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

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

3

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12

ACCEPTED MANUSCRIPT

13 **Abstract**

14

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

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

26

27

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

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29 1. Introduction

30 Blueberries are increasingly appreciated for their rich composition in flavonoids, phenolic acids, tannins and
31 anthocyanins giving them a great nutritional value. Anthocyanins are natural pigments, largely distributed in
32 nature and generally present in many fruit and vegetables. In particular, berries demonstrated to have a great
33 antioxidant activity, due to their high content in phenolic acids and flavonoids, which can cause a strong
34 antioxidant capacity in different products (Pellegrini et al., 2003). In addition, phenolic compounds may
35 exert beneficial effects on human health associated with the consumption of fruit and vegetables (Cheynier,
36 2012).

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

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

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

49 In order to improve the efficiency and stability of edible coatings it is essential to find adequate composition
50 of their formulations. The basic coating ingredients are polysaccharides, proteins and lipids, either as pure
51 substances or in combination. Edible coatings have high potential to carry active and functional ingredients
52 such as antimicrobial, antioxidant and antibrowning agents, colorants, nutrients that can enhance the
53 nutritional values and the stability of products during their shelf-life (Rojas-Graü, Tapia, & Martín-Belloso,
54 2008).

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

62 Procyanidins are one of the most abundant flavonoids present in grape seeds and skin. They are mainly
63 proanthocyanidins (condensed tannins) mostly constituted of oligomeric flavonoids as catechin, epicatechin,
64 epicatechin gallate and epigallocatechin (Souquet, Cheynier, Brossaud, & Moutounet, 1996). During food
65 processing and storage, plant phenolic compounds are converted to a variety of reaction products that could

66 contribute to the quality of plant-based foods, along with the genuine plant components (Cheyner, 2012).
67 Moreover, these bioactive compounds can be used to add value and to improve the nutritional functions of
68 numerous foodstuffs (dos Reis, de Oliveira, Hagen, Jablonski, Flôres, & de Oliveira Rios, 2015; Rodriguez-
69 Amaya, 2016; Martin & Ferreira, 2017). A lot of by-products from food processing could be a good source
70 for the recovery of polyphenols, protein and pectin, that can be used as natural ingredients and or additive in
71 food production (Kammerer, Kammerer, Valet, & Carle, 2014; Martins et al., 2017).
72 Nair, Saxena & Kaur (2018) investigated the effect of chitosan and alginate based coatings enriched with
73 pomegranate peel extract, showing that chitosan based coatings was more effective than alginate in
74 maintaining the postharvest quality of guava (*Psidium Guajava L.*). However, to the best of our knowledge,
75 investigations on the influence of coatings based on chitosan from mushrooms alone or enriched with
76 procyanidins, extracted from grape by-product, on fruit or vegetables quality have not been reported yet
77 Thus, the main aim of this research work was to evaluate the effect of the application of specific innovative
78 coatings on some quality characteristics (weight loss, pH, dry matter, colour and firmness), antioxidant
79 activity (ABTS and DPPH assays) and microbial growth of blueberry samples during storage at 4°C for 14
80 days.

81

82 2. Material and methods

83 2.1 Fruit material

84 Organic blueberries were purchased from local market. Berry fruits were kept for one day at $0 \pm 1^\circ\text{C}$ until
85 they were used. Fresh blueberries with similar colour and size and no damages were selected and these
86 berries were characterized by dry matter of 15.1 ± 0.3 g/100g.

87

88 2.2 Preparation of coating solutions

89 Two different coating solutions were prepared, each of them contained 1.5 % (w/w) of glycerol ($\geq 99.5\%$
90 Sigma-Aldrich, Germany) and 0.20 % (w/w) of Tween® 20 (Sigma-Aldrich, France) and solved in citric
91 acid solution 1% (Sigma- Aldrich, Germany). In a first solution, chitosan from mushrooms (C) provided by
92 Agrovin (Alcazar de San Juan, Spain) was added in the quantity of 1 % (w/w). The second coating solution
93 was prepared by combining chitosan from mushroom (1% w/w) and procyanidins extracted from grape seeds
94 (Chardonnay berries) (0.8 % w/w) (CP). The extraction of procyanidins was performed as follows: briefly,
95 200 g of dehydrated seeds were extracted with water-ethanol (1:1 w/w) for 2 hours under stirring at 200 rpm.
96 Extracts were rotary evaporated under vacuum at 35°C to remove ethanol. The resulting extracts were
97 washed with hexane to remove lipid-soluble substances, and then rotary evaporated to remove the residual
98 hexane. The aqueous fraction (about 75 mL) was applied to a Diaion HP-20 column (70x500 mm)
99 previously equilibrated with water, and rinsed with 10% ethanol. Procyanidins were eluted using 100 mL
100 water-ethanol 30:70 w/w, spray dried and stored at -30°C before their use.

101 The final concentration of procyanidins used for coating solution was chosen based on the higher antioxidant
102 activity and unchanged sensorial properties of fruit tested in preliminary trials by trained panel (data not

103 showed). Afterwards, all coating solutions were homogenised at 5000 rpm for 2 min in order to remove air
104 bubbles.

105

106 2.3 Sample preparation

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

118

119 2.4 Quality determinations

120 2.4.1 Weight loss, dry matter and pH

121 Weight loss (WL) of blueberry samples during 14 days of storage was measured by weighting fruits in all
122 trays per sample at the beginning of the storage and at every day of analysis; the results were calculated as
123 percentage loss of initial weight, following the standard AOAC method (1994).

124 Dry matter (DM) was determined gravimetrically by difference in weight before and after drying at 70 °C,
125 until constant weight was reached (AOAC International, 2002).

126 pH was measured at 20 °C with a pH meter CRISON GLP21 (Shinghai Shilu-Instruments, China).

127 For all treatment times and for each sample, DM was determined in triplicate from 9 blueberries and pH was
128 measured also in triplicate on the three different juice sub-samples obtained from 15 berries (fruit:water 1:1).

129 2.4.2 Colour

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

134

$$135 \quad h^\circ = \tan^{-1} \frac{b^*}{a^*} \quad (1)$$

136

137 where: a^* (red–green) and b^* (yellow–blue) are parameters of colour measurement (Vega-Gálvez et al.,
138 2012).

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

141

142 2.4.3 Texture

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

148

149 2.4.4 Antiradical activity (DPPH, ABTS assays)

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

154 The DPPH scavenging activity was based on the method proposed by Amarowicz, Naczki, & Shahidi (2000).
155 Briefly, 0.1 mL of extract was added to 2 mL of methanol and 0.25 mL of DPPH (Sigma-Aldrich, USA),
156 shaken with a vortex for 1 min and kept to the dark for 30 min. The absorbance was measured with a
157 spectrophotometer (Beckman Coulter DU 730 Life Science model) at 517 nm. Antioxidant activity was
158 quantified by plotting a Trolox calibration curve. Trolox concentration range was 0.001-1.500 mM ($r^2 =$
159 0.9980). The results were expressed as mmol Trolox/g of fruit.

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

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

167

168 2.4.5 Microbiological analysis

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

172

173 2.5 Data analyses

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

178

179 3. Results and discussion

180 3.1 Weight loss, dry matter and pH

181 The weight loss, dry matter and pH values of F and differently coated samples during 14 days of storage are
182 reported in Table 1. All the samples underwent a similar decrease of the weight during cold storage (around
183 4.5%); this could be due to the migration of water from the fruit to the environment. The weight loss of fruit
184 and vegetables is due to the water vapour pressure gradient that exists from different compartments in the
185 cell tissues (Yaman & Bayoindurlu, 2002). This result was in agreement with Carvalho et al. (2016), who
186 observed that the use of chitosan based coating with trans-cinnamaldehyde was not able to reduce the weight
187 loss of fresh-cut melon during 20 days of storage. Moreover, Mannozi et al. (2016) observed a progressive
188 decrease of weight loss, without seeing any significant differences between uncoated and differently coated
189 (polysaccharide-based coating) blueberry samples during storage.

190 For what concern the dry matter (Table 1), no relevant differences ($p < 0.05$) were found between C and CP
191 coated samples during the overall storage. In particular, only F sample underwent a slight decrease of dry
192 matter during 14 days of storage. The tendency to an increase of dry matter showed by CP sample during
193 storage could be due to the solutes gain caused by the presence of coatings (Carvalho, et al., 2016).

194 As reported in Table 1, F samples showed, in general, a decrease in pH already after 2 days of storage in
195 comparison to C and CP samples. However, all the blueberry samples showed a slight decreasing trend, even
196 though not significant, of the pH during the overall storage. This is probably due to the greater loss of water
197 and also it is possible that the loss of weight (up to 4 %) that occurred during the postharvest period
198 influenced these values (Hernández-Muñoz, Almenar, Del Valle, Velez, & Gavara, 2008; Chiabrande et al.,
199 2011)

200

201 3.2 Colour

202 Anthocyanins and other pigments derived from phenolic compounds are responsible for the colour of red
203 fruit and wines (Cheynier, 2012). Table 2 reported the lightness (L^*), a^* , b^* and hue angle (h°) values of
204 control and coated blueberry samples during 14 days of storage at 4 °C.

205 Immediately after coating (T0) C blueberry samples displayed lower L^* values than the F and CP ones. The
206 observed lower lightness of chitosan coated blueberry is probably due to the presence of coating that caused
207 changes in the surface properties (Hoagland & Parris, 1996). However, this behaviour has not been observed
208 in CP coated blueberries probably due to the presence of procyanidins.

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

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

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

229

230 3.3 Texture

231 Firmness is one of the most important critical quality parameter that influences the consumer acceptability of
232 fresh products. As shown in Fig. 1, in general, C and CP coated blueberry samples exhibited a higher ($p <$
233 0.05) firmness in comparison to F sample, immediately after coating at 0 day of storage, which can be
234 explained by the presence of coatings that provide rigidity to the skin of fruit (Duan, Wu, Strik, & Zhao,
235 2011). Generally, during the overall storage all the blueberry samples maintained similar texture values.
236 However, coated samples showed significantly ($p < 0.05$) higher values immediately after coating (T0) and
237 10th day of storage, compared to the uncoated ones. Moreover, the higher firmness of coated blueberry
238 samples could be explained by the thickness of the two different coating formulations. In fact, thickness of C
239 and CP coated blueberries measured in preliminary trials, ranged from 84 to 130 μm respectively.

240 The added procyanidins induced an increase in thickness and thus created more compact structure of
241 enriched coating formulation compared to chitosan one. In fact the procyanidins that might create a bridge
242 between chitosan and their free functional groups in the molecular structure (Zhang, Yang, Tang, Hu & Zou,
243 2008).

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

252

253 3.4 Antiradical activity (DPPH, ABTS assays)

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

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

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

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

267 Under both the analytical methods, the CP coated blueberries showed a higher antioxidant activity already at
268 0 day, in comparison to the C and the fresh ones. Its better retention during the overall storage period is
269 probably due to the presence of chitosan and procyanidins in the coatings that provide the enhancement of
270 antioxidant compounds. The use of procyanidins from grape by-products induced an improvement of the
271 nutritional value of coated blueberry fruit. Moreover, all blueberry samples showed similar behaviour, with
272 DPPH and ABTS antiradical activity method. It was possible to observe significant increase in antioxidant
273 activity in C coated sample at 6th and 10th day with respectively ABTS and DPPH methods. This is probably
274 due to the anthocyanins synthesis that occurs during ripening stage (Kalt, Forney, Martin, & Prior, 1999);
275 these results are in accordance with h^o colour data. For both analytical methods, studied C and CP based
276 coatings were able to delay the loss of antioxidant compounds. Chiabrando & Giacalone (2015) reported
277 similar results whit the application of chitosan on blueberries during 45 days of storage at 0 °C.

278

279 3.5 Microbiological analysis

280 In Table 3, the cell loads of total mesophilic aerobic bacteria, mould and yeasts during the storage at 4 °C are
281 reported. The chitosan coated samples (C) showed a significant lower cell load of mesophilic bacteria at the
282 1st day of storage compared to the other samples. However, at the 4th day of storage a decrease of mesophilic
283 aerobic bacteria was detected in all the considered samples and without significant differences between them.
284 At the end of storage (T14), an increase of the mesophilic bacteria was detected for all the considered
285 conditions without significant differences. However, the detected cell loads, except for samples F and CP
286 immediately after treatments never exceeded a cell load of 3.0 log cfu/g.

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

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

301 In addition, the obtained results showed, even if the microbial spoilage threshold ($>10^6$ cfu/g for yeast, and
302 $>10^7/10^8$ cfu/g mesophylic aerobic bacteria) (FSA of Ireland, 2016) was not reached in all the considered
303 samples, that in samples C there was a significant higher yeast and moulds inhibition compared to the other
304 samples. These results are in agreement with other studies that evidenced the antimicrobial and antifungal
305 activity of pectin, alginate and chitosan coatings on blueberry (Duan et al., 2011; Jiang, Sun, Jia, Wang, &
306 Huang, 2016; Mannozi et al., 2016).

307

308 4. Conclusions

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

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

321

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325

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 436 107, 891-897.

437 Figure captions

438 **Fig. 1.** Firmness (N) of uncoated (F) and coated blueberry samples (C and CP) during 14 days of storage at
 439 4°C.

440 Means with different lowercase letters means significant difference ($p < 0.05$) during time (days, in columns) and with
 441 capital letters means significant difference ($p < 0.05$) between samples at each day of storage (in rows).
 442

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

445 Means with different lowercase letters means significant difference ($p < 0.05$) during time (days, in columns) and with
 446 capital letters means significant difference ($p < 0.05$) between samples at each day of storage (in rows).
 447

448 **Table 1.** Weight loss (%), dry matter (%) and pH of uncoated (F) and coated blueberry samples (C and CP)
 449 during 14 days of storage at 4°C.
 450

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}

451

452 Data are reported as average values and standard deviations.

453

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

456

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

459

L^*						
	T0	T2	T4	T6	T10	T14
F	24.4 ± 0.3 ^{bcA}	25 ± 1 ^{bA}	24.8 ± 0.3 ^{bA}	23.6 ± 0.8 ^{ccA}	24.5 ± 0.6 ^{ccA}	26.1 ± 0.5 ^{aaA}
C	17.80 ± 0.03 ^{dc}	19.2 ± 0.2 ^{cc}	16.67 ± 0.5 ^{cc}	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^*						
	T0	T2	T4	T6	T10	T14
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^*						
	T0	T2	T4	T6	T10	T14
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°						
	T0	T2	T4	T6	T10	T14
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}

460

461

462 Data are reported as average values and standard deviations.

463

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

466

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

Mesophylic aerobic bacteria						
	T0	T2	T4	T6	T10	T14
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						
	T0	T2	T4	T6	T10	T14
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 ^{abB}	2.27±0.31 ^{bB}	2.06±0.24 ^{bB}	nd*	nd*	nd*
CP	3.12±0.18 ^{abB}	2.53±0.24 ^{bAB}	2.18±0.12 ^{bcAB}	1.29±0.26 ^{dA}	nd*	1.87±0.14 ^{cA}
Mould						
	T0	T2	T4	T6	T10	T14
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 ^{abB}	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

469

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

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

473

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

475

476

477 **Highlights**

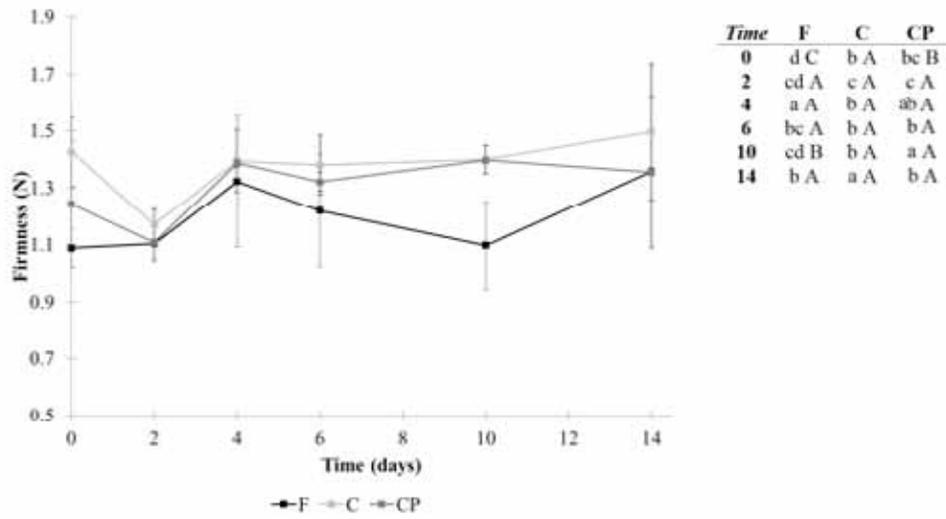
478 Quality parameters were maintained after the application of chitosan coatings

479 Procyanidin by-products enhanced the antioxidant activity of fresh blueberries

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

481

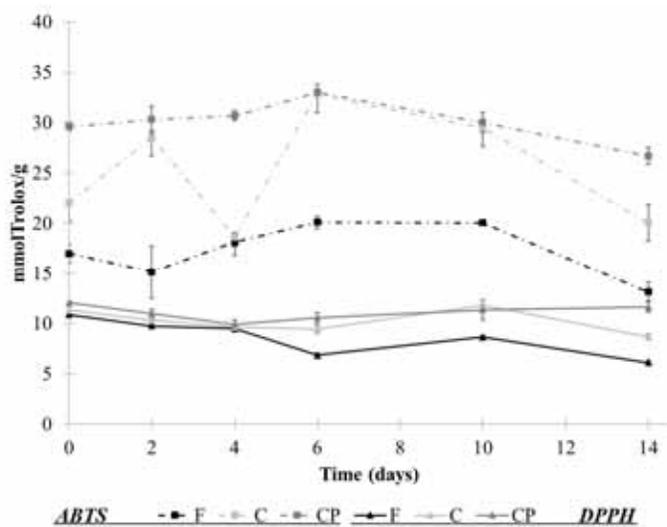
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