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1 **The efficacy of potassium sorbate-coated packaging to control postharvest gray mold**
2 **in raspberries, blackberries, and blueberries**

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10 **Abstract**

11 The aim of this work is to build on the success of *in vitro* studies of an active packaging,
12 produced by coating the surface of post-consumer recycled polyethylene terephthalate
13 (PCRPET) package with an aqueous silicone solution (2%, v/v) containing an antifungal
14 agent (potassium sorbate, KS). Antifungal efficacy was evaluated, *in vivo*, during the
15 storage of raspberries, blackberries and blueberries by examining their shelf life extension.
16 The packaging effectively delayed the growth of Botrytis by extending its lag-phase, which,
17 in turn, extended the shelf life of the berries by up to 3d. Among the three berries tested, the
18 packaging proved to be more advantageous in the case of raspberries, due to their
19 physiological characteristics and shorter shelf life. Based on sensory panel evaluations, it
20 was shown that the coating, containing KS, did not influence the packaging appearance and
21 transparency, and the fruit did not suffer from any off-flavor development.

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22 **Keywords:** Active packaging; *Botrytis cinerea*; fresh berries; shelf-life; potassium sorbate.

23 **1. Introduction**

24 There is a growing and impressive evidence base for the health benefits of fresh berry
25 consumption (Tulipani et al., 2008). Berries are a rich source of a wide variety of non-
26 nutritive and bioactive compounds such as flavonoids, phenolics, anthocyanins, phenolic
27 acids as well as nutritive compounds such as sugars, essential oils, carotenoids, vitamins,
28 and minerals (Nile and Park, 2014). However, a common problem with berries is their high
29 perishability due to rapid ripening and senescence, which hampers storage and marketing
30 (Han et al., 2004). The postharvest life of berries is also determined by their susceptibility
31 to water loss, softening, mechanical injuries, but primarily by the postharvest diseases of
32 gray mold and Rhizopus rot (Reddy et al., 2000; Perkins-Veazie et al., 2008).

33 Gray mold, caused by *B. cinerea*, is the most important postharvest disease in berries,
34 and has a considerably adverse economic impact. This mold is responsible for significant
35 loss, both before and after harvest, and is a major obstacle to long-distance transport and
36 storage because it can grow at temperatures as low as -0.5°C and spreads rapidly by means
37 of aerial mycelium among the berries (Crisosto and Mitchell, 2002).

38 Polyethylene terephthalate (PET) is the most commonly used packaging material,
39 worldwide, for marketing berries. In order to minimize polymer waste the use of recycled
40 PET is increasing (Dimitrov et al., 2013).

41 Junqueira-Gonçalves et al. (2014) described the development of an active packaging
42 designed with post-consumer recycled polyethylene terephthalate (PCRPE) and
43 incorporated potassium sorbate (KS) as an antifungal agent to fight *B. cinerea*. The
44 mechanical, thermal and optical properties of the packaging material were evaluated and its

45 efficacy against *B. cinerea* was demonstrated by employing a novel methodology that
46 mimics actual food contact conditions.

47 The aim of this work was to confirm the promise indicated in the earlier *in vitro* studies
48 and evaluate the antifungal efficacy of the potassium sorbate-coated packaging, *in vivo*,
49 during the storage of raspberries, blackberries and blueberries.

50 **2. Material and method**

51 *2.1. Materials*

52 The polymer used in this study was PCRPET (Typack S.A., Chile), approved by the
53 FDA (Food and Drug Administration) to be in contact with fresh fruits coated with KS
54 (Prinal, Chile) as the antifungal agent. The raspberries, blackberries and blueberries
55 employed were standard export quality, from different origins (Chile, Spain and Mexico)
56 and harvested in different years (2012 and/or 2013). These were delivered by two
57 marketing companies, one in Chile (Vital Berry Marketing S.A.) and another in England
58 (Berry World).

59 *2.2. Packaging preparation*

60 The incorporation of potassium sorbate on the surface of the PCRPET was carried out
61 during sheet manufacture by passing it through a bath containing an aqueous silicone
62 solution (2%, v/v) to which KS has been added at a concentration of 20% (w/v). Coating
63 PET with silicone is standard practice in the manufacture of PET packaging, which
64 prevents individual packaging pieces from adhering on high speed manufacturing lines. The
65 addition of KS to the silicone solution imparted antifungal properties to the PET surface, as
66 described by Junqueira-Gonçalves et al. (2014), with an active percentage of 0.005%. The
67 sheet was converted to clamshells by thermoforming (John Brown Inc., USA). Some

68 clamshells were also produced from sheets that were only coated with silicone but not KS,
69 and these constituted the control samples.

70 *2.3. Assessment of the shelf life of berries*

71 The fruit (125g per package) were carefully transferred to the control and active
72 clamshells, and stored in chambers maintained at 0, 5 and 10°C, simulating temperatures
73 employed during transportation and retailing. Seven replicate clamshells were used for each
74 condition (active and control) and the test was repeated twice.

75 The efficacy of the potassium sorbate-coated packaging on the berries shelf life was
76 evaluated by the incidence of gray mold-infected berries per clamshell, visually or with the
77 help of magnifying glasses. This test was carried out on the day the fruit arrived at the
78 laboratories after harvest. The specific conditions employed for each fruit study are shown
79 in Table 1.

80 *2.4. Sensory evaluation*

81 A sensory assessment was carried out by a trained panel (who were familiar with the
82 major sensory attributes of good quality berries: taste, aroma, texture and colour of the
83 samples), consisting of 8 women and 3 men, using an unstructured 15-point hedonic scale,
84 where 0 represents the worst, and 15 the best condition, meaning very good or very intense,
85 in order to evaluate the packaging appearance and also its influence on fruit aroma and
86 flavor. The raspberries and blueberries were stored at 5°C for 4 and 18d, respectively.
87 These fruits originated from the south of Chile and the storage studies commenced a day
88 after harvest (December, 2012). The samples (125g) were held at ambient temperature
89 (around 25°C) 30 min before the test, coded with 3-digit random numbers, and served
90 simultaneously under normal laboratory light.

91 2.5. *Statistical analysis*

92 Experimental values were statistically analyzed by one-way analysis of variance
93 (ANOVA) employing Statgraphics 5.1 software. Differences between pairs of means were
94 assessed on the basis of confidence intervals using the Tukey test. The least significance
95 difference was $P \leq 0.05$.

96 **3. Results and Discussion**

97 *3.1. Assessment of the shelf life of berries*

98 • Raspberries

99 The raspberries from the south of Chile were harvested in December 2012 and arrived
100 in Santiago by truck, one day after harvesting. The temperature of transport was less than
101 5°C. These fruit were stored at 10°C for 11d. The results of the packaging efficacy are
102 shown in Figure 1.

103 The raspberries from Spain were harvested in March 2013 and arrived in England by
104 truck, 3d after harvesting. The temperature of transport was less than 5°C. These fruit were
105 stored at 5°C for 7d. The results of the packaging efficacy are shown in Figure 2.

106 In both tests a significant difference ($P < 0.05$) between the control and the potassium
107 sorbate-coated packaging was observed. An extension in the shelf life of the raspberries of
108 2 or 3d were observed, especially in the case of the Chilean fruit because they were in
109 contact with the active packaging a few days sooner after harvesting when compared to the
110 Spanish fruit.

111 • Blackberries

112 The blackberries were from Mexico, harvested in March 2013 and arrived in England
113 by air freight, 1d after harvesting. The temperature of transport was 10°C. These fruit were
114 stored at 5°C for 23d. The results of the packaging efficacy are shown in Figure 3.

115 In the case of blackberries too, a significant difference ($P<0.05$) between the control
116 and the potassium sorbate-coated packaging was also observed and an extension in the fruit
117 shelf life of 1 or 2d was clear.

118 • Blueberries

119 The blueberries harvested in December 2012 were from the south of Chile, from two
120 different producers (Region of Los Lagos and Region of Araucania) and arrived in Santiago
121 by truck, 1d after harvesting. The temperature of transport was less than 5°C. These fruit
122 were stored at 0 and 10°C for 28d.

123 The fruit originally from the Region of Los Lagos had a low percentage of gray mold
124 during the entire storage time at 0 and 10°C; therefore it was not possible to evaluate the
125 difference between the packaging systems.

126 The fruit originally from the Region of Araucania also had a low percentage of gray
127 mold at 0°C, but at 10°C it was possible to observe the effect of the packaging on mold
128 growth (Fig. 4).

129 In this trial it was possible to observe a significant difference ($P<0.05$) between the
130 potassium sorbate-coated packaging and the control after 21d of storage.

131 The blueberries harvested in February 2013 were from the south of Chile (Region of
132 Bio Bio) and arrived in Santiago by truck, one day after harvesting. The temperature did
133 not exceed 5°C during transport, and the fruit were stored at 0°C for 49d.

134 The percentage of gray mold infected berries over the storage time was so low that it
135 was not possible to quantify statistically, any differences between the packaging systems.
136 The samples were then divided into two parts and stored for 4d under conditions that were
137 highly favorable for mold development: one part was stored at ambient temperature
138 ($24\pm 1^{\circ}\text{C}$, 60%RH) and the other part was stored in a humid chamber (95% RH, $20\pm 1^{\circ}\text{C}$).
139 Figure 5 depicts the results, which show that under both storage conditions gray mold
140 incidence in the potassium sorbate-coated packaging was marginally lower, although
141 statistically no significant differences ($P>0.05$) were found.

142 Antimicrobial active packaging is one of the most promising active food packaging
143 concepts for extending the shelf life of fresh produce. This technology can prevent
144 microbial growth on the product by means of interactions between the food and the
145 packaging materials (Almenar et al., 2008). In the case of the packaging used in this work
146 the additive is already available on the packaging surface and its action starts as soon as the
147 fruits are in contact with the packaging, and it is not necessary to wait for the antifungal
148 migration through the packaging structure.

149 Several research studies have shown that potassium sorbate can be used and is an
150 effective remedy for the postharvest treatment against *Penicilliumdigitatum* (Smilanick et
151 al., 2008), *Helminthosporiumsolani* (Hervieux et al., 2002), *Fusariumsambucinum*
152 (Mecteau et al., 2002), *Fusariumsolani* (El-Mogly et al., 2004), *Moniliafructigena*,
153 *Phytophthoracapsici*, *Rhizoctoniasolani* (Nikolov and Ganchev, 2011) and *B. cinerea*
154 (Nikolov and Ganchev, 2011).

155 Sofos (1989) reviewed antifungal activity of sorbates, and reported that a concentration
156 of 0.05–0.15% was needed to inhibit the growth of many fungi in foods, and this
157 concentration was influenced by pH and temperature.

158 In a previous study (Junqueira-Goncalves et al., 2014), an inhibitory effect of KS was
159 observed on the growth of *B. cinerea*, between 0.07 and 0.10% (w/v), when the initial
160 concentration of *B. cinerea* is 10^3 conidia/mL, and a specific migration concentration of KS
161 from the active packaging was found to be 46.37 ± 2.39 mg/kg, which is within the
162 compliance threshold for food legislation (60 mg/kg, Commission Regulation EU10/2011).
163 Thus, according to these results, the active concentration (46.37 ± 2.39 mg/kg) is between 5
164 and 20 times lower than the minimum inhibitory concentration stated above (0.05-0.15%).
165 This suggests an influence of KS on the lag-phase for the growth of Botrytis, i.e. KS acts
166 by delaying mold growth, but not inhibiting growth completely. The present study found
167 the fruit shelf life increased by up to 3d, and this is more important in the case of
168 raspberries due to their shorter shelf life.

169 Leistner (2000) hypothesizes that antimicrobial agents as well as all the measures taken
170 to preserve food products tend to temporarily or permanently disturb the homeostasis, i.e.
171 internal cell stability of the living organisms. When this occurs, the organism uses up all its
172 energy to overcome this disruption so it cannot multiply, remaining in the lag-phase or even
173 dying, before homeostasis is re-established.

174 Among the three berries tested, a superior efficacy of the potassium sorbate-coated
175 packaging was observed with raspberries, followed by blackberries and then blueberries.
176 Due to the fine skin and texture of the raspberries, it is easier to interact with the antifungal
177 agent on the packaging surface. The blueberries are covered in a natural protective coating
178 of powdery epicuticular wax, colloquially known as the "bloom" that makes the interaction
179 between the fruit and the potassium sorbate more difficult.

180 *3.3.Sensory Evaluation*

181 No significant difference ($P>0.05$) in appearance was detected between the fruits stored
182 in control and the active packaging, which means that the packaging transparency was not
183 affected by the KS addition on its surface, and also no aroma or flavor alterations were
184 found due to the KS addition (Table 2). The panellist group evaluated as satisfactory to
185 good, the aroma and flavour of the raspberries and blueberries without a significant
186 difference between the packaging types.

187 **4. Conclusions**

188 The potassium sorbate-coated packaging evaluated in this work proved to be efficient
189 in delaying the growth of *Botrytis* in the tested berries, by extending the mold lag-phase,
190 and thereby extending the shelf life by up to 3d.

191 The active packaging was effective at different storage temperatures.

192 Due to the physiological characteristics of raspberries and its shorter shelf life, these
193 fruit benefits more from the use of potassium sorbate-coated active packaging than
194 blackberries and blueberries, although the packaging is just as effective.

195 According to the sensory evaluation, the addition of KS did not affect the packaging
196 appearance, transparency, and the fruits did not suffer from off flavor development.

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