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

Maria Paula Junqueira-Gonçalves1*, Érica Alarcon2, Keshavan Niranjan3

1Department of Food Science and Technology, University of Santiago, Chile. Ecuador
3769, Estación Central, Santiago, Chile.
2Department of Chemical Engineering, University of Santiago, Alameda, 3363, Estación Central, Santiago, Chile.
3Department of Food and Nutritional Sciences, University of Reading, Whiteknights, PO Box 226, Reading RG6 6AP, UK.

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

*Corresponding author: Tel: +56-2-27184519; Fax: +56-2-27764796
E-mail: m paula.junqueira@usach.cl (M.P. Junqueira-Gonçalves)
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1. Introduction

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

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

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

Junqueira-Gonçalves et al. (2014) described the development of an active packaging designed with post-consumer recycled polyethylene terephthalate (PCRPET) and incorporated potassium sorbate (KS) as an antifungal agent to fight *B. cinerea*. The mechanical, thermal and optical properties of the packaging material were evaluated and its
efficacy against *B. cinerea* was demonstrated by employing a novel methodology that mimics actual food contact conditions.

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

2. Material and method

2.1. Materials

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

2.2. Packaging preparation

The incorporation of potassium sorbate on the surface of the PCRPET was carried out during sheet manufacture by passing it through a bath containing an aqueous silicone solution (2%, v/v) to which KS has been added at a concentration of 20% (w/v). Coating PET with silicone is standard practice in the manufacture of PET packaging, which prevents individual packaging pieces from adhering on high speed manufacturing lines. The addition of KS to the silicone solution imparted antifungal properties to the PET surface, as described by Junqueira-Gonçalves et al. (2014), with an active percentage of 0.005%. The sheet was converted to clamshells by thermoforming (John Brown Inc., USA). Some
clamshells were also produced from sheets that were only coated with silicone but not KS, and these constituted the control samples.

2.3. Assessment of the shelf life of berries

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

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

2.4. Sensory evaluation

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

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

3. Results and Discussion

3.1. Assessment of the shelf life of berries

- Raspberries

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

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

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

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

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

- Blueberries

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

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

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

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

The blueberries harvested in February 2013 were from the south of Chile (Region of Bio Bio) and arrived in Santiago by truck, one day after harvesting. The temperature did not exceed 5°C during transport, and the fruit were stored at 0°C for 49d.
The percentage of gray mold infected berries over the storage time was so low that it was not possible to quantify statistically, any differences between the packaging systems. The samples were then divided into two parts and stored for 4d under conditions that were highly favorable for mold development: one part was stored at ambient temperature (24±1°C, 60%RH) and the other part was stored in a humid chamber (95% RH, 20±1°C). Figure 5 depicts the results, which show that under both storage conditions gray mold incidence in the potassium sorbate-coated packaging was marginally lower, although statistically no significant differences (P>0.05) were found.

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

Several research studies have shown that potassium sorbate can be used and is an effective remedy for the postharvest treatment against *Penicillium digitatum* (Smilanick et al., 2008), *Helminthosporium solani* (Hervieux et al., 2002), *Fusarium sambucinum* (Mecteauet al., 2002), *Fusarium solani* (El-Mogyet al., 2004), *Monilia fructigena*, *Phytophthora capsici*, *Rhizoctonia solani* (Nikolov and Ganchev, 2011) and *B. cinerea* (Nikolov and Ganchev, 2011).

Sofos (1989) reviewed antifungal activity of sorbates, and reported that a concentration of 0.05–0.15% was needed to inhibit the growth of many fungi in foods, and this concentration was influenced by pH and temperature.
In a previous study (Junqueira-Goncalves et al., 2014), an inhibitory effect of KS was observed on the growth of *B. cinerea*, between 0.07 and 0.10% (w/v), when the initial concentration of *B. cinerea* is $10^3$ conidia/mL, and a specific migration concentration of KS from the active packaging was found to be $46.37\pm2.39$ mg/kg, which is within the compliance threshold for food legislation (60 mg/kg, Commission Regulation EU10/2011). Thus, according to these results, the active concentration ($46.37\pm2.39$ mg/kg) is between 5 and 20 times lower than the minimum inhibitory concentration stated above (0.05-0.15%).

This suggests an influence of KS on the lag-phase for the growth of Botrytis, i.e. KS acts by delaying mold growth, but not inhibiting growth completely. The present study found the fruit shelf life increased by up to 3d, and this is more important in the case of raspberries due to their shorter shelf life.

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

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

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

4. Conclusions

The potassium sorbate-coated packaging evaluated in this work proved to be efficient in delaying the growth of Botrytis in the tested berries, by extending the mold lag-phase, and thereby extending the shelf life by up to 3d. The active packaging was effective at different storage temperatures. Due to the physiological characteristics of raspberries and its shorter shelf life, these fruit benefits more from the use of potassium sorbate-coated active packaging than blackberries and blueberries, although the packaging is just as effective. According to the sensory evaluation, the addition of KS did not affect the packaging appearance, transparency, and the fruits did not suffer from off flavor development.

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