional functions of numerous foodstuffs (dos Reis, de Oliveira, Hagen, Jablonski, Flôres, & de Oliveira Rios, 2015; Rodriguez-Amaya, 2016; Martin & Ferreira, 2017). A lot of by-products from food processing could be a good source for the recovery of polyphenols, protein and pectin, that can be used as natural ingredients and or additive in food production (Kammerer, Kammerer, Valet, & Carle, 2014; Martins et al., 2017).

Nair, Saxena & Kaur (2018) investigated the effect of chitosan and alginate based coatings enriched with pomegranate peel extract, showing that chitosan based coatings was more effective than alginate in maintaining the postharvest quality of guava (*Psidium Guajava L.*). However, to the best of our knowledge, investigations on the influence of coatings based on chitosan from mushrooms alone or enriched with procyanidins, extracted from grape by-product, on fruit or vegetables quality have not been reported yet Thus, the main aim of this research work was to evaluate the effect of the application of specific innovative coatings on some quality characteristics (weight loss, pH, dry matter, colour and firmness), antioxidant activity (ABTS and DPPH assays) and microbial growth of blueberry samples during storage at 4°C for 14 days.

2. Material and methods

2.1 Fruit material

Organic blueberries were purchased from local market. Berry fruits were kept for one day at 0 ± 1°C until they were used. Fresh blueberries with similar colour and size and no damages were selected and these berries were characterized by dry matter of 15.1 ± 0.3 g/100g.

2.2 Preparation of coating solutions

Two different coating solutions were prepared, each of them contained 1.5 % (w/w) of glycerol (≥ 99.5% Sigma-Aldrich, Germany) and 0.20 % (w/w) of Tween® 20 (Sigma-Aldrich, France) and solved in citric acid solution 1% (Sigma- Aldrich, Germany). In a first solution, chitosan from mushrooms (C) provided by Agrovin (Alcazar de San Juan, Spain) was added in the quantity of 1 % (w/w). The second coating solution was prepared by combining chitosan from mushroom (1% w/w) and procyanidins extracted from grape seeds (Chardonnay berries) (0.8 % w/w) (CP). The extraction of procyanidins was performed as follows: briefly, 200 g of dehydrated seeds were extracted with water-ethanol (1:1 w/w) for 2 hours under stirring at 200 rpm. Extracts were rotary evaporated under vacuum at 35°C to remove ethanol. The resulting extracts were washed with hexane to remove lipid-soluble substances, and then rotary evaporated to remove the residual hexane. The aqueous fraction (about 75 mL) was applied to a Diaion HP-20 column (70×500 mm) previously equilibrated with water, and rinsed with 10% ethanol. Procyanidins were eluted using 100 mL water-ethanol 30:70 w/w, spray dried and stored at -30°C before their use.

The final concentration of procyanidins used for coating solution was chosen based on the higher antioxidant activity and unchanged sensorial properties of fruit tested in preliminary trials by trained panel (data not showed). Afterwards, all coating solutions were homogenised at 5000 rpm for 2 min in order to remove air bubbles.

2.3 Sample preparation

Blueberry fruits were surface disinfected by immersion in 200 ppm sodium hypochlorite water solution; successively they were washed in distilled water and dried on the surface with absorbing paper. Whole blueberry fruits were dipped in the coating solutions in two different steps (each one of 30 s), the first dipping was followed by drying step for 60 min at 25 ± 1 °C and the second one for 30 min at the same temperature. Blueberries dipped in distilled water with the same procedure were used as control. Afterwards, coated berry samples were placed in plastic trays (PET), closed in micro-perforated bags (PLA) to maintain aerobic conditions limiting fruit dehydration, and stored at 4°C for 14 days. All blueberries samples were analysed at 0, 2, 4, 6, 10 and 14 days of storage. Three samples were obtained as a total: 2 differently coated blueberry samples (C and CP) and one uncoated sample (F). For each sample, 720 berries were used. For every sampling time 3 trays were prepared, containing 40 blueberries randomly categorized and used for analytical determinations.

2.4 Quality determinations

2.4.1 Weight loss, dry matter and pH

Weight loss (WL) of blueberry samples during 14 days of storage was measured by weighting fruits in all trays per sample at the beginning of the storage and at every day of analysis; the results were calculated as percentage loss of initial weight, following the standard AOAC method (1994).
Dry matter (DM) was determined gravimetrically by difference in weight before and after drying at 70 °C, until constant weight was reached (AOAC International, 2002).
pH was measured at 20 °C with a pH meter CRISON GLP21 (Shinghai Shilu-Instruments, China).
For all treatment times and for each sample, DM was determined in triplicate from 9 blueberries and pH was measured also in triplicate on the three different juice sub-samples obtained from 15 berries (fruit:water 1:1).

2.4.2 Colour

Surface colour of blueberry fruits, were measured using a spectrophotocolorimeter HUNTERLAB ColorFlexTM, mod. A60-1010-615 (Reston, Virginia). For each sample, L*, a* and b* parameters from CIELAB scale were measured. Hue angle (h°), which is the hue in the CIELAB colour wheel, was calculated by the following equation:

$$h° = \tan^{-1} \frac{b^*}{a^*} \quad (1)$$

where: a* (red–green) and b* (yellow–blue) are parameters of colour measurement (Vega-Gálvez et al., 2012).

The analyses were carried out in twelve repetitions from randomly selected blueberries from each sample at each storage day.

2.4.3 Texture

Firmness evaluation was conducted with penetration test by means of Texture Analyser mod. TA-HDi500 (Stable Micro Systems, Surrey, Godalming, UK), equipped with a 50 N load cell and a 2 mm diameter stainless steel probe. Test speed was 0.5 mm s^{-1} and ended when a maximum deformation of 80% was reached. Results were expressed as average of twelve measurements performed on twelve blueberries from each sample at each storage day.

2.4.4 Antiradical activity (DPPH, ABTS assays)

The extraction was performed by mixing 0.5 g of freeze-dried sample whit 10 mL of methanol 60% (w/w) in centrifuge tube. The mixture was vortexed for 2 min, agitated for 10 min and centrifuged for 10 min at 18600 rpm in a centrifuge (Beckman) set at 4°C. The supernatants were collected and used to evaluate the antiradical activity by DPPH and ABTS assays.

The DPPH scavenging activity was based on the method proposed by Amarowicz, Naczk, & Shahidi (2000). Briefly, 0.1 mL of extract was added to 2 mL of methanol and 0.25 mL of DPPH (Sigma-Aldrich, USA), shaken with a vortex for 1 min and kept to the dark for 30 min. The absorbance was measured with a spectrophotometer (Beckman Coulter DU 730 Life Science model) at 517 nm. Antioxidant activity was quantified by plotting a Trolox calibration curve. Trolox concentration range was 0.001-1.500 mM (r^2 = 0.9980). The results were expressed as mmol Trolox/g of fruit.

The $ABTS^{+\bullet}$ scavenging activity was carried out following the method proposed by Re, Pellegrini, Proteggente, Pannala, Yang, & Rice-Evans (1999). 30 μL of extract were added to 3 mL of diluted $ABTS^{+\bullet}$ solution (Sigma-Aldrich, USA) after mixing and the absorbance was measured with a spectrophotometer (Beckman Coulter DU 730 Life Science model) at 734 nm every 30 s for a total time of 6 min; the results were expressed as mmol Trolox/g of fruit. Antioxidant activity was quantified by plotting a Trolox calibration curve. Trolox concentration range was 0.001-1.500 mM (r^2 = 0.9853).

The values provided are the average of three replicates from each sample at each day of storage.

2.4.5 Microbiological analysis

The cell loads of mesophylic aerobic bacteria, lactic acid bacteria, yeasts, moulds and total coliforms were monitored in all samples over the storage, according to the method reported by Mannozzi et al. (2016). The values obtained are the average of three independent sub-samples for each sample.

2.5 Data analyses

Analysis of variance (ANOVA) and the test of mean comparison, according to Fisher's least significant difference (LSD) were carried out on analytical replicates for F, C and CP blueberry samples. Level of significance was $p < 0.05$. The statistical software used was STATISTICA v 8.0 (StatSoft, Tulsa, Okhlaoma).

3. Results and discussion

3.1 Weight loss, dry matter and pH

The weight loss, dry matter and pH values of F and differently coated samples during 14 days of storage are reported in Table 1. All the samples underwent a similar decrease of the weight during cold storage (around 4.5%); this could be due to the migration of water from the fruit to the environment. The weight loss of fruit and vegetables is due to the water vapour pressure gradient that exists from different compartments in the cell tissues (Yaman & Bayoındırlı, 2002). This result was in agreement with Carvalho et al. (2016), who observed that the use of chitosan based coating with trans-cinnamaldehyde was not able to reduce the weight loss of fresh-cut melon during 20 days of storage. Moreover, Mannozzi et al. (2016) observed a progressive decrease of weight loss, without seeing any significant differences between uncoated and differently coated (polysaccharide-based coating) blueberry samples during storage.
For what concern the dry matter (Table 1), no relevant differences ($p < 0.05$) were found between C and CP coated samples during the overall storage. In particular, only F sample underwent a slight decrease of dry matter during 14 days of storage. The tendency to an increase of dry matter showed by CP sample during storage could be due to the solutes gain caused by the presence of coatings (Carvalho, et al., 2016).
As reported in Table 1, F samples showed, in general, a decrease in pH already after 2 days of storage in comparison to C and CP samples. However, all the blueberry samples showed a slight decreasing trend, even though not significant, of the pH during the overall storage. This is probably due to the greater loss of water and also it is possible that the loss of weight (up to 4 %) that occurred during the postharvest period influenced these values (Hernández-Muñoz, Almenar, Del Valle, Velez, & Gavara, 2008; Chiabrando et al., 2011)

3.2 Colour

Anthocyanins and other pigments derived from phenolic compounds are responsible for the colour of red fruit and wines (Cheynier, 2012). Table 2 reported the lightness (L*), a*, b*and hue angle (h°) values of control and coated blueberry samples during 14 days of storage at 4 °C.
Immediately after coating (T0) C blueberry samples displayed lower L* values than the F and CP ones. The observed lower lightness of chitosan coated blueberry is probably due to the presence of coating that caused changes in the surface properties (Hoagland & Parris, 1996). However, this behaviour has not been observed in CP coated blueberries probably due to the presence of procyanidins.

In C and CP coated blueberry samples a significant decrease of a* values ($p<0.05$) until the 6th day of storage was observed, then the values increased again. For the b* values, both coated blueberry samples exhibited higher values compared to the F one during the overall storage. C blueberry coated sample displayed significantly higher b* values ($p<0.05$) in comparison to CP sample starting from the 2nd day of storage.

The h° values for all blueberry samples tended to decrease significantly ($p<0.05$) mostly during the first six days of storage, after this time the values raised again. The reduction of hue colour could be due to the oxidation reactions between polyphenol compounds that can cause loss of anthocyanins during cold storage of blueberry (Reque, Steffens, Jablonski, Flôres, Rios, & de Jong, 2014). Castañeda-Ovando, de Lourdes Pacheco-Hernández, Páez-Hernández, Rodríguez, & Galán-Vidal (2009) reported that the increased of the polymeric colour is probably due to the co-pigmentation phenomenon which promotes the formation of polymers occurred from the condensation of anthocyanins and other phenolic compounds and also the increase of hue values at the end of storage might be caused by a possible anthocyanins synthesis during ripening.

The h° results are in agreement with those observed by Mannozzi, et al. (2016) who studied the effects of different polysaccharide based coatings such as alginate, pectin and the combination of them on blueberry fruits. In fact, also in their work h° values are highest for all coated blueberry samples compared to control one. However, h° values were in the range from 140 to 179 for all coated blueberry samples, this discrepancy could be explained by the different biopolymer used into the coatings and also strongly depends on the raw materials properties.

3.3 Texture

Firmness is one of the most important critical quality parameter that influences the consumer acceptability of fresh products. As shown in Fig. 1, in general, C and CP coated blueberry samples exhibited a higher ($p < 0.05$) firmness in comparison to F sample, immediately after coating at 0 day of storage, which can be explained by the presence of coatings that provide rigidity to the skin of fruit (Duan, Wu, Strik, & Zhao, 2011). Generally, during the overall storage all the blueberry samples maintained similar texture values. However, coated samples showed significantly ($p< 0.05$) higher values immediately after coating (T0) and 10^{th} day of storage, compared to the uncoated ones. Moreover, the higher firmness of coated blueberry samples could be explained by the thickness of the two different coating formulations. In fact, thickness of C and CP coated blueberries measured in preliminary trials, ranged from 84 to 130 µm respectively.

The added procyanidins induced an increase in thickness and thus created more compact structure of enriched coating formulation compared to chitosan one. In fact the procyanidins that might create a bridge between chitosan and their free functional groups in the molecular structure (Zhang, Yang, Tang, Hu & Zou, 2008).

Blueberries are usually subjected to loss of firmness during postharvest, which subsequently tends to decrease fruit quality and shelf life (Li et al., 2011). Previous works showed that edible coatings were able to increase/improve firmness maintenance of blueberries (Duan et al., 2011; Mannozzi, et al., 2016). In general, it is expected that water loss leads to raise firmness during postharvest storage (Chiabrando et al., 2011). It has been well established that the loss of firmness is due to enzymatic hydrolysis of the cell wall and also due to the cell turgor loss promoted by transpiration, that cause softening of the fresh fruit tissues. Moreover, Yaman et al. (2002) reported that coated cherries better retain the firmness values when stored at cold storage temperature, as obviously expected.

3.4 Antiradical activity (DPPH, ABTS assays)

Blueberry fruits have a high antioxidant activity, especially due to their natural phenolic compounds and anthocyanin content, and for this reason could be one of the uppermost antioxidant resources among fruits and vegetables (Cheynier, 2012).

DPPH method seems to be more prone to detect flavanones, while ABTS method seems to be more suitable to detect the radical scavengers such as vitamin C (Del Caro, Piga, Vacca, & Agabbio, 2004). Nevertheless, these two methods are a useful tool to determine the antiradical scavenging activity of different fruits (Gil, Tomás-Barberán, Hess-Pierce, Holcroft, & Kader, 2000).

In Figure 2, the results of antioxidant activity, obtained with DPPH and ABTS antiradical activity methods, of uncoated and differently coated blueberries during storage are showed.

The antioxidant activity of blueberry fruits detected by using DPPH method was lower compared to that obtained with the radical ABTS. Despite DPPH scavenging activity is recommended as accurate and simple method for the detection of antioxidant activity of fruit and vegetable, it is less sensitive to the activity of hydrophilic antioxidant compounds (Gil et al., 2000).

Under both the analytical methods, the CP coated blueberries showed a higher antioxidant activity already at 0 day, in comparison to the C and the fresh ones. Its better retention during the overall storage period is probably due to the presence of chitosan and procyanidins in the coatings that provide the enhancement of antioxidant compounds. The use of procyanidins from grape by-products induced an improvement of the nutritional value of coated blueberry fruit. Moreover, all blueberry samples showed similar behaviour, with DPPH and ABTS antiradical activity method. It was possible to observe significant increase in antioxidant activity in C coated sample at 6th and 10th day with respectively ABTS and DPPH methods. This is probably due to the anthocyanins synthesis that occurs during ripening stage (Kalt, Forney, Martin, & Prior, 1999); these results are in accordance with h° colour data. For both analytical methods, studied C and CP based coatings were able to delay the loss of antioxidant compounds. Chiabrando & Giacalone (2015) reported similar results whit the application of chitosan on blueberries during 45 days of storage at 0 °C.

3.5 Microbiological analysis

In Table 3, the cell loads of total mesophilic aerobic bacteria, mould and yeasts during the storage at 4 °C are reported. The chitosan coated samples (C) showed a significant lower cell load of mesophilic bacteria at the 1st day of storage compared to the other samples. However, at the 4th day of storage a decrease of mesophilic aerobic bacteria was detected in all the considered samples and without significant differences between them. At the end of storage (T14), an increase of the mesophilic bacteria was detected for all the considered conditions without significant differences. However, the detected cell loads, except for samples F and CP immediately after treatments never exceeded a cell load of 3.0 log cfu/g.

As shown in Table 3, yeasts resulted significantly lower in samples C and CP immediately after treatments. During storage, CP samples showed yeast loads not significantly different in comparison to the samples F. Contrarily, yeast loads in samples C resulted significantly lower than control samples during the whole period of refrigerated storage, and after six days resulted under the detection limit. A similar trend was evidenced for mould cell loads (Table 4). Lactic acid bacteria and total coliform cell loads resulted under the detection limit, independently from the coating adopted, during the whole storage period (data not shown).

The microbiological results obtained showed that all the considered samples did not reach a significant microbial spoilage during 14 days of storage at 4 °C (FSA of Ireland, 2016). On the other hand, it is widely reported that berries are rich in phenolic compounds that can have an antimicrobial activity (Lacombe, Wu, Tyler, & Edwards, 2010). In particular, Lacombe, Wu, White, Tadepalli, & Andre (2012) showed a strong antimicrobial activity of phenolic compounds from North American lowbush blueberries against the growth of *E. coli O157:H7*. Moreover, Shen et al. (2014) showed a significant growth inhibition of *Listeria monocytogenes* to blueberry extracts from 4 different cultivars, indicating the potential of blueberry as natural antimicrobials in food products.

In addition, the obtained results showed, even if the microbial spoilage threshold (>10^6 cfu/g for yeast, and >$10^7/10^8$ cfu/g mesophylic aerobic bacteria) (FSA of Ireland, 2016) was not reached in all the considered samples, that in samples C there was a significant higher yeast and moulds inhibition compared to the other samples. These results are in agreement with other studies that evidenced the antimicrobial and antifungal activity of pectin, alginate and chitosan coatings on blueberry (Duan et al., 2011; Jiang, Sun, Jia, Wang, & Huang, 2016; Mannozzi et al., 2016).

4. Conclusions

The used innovative coatings (chitosan and chitosan+procyanidin) showed a positive effect mainly on maintaining the firmness and increasing the antioxidant activity (DPPH and ABTS methods) of blueberry samples. The use of procyanidins from grape by-product contributed to add value of coated organic blueberry fruit. In addition, the obtained results showed, even if the microbial spoilage threshold was not reached in all the considered samples, that the chitosan-based coated samples had a significant higher yeast and moulds inhibition compared to the uncoated ones. In general results from this study demonstrated the efficacy of the new type of coating ingredients (chitosan alone and with natural procyanidins) to maintain the overall quality of fresh blueberries during storage. Up to now, the use of chitosan is not allowed by the

European regulation for organic production. However, obtained results could help to develop a new regulation that could consider the use of chitosan extracted from mushrooms as a valid opportunity for its application on organic fruits, since it is not a potential allergenic compound as happen for the one extracted from crustaceans (Vo & Kim, 2014).

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Figure captions

Fig. 1. Firmness (N) of uncoated (F) and coated blueberry samples (C and CP) during 14 days of storage at 4°C.

Means with different lowercase letters means significant difference ($p<0.05$) during time (days, in columns) and with capital letters means significant difference ($p<0.05$) between samples at each day of storage (in rows).

Fig. 2. Antiradical activity with DPPH method (▲) and ABTS method (■) of uncoated (F) and coated blueberry samples (C and CP) during 14 days of storage at 4°C.

Means with different lowercase letters means significant difference ($p<0.05$) during time (days, in columns) and with capital letters means significant difference ($p<0.05$) between samples at each day of storage (in rows).

Table 1. Weight loss (%), dry matter (%) and pH of uncoated (F) and coated blueberry samples (C and CP) during 14 days of storage at 4°C.

Weight loss (%)					
	T2	**T4**	**T6**	**T10**	**T14**
F	-0.89 ± 0.03^{aA}	-1.23 ± 0.06^{aA}	-2.1 ± 0.4^{bA}	-3.80 ± 0.06^{cA}	-4.5 ± 0.3^{dA}
C	-0.87 ± 0.06^{aA}	-1.42 ± 0.05^{bA}	-2.1 ± 0.2^{cA}	-3.5 ± 0.1^{dA}	-4.5 ± 0.1^{eA}
CP	-0.8 ± 0.2^{aA}	-1.2 ± 0.3^{aA}	-2.37 ± 0.04^{bA}	-3.2 ± 0.4^{cA}	-4.4 ± 0.4^{dA}

Dry matter (%)						
	T0	**T2**	**T4**	**T6**	**T10**	**T14**
F	15.1 ± 0.1^{aA}	15.1 ± 0.1^{aA}	14.42 ± 0.09^{cB}	15.50 ± 0.02^{aA}	15.1 ± 0.3^{aA}	14.5 ± 0.2^{bB}
C	14.8 ± 0.7^{bcB}	14.8 ± 0.7^{bcA}	15.8 ± 0.2^{abA}	15.7 ± 0.1^{bA}	15.9 ± 0.2^{aA}	14.6 ± 0.1^{cB}
CP	15.0 ± 0.7^{aA}	15.04 ± 0.04^{aA}	15.5 ± 0.9^{aA}	15.0 ± 0.6^{aA}	15.46 ± 0.05^{aA}	15.34 ± 0.03^{aA}

pH						
	T0	**T2**	**T4**	**T6**	**T10**	**T14**
F	3.43 ± 0.09^{aA}	3.16 ± 0.05^{cB}	3.22 ± 0.05^{bcB}	3.26 ± 0.04^{bA}	3.19 ± 0.03^{bcA}	3.29 ± 0.08^{aA}
C	3.33 ± 0.11^{aA}	3.35 ± 0.07^{aA}	3.36 ± 0.02^{aA}	3.32 ± 0.05^{aA}	3.34 ± 0.09^{aA}	3.40 ± 0.18^{aA}
CP	3.39 ± 0.23^{aA}	3.29 ± 0.15^{aAB}	3.42 ± 0.09^{aB}	3.24 ± 0.08^{aA}	3.30 ± 0.09^{aA}	3.27 ± 0.10^{aA}

Data are reported as average values and standard deviations.

Means followed by different lowercase letters means significant different ($p<0.05$) during time (days, in rows) and with capital letters means significant difference ($p<0.05$) between samples at each day of storage (in columns).

Table 2. Lightness (L*), a*, b* and hue angle (h°) values of uncoated (F) and coated blueberry samples (C and CP) during 14 days of storage at 4 °C.

	T0	T2	T4	T6	T10	T14
L*						
F	24.4 ± 0.3^{bcA}	25 ± 1^{bA}	24.8 ± 0.3^{bA}	23.6 ± 0.8^{cA}	24.5 ± 0.6^{cA}	26.1 ± 0.5^{aA}
C	17.80 ± 0.03^{dC}	19.2 ± 0.2^{cC}	16.67 ± 0.5^{eC}	17.5 ± 0.1^{dC}	20.1 ± 0.1^{bC}	20.9 ± 0.4^{aB}
CP	23 ± 1^{bB}	23.9 ± 0.2^{bB}	20.6 ± 0.3^{dB}	21.7 ± 0.2^{cB}	21 ± 1^{cB}	26.2 ± 0.6^{aA}
a*						
F	-0.2 ± 0.1^{aB}	-0.6 ± 0.1^{bB}	-0.87 ± 0.04^{cA}	-0.7 ± 0.2^{cA}	-0.72 ± 0.04^{bcB}	-0.70 ± 0.09^{bcA}
C	0.46 ± 0.07^{aA}	-0.45 ± 0.07^{cB}	-1.0 ± 0.1^{dA}	-0.9 ± 0.4^{dAB}	-0.1 ± 0.2^{bA}	-0.5 ± 0.1^{bcA}
CP	-0.06 ± 0.06^{aB}	-0.27 ± 0.05^{bA}	-0.97 ± 0.06^{dA}	-1.1 ± 0.1^{dB}	-0.2 ± 0.1^{abA}	-0.5 ± 0.1^{cA}
b*						
F	-4.28 ± 0.06^{cB}	-5.11 ± 0.09^{dC}	-4.2 ± 0.2^{bcC}	-3.2 ± 0.3^{aC}	-3.9 ± 0.1^{bC}	-4.1 ± 0.2^{bcC}
C	-2.7 ± 0.2^{cA}	-1.8 ± 0.6^{bA}	-1.9 ± 0.1^{bA}	-0.7 ± 0.4^{aA}	-1.5 ± 0.2^{bA}	-1.6 ± 0.1^{bA}
CP	-2.8 ± 0.2^{bcA}	-3.11 ± 0.08^{cB}	-3.2 ± 0.2^{cB}	-2.6 ± 0.4^{abB}	-2.5 ± 0.1^{aB}	-2.6 ± 0.4^{abB}
h°						
F	88 ± 6^{aB}	83 ± 4^{bAB}	78 ± 4^{cdA}	76 ± 11^{dAB}	80 ± 7^{cB}	80 ± 5^{cA}
C	102 ± 15^{bA}	78 ± 10^{bB}	66 ± 9^{aC}	79 ± 12^{bA}	82 ± 14^{bB}	81 ± 14^{bA}
CP	89 ± 14^{aB}	87 ± 23^{aA}	73 ± 7^{cB}	71 ± 9^{cB}	86 ± 9^{abA}	80 ± 8^{bA}

Data are reported as average values and standard deviations.

Means followed by different lowercase letters means significant different ($p<0.05$) during time (days, in rows) and with capital letters means significant difference ($p<0.05$) between samples at each day of storage (in columns).

Table 3. Mesophylic aerobic bacteria, yeast and mould count of uncoated (F) and coated blueberry samples (C and CP) during 14 days of refrigerated storage at 4 °C

	T0	T2	T4	T6	T10	T14
Mesophylic aerobic bacteria						
F	3.31 ± 0.18^{aA}	2.79 ± 0.19^{bA}	2.11 ± 0.31^{cA}	2.12 ± 0.18^{cA}	2.18 ± 0.33^{cA}	2.97 ± 0.24^{abA}
C	2.70 ± 0.22^{abB}	2.49 ± 0.13^{bcA}	2.41 ± 0.25^{bcdAB}	2.04 ± 0.23^{dA}	2.12 ± 0.14^{cdA}	2.96 ± 0.26^{aA}
CP	3.34 ± 0.21^{aA}	2.75 ± 0.24^{bA}	2.50 ± 0.15^{bB}	2.57 ± 0.17^{bB}	2.70 ± 0.24^{bB}	2.89 ± 0.31^{bA}
Yeast						
F	3.61 ± 0.33^{aA}	2.97 ± 0.26^{bA}	2.65 ± 0.31^{bA}	1.68 ± 0.33^{cA}	nd*	1.57 ± 0.25^{cA}
C	2.85 ± 0.21^{aB}	2.27 ± 0.31^{bB}	2.06 ± 0.24^{bB}	nd*	nd*	nd*
CP	3.12 ± 0.18^{aB}	2.53 ± 0.24^{bAB}	2.18 ± 0.12^{bcAB}	1.29 ± 0.26^{dA}	nd*	1.87 ± 0.14^{cA}
Mould						
F	2.39 ± 0.38^{aAB}	1.73 ± 0.26^{bA}	nd*	1.47 ± 0.19^{bA}	1.30 ± 0.22^{bB}	nd*
C	2.03 ± 0.17^{aB}	1.53 ± 0.15^{bA}	nd*	nd*	1.16 ± 0.27^{bB}	nd*
CP	2.82 ± 0.25^{aA}	1.81 ± 0.22^{bcA}	1.18 ± 0.24^{d}	1.64 ± 0.23^{cA}	2.07 ± 0.17^{bcA}	1.18 ± 0.23^{d}

Counts are expressed in Log cfu/g (± standard deviation).

Means followed by different lowercase letters means significant different ($p<0.05$) during time (days, in rows) and with capital letters means significant difference ($p<0.05$) between samples at each day of storage (in columns).

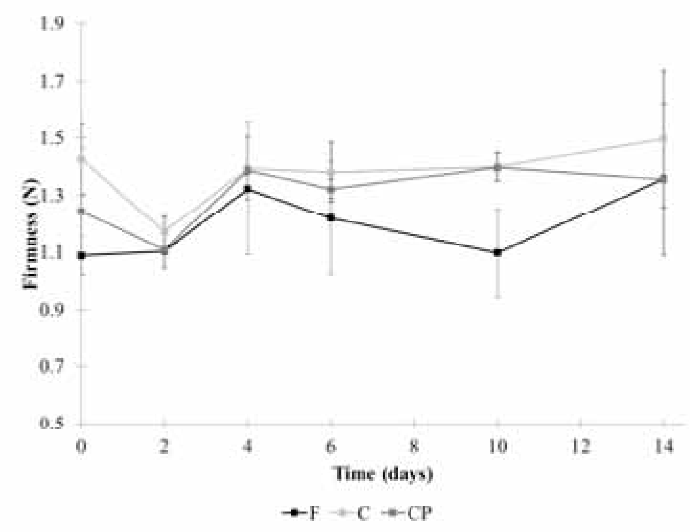
* under the detection limit (1 Log cfu/g)

Highlights

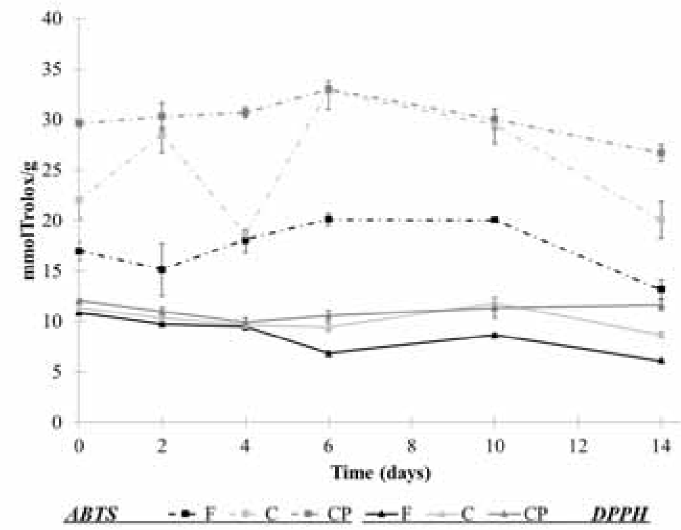
Quality parameters were maintained after the application of chitosan coatings

Procyanidin by-products enhanced the antioxidant activity of fresh blueberries

Chitosan coating of blueberries delayed the yeast and mold growth during storage



Time	F	C	CP
0	d C	b A	bc B
2	cd A	c A	c A
4	a A	b A	ab A
6	bc A	b A	b A
10	cd B	b A	a A
14	b A	a A	b A



ABTS

Time	F	C	CP
0	bc C	d B	abc A
2	cd B	b A	bc A
4	ab B	c B	ab A
6	a B	a A	a A
10	a B	b A	ab A
14	d C	c B	c A

DPPH

Time	F	C	CP
0	a B	b B	a A
2	b C	c B	ab A
4	b A	d A	c A
6	d C	d B	bc A
10	c B	a A	ab A
14	c C	c B	ab A