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Abstract

Edible films or coatings could be used as an alternative way of conservation, because of their ability to reduce respiration and transpiration rate, maintain firmness and generally delay fruit senescence. The aim of this research was to evaluate the influence of different types of coating: sodium alginate (Al), pectin (Pe) and sodium alginate plus pectin (Al + Pe), on some blueberries quality characteristics, cell viability and microbial growth during 14 days of storage at 4°C.

Blueberry samples differently coated did not show significant differences in weight loss, pH, soluble solid and dry matter content. However, the application of Al, Pe and Al + Pe improved the firmness of blueberry samples as compared to the uncoated one. Changes in the surface reflection properties in the coated blueberries induced a general lower lightness and a more intense blue hue colour than the control sample. The microbiological results indicated that the coating of blueberry, in particular with Al or Pe, significantly reduced the growth kinetics of yeasts and mesophilic aerobic bacteria.

Keywords

Fruit, quality, storage, alginate, pectin

1. Introduction

Blueberries are appreciated for their rich composition in bioactive compounds such as flavonoids, phenolic acids, tannins and anthocyanins giving them nutraceutical properties. However, fresh fruit deteriorate rapidly due to loss of water and juice (product of superficial lesions), mould and/or putrefaction (Yang et al., 2014). The shelf-life of fresh blueberries usually is in the range of 10-40 days depending on different factors such as fruit maturity, cultivar, harvest method and storage conditions (Abugoch et al., 2016). Various technologies are used to reduce spoilage, extend the shelf-life and retain the nutritional value of fruit products; among this group particular attention can be given to refrigeration, UV irradiation, ozonation and modified packaging atmosphere (Duan, Wu, Strik, & Zhao, 2011). The use of edible films or coatings represents an alternative way of 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). The efficiency and stability of edible coatings or films depend on their compositions. Edible films and coatings are generally based on biological materials such as proteins, lipids and polysaccharides, alone or, more often, in combination.

Sodium alginate is a natural linear polysaccharide obtained from brown seaweeds and has many important physical and biological properties, such as moisture retention, gel-forming capability, good biocompatibility, low price and high availability (Pei, Chen, Li, & Zhou, 2008).

Pectin is a complex of acidic polysaccharides that form an interpenetrating network in the plant cell wall; it is one of the most important citrus by-products that are industrially extracted from apple pomace and citrus peels. Generally it is used to increase viscosity and gel strength of food products (Krochta & Mulder-Johnston, 1997).

Some studies confirm that the application of edible coatings on fruit surface can increase the shelf-life of different fruits, for example raspberries (Tezotto-Uliana et al., 2014) and tropical fruits

(Cerqueira, Lima, Teixeira, Moreira, & Vicente, 2009). However, there are few works about coatings effects on blueberries (Duan et al., 2011; Chiabrando & Giacalone, 2015). In both papers, the authors showed that the use of alginate coating on berries had a positive effect on firmness, titratable acidity and maintained surface lightness of coated fruit products. However, to the best of our knowledge there are no papers presented in the literature on the effect of pectin-based coating on blueberries.

Although edible films are not intended to completely replace conventional packages, the efficiency of food protection can be improved by combining both actions. The objectives of this study were to investigate the effectiveness of sodium alginate, pectin and both of these polysaccharides based coatings in improving some qualitative characteristics of blueberry fruits during shelf-life.

2. Material and methods

2.1. Fruit material

Organic blueberries were purchased once from local market. Berry fruits were kept at 0 ± 1°C until they were used, for no longer than one week, as suggested by Perkins-Veazie, Clark, Collins, & Magee, 1995 and Jackson, Sanford, Lawrence, McRae, & Stark, 1999. Fresh blueberries with the same colour and size and no damages were selected for the experiments.

2.2. Preparation of coating solutions

Three different coating solutions were prepared, each of them contained 15 g/kg of glycerol (≥ 99.5% Sigma-Aldrich, St. Louis, MO USA) and 2 g/kg of Tween® 20 (Sigma-Aldrich, St. Louis, MO USA) and solved in distilled water. In a first solution, sodium alginate (Al) (Sigma-Aldrich, St. Louis, MO USA) was added in the quantity of 20 g/kg. The second one was enriched by 20 g/kg of pectin (Pe) from citrus peel (Galacturonic acid ≥ 74.0% Sigma, St. Louis, MO USA), and the third one was prepared by combination of Sodium Alginate and Pectin (Al + Pe) in equals amounts of 10 g/kg + 10 g/kg. Afterwards, all coating solutions were homogenised at 5000 rpm for 2 min in order to remove air bubbles.

96

97 2.3. Sample preparation

98 Blueberry fruits were sanitized with sodium hypochlorite water solution (0.2 g/kg), rinsed in
99 distilled water and dried with absorbing paper. Whole fruits were dipped in coating solutions, in
100 two process steps, each one of 30 sec duration. The berry samples were drained in a ventilated oven
101 at 25 ± 1 °C for 30 min following the first step dipping, and for 60 min following the second step
102 dipping. Blueberries dipped in distilled water with the same procedures were used as control.
103 Coated berry samples were then placed in plastic trays (PET) closed in micro-perforated bags
104 (PLA) and stored at 4 °C for 14 days. Coated samples and control ones were analysed at 0, 2, 4, 6,
105 10 and 14 days of storage. Totally 4 samples were obtained: 3 differently coated blueberry samples
106 (Al, Pe, Al+Pe) and 1 not coated control sample. For each sample 540 blueberries were used. Three
107 trays for every sampling time were made, containing 30 blueberries each, from which fruits were
108 taken randomly from the three trays and used for analytical determinations.

109

110 2.4. Quality determinations

111 2.4.1. Weight loss, Dry matter, pH and Soluble solid content

112 Weight loss (WL) of blueberry samples during storage was measured by weighting fruits in the
113 trays before storage and at every day of analysis, following the standard method of AOAC (1994).
114 Dry matter content was determined gravimetrically by difference in weight before and after drying
115 at 70 °C, until a constant weight was reached (AOAC International, 2002).
116 pH was determined at 20 °C with a pH meter CRISON GLP21 (Shinghai Shilu-Instruments, China).
117 Soluble solid content (SSC) analysis were performed at 20 °C by measuring the refractive index of
118 blueberry juice with digital hand refraktometer mod. DR301-95 (Kruess, Germany).
119 For each treatment-time condition, dry matter was determined in triplicate from 8 blueberries from
120 each tray; pH and SSC were determined also in triplicate on three different juice samples each
121 obtained from 10 berries from each tray, after filtering through Whatman #1 filter paper.

122

123 2.4.2. Colour and Texture

124 Surface colour of blueberry was measured using spectrophotocolorimeter HUNTERLAB
125 ColorFlexTM, mod. A60-1010-615 (Reston, Virginia). For each sample L*, a* and b* parameters
126 from CIELAB scale were measured and Hue angles (h°) index was calculated.
127 Penetration test was performed with a Texture Analyser mod. TA-HDi500 (Stable Micro Systems,
128 Godalming, UK) equipped with a 50 N load cell and a 2 mm diameter stainless steel probe.
129 Penetration test speed was 0.5 mm s^{-1}, the test ended when a maximum deformation of 80% was
130 reached. Results were expressed as average of 12 measurements carried out on 12 blueberries for
131 each treatment-time condition.

132

133 2.4.3. Cell viability

134 The cell viability test was performed on blueberries slices obtained from 9 different blueberries
135 using fluorescein diacetate (FDA, Sigma-Aldrich, USA, λ_{ex} = 495 nm, λ_{em} = 518 nm), as described
136 by Tylewicz, Romani, Widell, & Galindo, (2013). Viable cells could be easily identified by a bright
137 fluorescence. Observations were performed under a fluorescent light in a Nikon upright microscope
138 (Eclipse Ti-U, Nikon Co, Tokyo, Japan) equipped with a Nikon digital video camera (digital sight
139 DS-Qi1Mc, Nikon Co, Tokyo, Japan) at a magnification of 4 ×.

140

141 2.4.4. Microbial growth

142 The total loads of mesophylic aerobic bacteria, lactic acid bacteria, yeasts, moulds and total
143 coliforms were evaluated according to the methods reported by Siroli et al., (2015). Briefly, 10 g
144 portion of each sample were used (around 6 berries), suspended in 90 ml of sterile saline solution (9
145 g/l NaCl) and homogenized using a Stomacher for 2 min at room temperature; serial dilutions were
146 made. The microbiological analyses were performed in triplicate immediately after treatments and
147 during storage.

148

149 2.4.5. Data analyses

150 Analysis of variance (ANOVA) and the test of mean comparison, according to Fisher's least
151 significant difference (LSD) were applied on all obtained data. Level of significance was $p < 0.05$.
152 The statistical software used was STATISTICA, v 8.0 (StatSoft, Tulsa, Okhlaoma).

153

154 **3. Results and discussion**

155 3.1 Weight loss, Dry matter, pH and Soluble solid content

156 The fruits weight loss during storage usually is caused by the migration of the water from the fruit
157 to the surrounding environment. As reported in Table 1, all samples underwent a slight loss of
158 weight during 14 days of storage. Coated samples did not show any significant differences in
159 weight loss as compared to the control. These results are probably due to a slight loss of water
160 undergone by samples. The moisture loss of fresh fruit and vegetables is due to the gradient of
161 water vapor pressure that occurs from different locations in the cell tissues (Yaman & Bayoındırlı,
162 2002). The cold storage conditions (temperature and relative humidity) could have an effect on the
163 difference of vapor pressure between blueberries and the environment resulting in non-significant
164 weight losses. In fact, as reported by Nunes (2015) the weight loss up to 4-5% does not significantly
165 influence the freshness of the fruit.
166 As reported in Table 2, no significant differences ($p < 0.05$) on dry matter and pH were detected,
167 among control and differently coated samples at each considered storage time. Concerning the SSC,
168 significant differences ($p < 0.05$), even if slight, were observed only at 10 days of storage; in
169 particular Al and Al + Pe presented higher SSC values as compared to the control and Pe coated
170 blueberry fruits. As a general trend dry matter, pH and SSC tended to increase during storage in
171 both control and coated fruit samples. pH and SSC showed the same behaviour increasing with
172 longer storage time, similar results have been provided by Duan et al (2011). The increase of pH

and SSC is probably due to metabolic processes and reactions during post-harvest storage, which continue to converting starch and acids into the sugar.

3.2 Colour and Texture

In Table 3 colour data (lightness - L* and Hue angles - h°) of blueberry samples during 14 days of storage at 4 °C are reported. Coating induced a general lower lightness and a more intense blue hue colour in blueberry samples as compared with the control one ($p < 0.05$), probably due to the glossy effect of coating. The observed lower luminosity value of coated samples could be caused by the modifications in the surface reflection properties (Hoagland & Parris, 1996). L* values of control and coated samples tended to increase during the first days of storage, then remained relatively stable and decreased after the sixth storage day.

The visual perception of the intensity of blue colour was always more intense in the coated than in the control samples, as indicated from their highest hue values. Moreover, the blueberry samples showed a general decrease in hue values from 0 to 10 days that tended to increase on 14^{th} day. The h° decrease of blueberries during the first period of storage is probably caused by oxidation or condensation reactions of phenolic compounds resulting in loss of anthocyanins during cold storage (Reque et al., 2014). Moreover, the increase of hue values at the end of storage might be caused by a possible anthocyanins synthesis during ripening as also observed by the higher pH and SSC values (Table 2).

As shown in Fig. 1 blueberry coated samples presented a significantly ($p < 0.05$) higher firmness compared to control sample until the first 10 days of storage. After this period, texture of blueberries coated samples decreased, reaching the same value of control one (1.75N). The higher firmness values of coated samples are probably due to the presence of coating that provide a structural rigidity to the surface of fruit (Duan et al., 2011). Pe and Pe + Al showed the same behaviour of the Al based coating. This result of Al coating was in agreement with Rojas-Graü, Tapia, & Martín-Belloso, (2008) on fresh-cut apple and Fan et al., (2009) on strawberry fruits.

198 Moreover, the retention of firmness could be explained by the delay of pectin and proto-pectin
199 degradation, involved in maintaining the structural integrity of the fruits (Thompson, 1996).
200
201 3.3 Cell viability
202 Figure 2 shows the micrographs resulted from microscopic observations of control (a) and Al + Pe
203 coated blueberry samples (b) after 14 days of storage. The pictures demonstrate that cell viability in
204 all tissues is preserved until 14 days of storage both in case of control and coated samples. The
205 results provide evidence that cell viability (viable cells could be identified by a bright fluorescence
206 on the Figure) can be preserved in blueberries also after the application of coating. If the protoplasts
207 of the cells did not retain the FDA, this means disruption of the plasma membrane (cell lysis) or
208 loss of membrane semi permeability (Halperin & Koster, 2006). These results provide versatile tool
209 to conduct study of the metabolism of blueberry tissues that was maintained despite storage and the
210 application of different types of coatings.
211
212 3.4 Microbial growth
213 As reported in Table 4, yeasts were detected only in control sample after 2 and 4 days of storage
214 while in all the coated samples the yeast cell loads were below the detection limit. In samples
215 coated with Al or Pe yeasts were detected only after 10 days of storage. However, at the end of
216 storage the yeast loads of coated samples were 1.09-1.38 logarithmic cycles lower than control
217 samples.
218 Significant differences were also evidenced in the total aerobic mesophilic cell loads among the
219 samples during the storage period (Table 5). In fact, in this case only the control sample showed
220 mesophilic cell loads above the detection limit after 2 days of storage. Samples coated with Al or Pe
221 showed mesophilic cell loads from the sixth days of storage, significantly lower than the controls
222 and samples coated with Al + Pe. Finally, no significant differences were found for lactic acid

bacteria and total coliform cell loads in relation to the coating adopted, whose loads resulted below 2.0 log CFU/g, during the whole period of storage. The microbiological results indicate that the coating of blueberry, in particular with Al or Pe, significantly reduce the growth kinetics of yeasts and mesophilic aerobic bacteria that play a dominant role in the spoilage of minimally processed fruits (Siroli et al., 2014) .

4. Conclusions

The use of coating showed a positive effect mainly on firmness and microbial growth of treated blueberries samples. The firmness was maintained until 10 storage days also for the Pe and Al + Pe coated blueberries. Furthermore, the application of coatings reduced the growth kinetics of yeasts and mesophilic aerobic bacteria, in particular with the application of Al and Pe based coatings. Results from this study indicate the possibility of using edible coatings to develop ready-to-eat fresh blueberries with no reduction in their shelf-life. Further researches will focus on the effect of these edible coatings on blueberry bioactive compounds and sensorial properties.

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299 blueberries. *Postharvest Biology and Technology, 92*, 46-53.

300 **Figure captions**

301 **Fig. 1** Firmness (N) of control (Control ○) and differently coated blueberry samples (sodium
302 alginate - Al ◊; pectin - Pe □; sodium alginate plus pectin – Al + Pe Δ) during 14 days of storage at
303 4°C.

304 **Fig. 2** Cell viability for (a) control (Control) and sodium alginate (Al), pectin (Pe) and sodium
305 alginate plus pectin (Al + Pe), coated blueberry samples (b) at 14 days of storage after treatment
306 using fluorescein diacetate (FDA) marker. Bar = 100 µm

307

Table 1. Weight loss (%) of control (Control) and sodium alginate (Al), pectin (Pe) and sodium alginate plus pectin (Al + Pe) coated blueberry samples during 14 days of storage at 4°C.

	Weight loss (%)				
	T2	T4	T6	T10	T14
Control	-1.1 ± 0.1[a]	-1.2 ± 0.3[a]	-2.3 ± 0.3[a]	-3.9 ± 0.2[a]	-5.9 ± 0.8[a]
Al	-1.05 ± 0.05[a]	-1.8 ± 0.4[a]	-2.34 ± 0.05[a]	-4.2 ± 0.2[a]	-6 ± 1[a]
Pe	-0.83 ± 0.07[a]	-1.5 ± 0.2[a]	-2.2 ± 0.3[a]	-4.0 ± 0.5[a]	-5.5 ± 0.2[a]
Al+Pe	-2 ± 1[a]	-2.2 ± 0.1[a]	-2.3 ± 0.5[a]	-4.1 ± 0.6[a]	-5.6 ± 0.3[a]

Data are reported as average values and standard deviations obtained from three replicates for each treatment-time conditions.

Values with different letter within the column are significantly different ($p < 0.05$).

Table 2. Dry matter (g/kg) pH and soluble solid content (°Bx) of control (Control) and sodium alginate (Al), pectin (Pe) and sodium alginate plus pectin (A l+ Pe) coated blueberry samples during 14 days of storage at 4°C.

Dry Matter (g/kg)						
	T0	T2	T4	T6	T10	T14
Control	178.2 ± 0.4[a]	193.0 ± 0.6[a]	183 ± 2[a]	204.5 ± 0.1[a]	199 ± 1[a]	198 ± 1[a]
Al	177.8 ± 0.8[a]	180 ± 2[a]	194.8 ± 0.7[a]	194.30 ± 0.02[a]	183.0 ± 0.1[a]	202 ± 1[a]
Pe	185.8 ± 0.6[a]	179.9 ± 0.7[a]	195.9 ± 0.2[a]	204.9 ± 0.4[a]	196.2 ± 0.2[a]	194.3 ± 0.6[a]
Al+Pe	185.4 ± 0.2[a]	186.5 ± 0.8[a]	190.7 ± 0.5[a]	184.32 ± 0.06[a]	193.5 ± 0.4[a]	188.0 ± 0.6[a]
pH						
	T0	T2	T4	T6	T10	T14
Control	3.49 ± 0.00[a]	4.09 ± 0.03[a]	3.5 ± 0.2[a]	3.7 ± 0.5[a]	3.7 ± 0.2[a]	4.1 ± 0.1[a]
Al	3.47 ± 0.07[a]	3.9 ± 0.2[a]	3.8 ± 0.8[a]	3.35 ± 0.08[a]	3.4 ± 0.2[a]	4.03 ± 0.05[a]
Pe	3.28 ± 0.04[a]	3.8 ± 0.1[a]	3.4 ± 0.2[a]	3.52 ± 0.00[a]	3.38 ± 0.07[a]	4.0 ± 0.1[a]
Al+Pe	3.55 ± 0.04[a]	3.8 ± 0.2[a]	3.5 ± 0.2[a]	3.31 ± 0.02[a]	3.6 ± 0.3[a]	3.58 ± 0.02[a]
SSC						
	T0	T2	T4	T6	T10	T14
Control	13.4 ± 0.7[a]	13 ± 2[a]	15.0 ± 0.2[a]	15.2 ± 0.2[a]	12.7 ± 0.9[b]	15 ± 2[a]
Al	12.6 ± 0.7[a]	15 ± 2[a]	15 ± 3[a]	14.6 ± 0.1[a]	15.1 ± 0.9[a]	15 ± 2[a]
Pe	13 ± 2[a]	15 ± 1[a]	13 ± 1[a]	13.1 ± 0.5[a]	11.6 ± 0.4[b]	18 ± 1[a]
Al+Pe	13 ± 2[a]	14 ± 1[a]	14 ± 1[a]	15.6 ± 0.2[a]	15.0 ± 0.4[a]	17 ± 1[a]

Data are reported as average values and standard deviations obtained from three replicates for each treatment-time conditions.

Values with different letter within the column are significantly different ($p < 0.05$).

Table 3.Lightness-L* and Hue angles- h° of control (Control) and sodium alginate (Al), pectin (Pe) and sodium alginate plus pectin (Al + Pe) coated blueberry samples during 14 days of storage at 4°C.

	L*					
	T0	T2	T4	T6	T10	T14
Control	21 ± 1^{a}	28.4 ± 0.1^{a}	31.5 ± 0.8^{a}	30.5 ± 0.5^{a}	28.5 ± 0.6^{a}	29 ± 1^{a}
Al	19.33 ± 0.07^{a}	18.9 ± 0.1^{b}	22.74 ± 0.05^{c}	22.2 ± 0.6^{c}	19.4 ± 0.6^{b}	16.48 ± 0.00^{c}
Pe	14 ± 2^{b}	19.5 ± 0.5^{b}	23.2 ± 0.4^{bc}	26.0 ± 0.2^{b}	19.3 ± 0.2^{b}	19.59 ± 0.02^{b}
Al+Pe	15.3 ± 0.6^{b}	15.9 ± 0.8^{c}	24.9 ± 0.2^{b}	25.6 ± 0.5^{b}	17.6 ± 1.4^{b}	19.9 ± 0.4^{b}
	h°					
	T0	T2	T4	T6	T10	T14
Control	100 ± 11^{b}	90 ± 3^{c}	97 ±5^{c}	93 ±4^{b}	72 ± 6^{c}	89 ± 6^{c}
Al	140 ± 11^{a}	126 ± 10^{ab}	117 ±7^{b}	102 ± 9^{b}	75 ± 6^{b}	145 ± 11^{b}
Pe	145 ± 11^{a}	139 ± 7^{a}	128 ± 5^{a}	134 ± 6^{a}	87 ±6^{a}	151 ± 11^{b}
Al+Pe	154 ± 11^{a}	123 ± 9^{b}	111 ± 7^{b}	126 ± 5^{a}	85 ±5^{ab}	179 ± 11^{a}

Data are reported as average values and standard deviations obtained from twelve replicates for each treatment-time conditions.

Values with different letter within the column are significantly different ($p < 0.05$).

Table 4. Yeast count of control (Control) and sodium alginate (Al), pectin (Pe) and sodium alginate plus pectin (Al + Pe) coated blueberry samples.

	T0	T2	T4	T6	T10	T14
Control	nd*	2.2 ± 0.3[a]	3.2 ± 0.2[a]	3.5 ± 0.3[a]	3.6 ± 0.3[a]	3.3 ± 0.3[a]
Al	nd*	nd*	nd*	nd*	2.1 ± 0.2[b]	2.0 ± 0.2[b]
Pe	nd*	nd*	nd*	nd*	1.8 ± 0.3[b]	1.9 ± 0.2[b]
Al+Pe	nd*	nd*	nd*	2.2 ± 0.2[b]	2.2 ± 0.2[b]	2.2 ± 0.2[b]

Counts are expressed in log10 cfu/g (± standard deviation). Means followed by different letters are significantly different ($p<0.05$) and are obtained from three replicates for each treatment-time conditions.

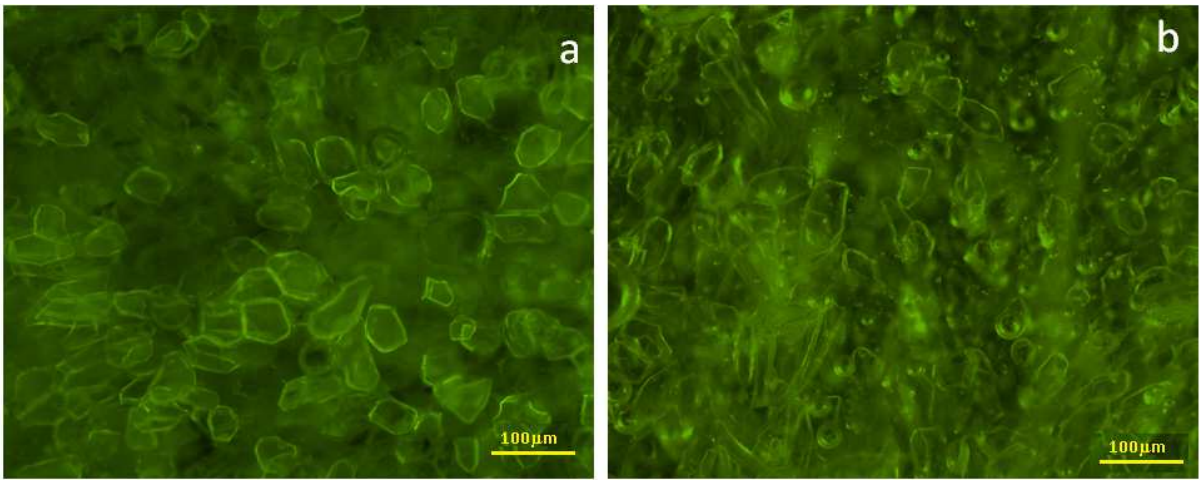
* under the detection limit (1 log10 cfu/g)

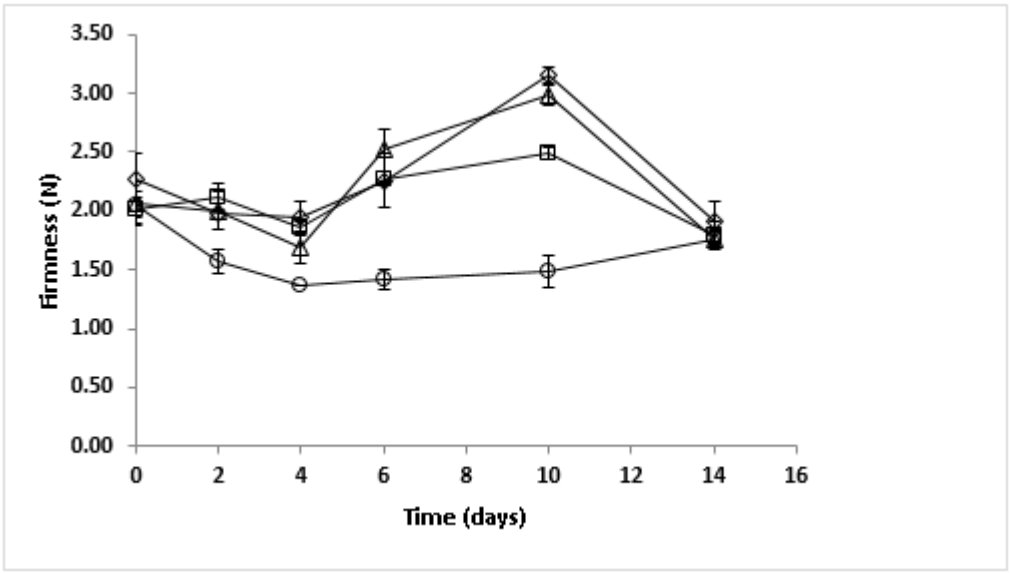
Table 5. Mesophylic aerobic bacteria of control (Control) and sodium alginate (Al), pectin (Pe) and sodium alginate plus pectin (Al + Pe) coated blueberry samples.

	T0	T2	T4	T6	T10	T14
Control	nd*	2.3 ± 0.3[a]	2.9 ± 0.3[a]	3.1 ± 0.3[a]	4.1 ± 0.3[a]	4.5 ± 0.3[a]
Al	nd*	nd*	1.5 ± 0.3[b]	1.9 ± 0.3[b]	2.0 ± 0.2[b]	2.6 ± 0.3[b]
Pe	nd*	nd*	nd*	2.1 ± 0.3[b]	2.2 ± 0.3[b]	2.7 ± 0.2[b]
Al+Pe	nd*	nd*	1.5 ± 0.3[b]	3.0 ± 0.3[a]	3.6 ± 0.4[a]	4.2 ± 0.4[a]

Counts are expressed in log10 cfu/g (± standard deviation). Means followed by different letters are significantly different ($p<0.05$) and are obtained from three replicates for each treatment-time conditions.

* under the detection limit (1 log10 cfu/g)


a
100µm
b
100µm


3.50
3.00
2.50
2.00
1.50
1.00
0.50
0.00
Firmness (N)
0
2
4
6
8
10
12
14
16
Time (days)

Coating induced a decrease of lightness and an increase of blue colour in berries.

Firmness of blueberries was improved by application of coating.

Alginate/pectin coating reduced the growth of yeasts and mesophilic aerobic bacteria.