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Research Papers

Starch-glycerol monostearate edible coatings formulated with sodium benzoate control postharvest citrus diseases caused by *Penicillium digitatum* and *Penicillium italicum*

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Summary. The curative antifungal activity of edible composite coatings (ECs) based on pregelatinized potato starch-glycerol monostearate (PPS-GMS) formulated with or without sodium benzoate (SB) to control green mould (caused by *Penicillium digitatum*) and blue mould (*P. italicum*) was assessed on 'Orri' mandarins, 'Valencia' oranges and 'Fino' lemons. These fruit were artificially inoculated with *P. digitatum* or *P. italicum*, treated by immersion in coating emulsions and compared to uncoated control fruit immersed in water and fruit immersed in 2% SB (w/v) aqueous solution. Treated fruit were then stored at either 20°C or commercial low temperature (5°C for mandarins and oranges, 12°C for lemons). Coatings without SB did not exhibit antifungal activity, whereas coatings containing 2% SB reduced incidence and severity of green and blue moulds, in comparison to the controls, on all citrus species and in all storage conditions, without differing from the application of 2% SB alone. For example, incidence reduction on 'Fino' lemons was from 99 to 0% after 7 d at 20°C, and from 99 to 30% after 2 weeks at 12°C. None of the treatments was phytotoxic. These results indicate that applications of SB as antifungal ingredient of PPS-GMS based ECs is a promising non-polluting alternative to control *Penicillium* postharvest decay of citrus, and these ECs are effective substitutes for conventional waxes amended with synthetic fungicides.

Keywords. Green mould, blue mould, alternative disease control, antifungal fruit coatings, GRAS salts.

INTRODUCTION

Fungal pathogens are one of the main factors contributing to citrus spoilage and quality deterioration during postharvest fruit handling, leading to significant economic losses (Zacarias *et al.*, 2020). Green mould (GM;

see Table 1 for definitions of abbreviations used in this paper) and blue mould (BM), caused, respectively, by *Penicillium digitatum* (Pers.: Fr.) Sacc. and *Penicillium italicum* Wehmer, are the most important postharvest citrus diseases, particularly in Mediterranean climate regions. These fungi are strict wound pathogens that infect citrus fruit through rind injuries caused during harvest, transportation, and postharvest handling and commercialization (Palou, 2014; Smilanick *et al.*, 2020).

Treatments with synthetic fungicides applied as aqueous solutions or added to waxes have been traditionally used to reduce postharvest citrus decay to commercially acceptable levels (Erasmus *et al.*, 2013; Njombolwana *et al.*, 2013). However, due to legislative restrictions and consumer trends, the citrus industry demands safer approaches to control postharvest diseases. Alternative control methods include different physical treatments, antimicrobial antagonists used as biocontrol agents, and low-toxicity chemicals classified as food additives or generally recognized as safe (GRAS) compounds. These compounds include organic and inorganic salts, chitosan, essential oils and other plant extracts (Moscoso-Ramírez *et al.*, 2013; Palou *et al.*, 2016; Palou, 2018; Papoutsis *et al.*, 2019; Sapper *et al.*, 2019).

Among the different disease management alternatives, GRAS salts present important advantages, including high water solubility, availability, and general low cost (Palou, 2018). Thus, their potential to control citrus postharvest decay as aqueous solutions or as ingredients of composite edible coatings (ECs) is an active research field (Palou *et al.*, 2015; Montesinos-Herrero *et al.*, 2016). The effectiveness of GRAS salts, including benzoates, bicarbonates, carbonates, metabisulfites, parabens, silicates, and sorbates, for control of major postharvest citrus diseases has been demonstrated in previous studies (Palou *et al.*, 2002; Smilanick *et al.*, 2008; Valencia-Chamorro *et al.*, 2009a; Askarne *et al.*, 2013; Moscoso-Ramírez *et al.*, 2013; Youssef *et al.*, 2014; Montesinos-Herrero *et al.*, 2016; Guimarães *et al.*, 2019; Martínez-Blay *et al.*, 2020a; 2020b). We have found that, among these salts, aqueous solutions of sodium benzoate (SB) had substantial curative activity against citrus GM and BM (Montesinos-Herrero *et al.*, 2016). Therefore, we have evaluated ECs containing this salt as an antifungal ingredient.

Since ECs on fruit act as water and gas barriers, the use of ECs formulated with antifungal GRAS ingredients allows coating the fruit directly with a thin layer of edible material to provide antifungal activity, maintain fruit physicochemical quality and extend shelf life (Janjarasskul and Krochta, 2010; Valencia-Chamorro *et al.*, 2011a; Palou *et al.*, 2015; Sapper and Chiralt, 2018). In addition, postharvest use of ECs containing GRAS

compounds may facilitate slow diffusion of active ingredient from coating matrices, compared to application of aqueous solutions (Palou *et al.*, 2015; Palou, 2018). We have previously demonstrated that ECs based on hydroxypropyl methylcellulose (HPMC) containing antifungal GRAS salts reduced brown rot in plums (Karaca *et al.*, 2014; Gunaydin *et al.*, 2017) and *Alternaria* black spot and gray mould in cherry tomatoes (Fagundes *et al.*, 2013; 2015), while the physicochemical and sensory qualities of the fruit were maintained. Furthermore, on citrus fruit, these ECs controlled GM and BM (Valencia-Chamorro *et al.*, 2008; 2009a; 2009b; 2010; 2011b), *Diplodia* stem-end rot (Guimarães *et al.* 2019) and postharvest anthracnose (Martínez-Blay *et al.*, 2020a), while fruit quality was preserved during cold storage. However, the effectiveness and stability of the ECs depended on their composition, and the incorporation of antifungal GRAS salts greatly changed the original coating matrix properties. This indicated the need to optimize the formulations for each target pathogen and fruit species or cultivar (Valencia-Chamorro *et al.*, 2011a; Palou *et al.*, 2015).

In addition to HPMC, starch has been reported as a promising polysaccharide for ECs due to its biodegradability, biocompatibility, availability and low cost, creating odourless, tasteless and transparent films with good fruit preservation properties (Acosta *et al.*, 2015; Sapper and Chiralt, 2018). In addition, some studies have reported the antifungal activity of starch-based ECs amended with GRAS ingredients such as essential oils (Sapper *et al.*, 2019), natamycin-cyclodextrin complex (Yang *et al.*, 2019), biocontrol agents (Marín *et al.*, 2016), lactic acid bacteria (Marín *et al.*, 2019) and potassium sorbate (Mehyar *et al.*, 2011) to control different postharvest diseases in apple, cucumbers, grapes, persimmon or tomatoes. However, no information is available regarding the utilization of GRAS salts as ingredients of starch-based ECs to control major citrus postharvest diseases. Considering the importance of factors such as coating composition (i.e., type of ingredients and relative content) on coating performance, we have developed and optimized ECs formulated with SB as the antifungal ingredient and different ratios of pregelatinized potato starch (PPS), glyceryl monostearate (GMS) and glycerol as hydrophobic and plasticizer components to maintain the physicochemical and sensory quality of 'Orri' mandarins during storage (Soto-Muñoz *et al.*, 2021). From that research, two antifungal ECs were selected as promising treatments to maintain quality, reduce decay and extend postharvest life of mandarins.

The objective of the present study was to assess the efficacy of the optimized antifungal PPS-based ECs containing SB for control of GM and BM on mandarins,

oranges and lemons. Curative activity of the ECs was assessed on fruit artificially inoculated with the postharvest pathogens and stored at either 20°C or commercial low temperatures (5°C for mandarins and oranges, 12°C for lemons).

MATERIALS AND METHODS

Abbreviations used in this paper are presented in Table 1.

Fruit

Experiments were conducted with ‘Orri’ mandarins (*Citrus reticulata* Blanco), ‘Valencia’ oranges (*Citrus sinensis* (L.) Osbeck) and ‘Fino’ lemons (*Citrus limon* (L.) Osbeck). Commercially mature fruit were collected from citrus orchards in the Valencia area (Spain), and were used the same day or stored [5°C, 90% relative humidity (RH)] for up to 1 week before use. No commercial postharvest treatments were applied to the fruit before the experiments. Fruit were selected for uniformity of size and shape, and diseased or mechanically damaged fruit were discarded. Selected fruit were surface disinfected (4-min dips in 0.5% sodium hypochlorite solution), rinsed with tap water, allowed to air dry at room temperature, and then randomized before each experiment.

Fungal inoculations

The fungus strains NAV-7 of *P. digitatum* and MAV-1 of *P. italicum* were obtained from decayed citrus fruit

from local packhouses in the Valencia region. These strains were isolated, identified and maintained in the culture collection of postharvest pathogens of the IVIA CTP, after being selected for their aggressiveness and uniform behaviour on fruit of the most commercially important citrus cultivars. These isolates were deposited in the Spanish Type Culture Collection (CECT, University of Valencia, Valencia, Spain) with the accession numbers CECT 21108 for NAV-7 and CECT 21109 for MAV-1. Prior to the experiments, the two isolates were incubated on potato dextrose agar (PDA) (Scharlab S.L.) in Petri dishes at 25°C for 7–14 d.

For fruit inoculations, conidia from 7- to 14-d-old cultures of *P. digitatum* or *P. italicum* were taken from the PDA surfaces with sterilized inoculation loops and each transferred to a sterile aqueous solution of 0.05% Tween® 80 (Panreac-Química S.A.). Conidium suspensions were then filtered through two layers of cheesecloth. Conidium numbers in suspensions were measured with a hemocytometer, and dilutions with sterile water were made to obtain an inoculum density of 10⁵ conidia mL⁻¹. Each pathogen was wound-inoculated onto different sets of fruit. For each inoculation, the tip of a stainless steel rod (1 mm wide, 2 mm long) was immersed in the conidium suspension and then inserted in the fruit rind. Each fruit was inoculated at one point in the equatorial zone. Inoculated fruit were kept at 20°C and 90% RH for 24 h before application of fruit coatings.

Preparation of edible fruit coatings

The ECs were prepared by combining PPS as biopolymer (Quimidroga, S.A.), GMS as lipidic component (Italmatch Chemicals Spa) and glycerol as plasticizer (Panreac-Química S.A.) suspended in water. SB (Sigma-Aldrich Química S.A.) was added as antifungal GRAS salt in the formulations at 2% (w/v). These ingredients were combined in different proportions to prepare four different ECs designated as F10, F6, F10/SB and F6/SB based on the optimized stable emulsions described by Soto-Muñoz *et al.* (2021), where F10 and F6 were the PPS-GMS-based ECs formulated without GRAS salt and F10/SB and F6/SB were the same ECs formulated with 2% SB. The proportions of each component and the characteristics of these ECs are detailed in Table 2. In all formulations, sunflower lecithin (LEC) and diacetyl tartaric acid esters of mono-diglycerides (DATEM) (Lase-nor S.A.) were also incorporated as emulsifiers at the ratio GMS:emulsifier of 2:1 (dry basis, db).

For each preparation, a PPS solution (5%, w/w) was first stirred at 65°C for 30 min, and then kept under magnetic stirring at 25°C overnight. The required

Table 1. Abbreviations used in this paper.

Abbreviation	Definition
GM	Green mould
BM	Blue mould
ECs	Edible coatings
GRAS	Generally recognized as safe
SB	Sodium benzoate
HPMC	Hydroxypropyl methylcellulose
PPS	Pregelatinized potato starch
GMS	Glyceryl monostearate
RH	Relative humidity
PDA	Potato dextrose agar

Table 2. Composition and characteristics of the edible fruit coatings evaluated in this study.

Composition and characteristics ^a	Edible coating			
	F10	F6	F10/SB	F6/SB
SB concentration (% w.b.)	-	-	2.0	2.0
Solid content (%)	3.9	3.5	5.9	5.5
PPS (% d.b.)	48.3	28.6	32.0	18.2
GMS (% d.b.)	13.2	28.6	8.8	18.2
Glycerol (% d.b.)	25.2	14.3	16.7	9.1
pH	3.3	3.3	5.8	5.4
Viscosity (cP)	55.1	48.6	33.6	11.2

^a SB, sodium benzoate; PPS, pregelatinized potato starch; GMS, glyceryl monostearate (GMS); w.b., wet basis; d.b., dry basis.

amount of SB aqueous solution (10% w/w), the emulsifiers and water were then added to the PPS solution, GMS and glycerol and the resulting emulsion was heated to 90°C. Once the compounds were melted, samples were homogenized using a high-shear probe mixer (Ultra-Turrax IKA® model T25; IKA-Werke) for 1 min at 12,000 rpm followed by 3 min at 22,000 rpm. Emulsions were then cooled under agitation to a temperature below 25°C by placing them in an ice/waterbath under constant agitation for 25 min. The emulsions were kept overnight at 5°C before use.

Assessment of curative activity of fruit coatings

‘Orri’ mandarins and ‘Fino’ lemons were artificially inoculated with each pathogen (as above) and incubated at 20°C for 24 h. Inoculated fruit were then individually treated by immersion (10 s at 20°C) in relevant coating emulsions, drained and allowed to air-dry at room temperature. Curative activity of ECs was assessed since the disease control treatments were applied to already infected fruit. Treatments applied were: control = uncoated (immersion in water at 20°C for 10 s); F10 coating; F6 coating; SB (immersion in 2% (w/v) SB aqueous solution at 20°C for 10 s); F10/SB coating, or F6/SB coating. For each pathogen, each treatment was applied to four replicates of five fruit each. Treated fruit were randomly placed on cavity sockets in plastic trays and incubated at 20°C and 90% RH. Incidence of GM and BM were assessed as the percentage of decayed fruit, and disease severity was determined as individual lesion diameter (mm). All wounds, including asymptomatic wounds (diam. = 0 mm), were considered. Disease inci-

dence and severity were assessed after 4 and 7 d incubation at 20°C. Experiments were repeated once, and average data are presented.

Effectiveness of fruit coatings during cold storage

‘Orri’ mandarins, ‘Valencia’ oranges and ‘Fino’ lemons were inoculated and treated as described above (the six different treatments were applied to each of the three fruit species). For each host and disease (GM or BM), each treatment was applied to four replicates of ten fruit each. Treated fruit were randomly placed on cavity sockets in plastic trays and then cold-stored in recommended commercial conditions. The mandarins and oranges were stored at 5°C, and the lemons were kept at 12°C to avoid chilling injury (Ladaniya, 2008; Zacarias *et al.*, 2020). RH was 90% in all cases. Incidence and severity of GM and BM were assessed as described above. These parameters were evaluated for mandarins after 2, 3, 4 and 5 weeks of cold storage, for oranges after 4, 6 and 10 weeks, and for lemons after 1, 2 and 3 weeks. Every trial was conducted twice, and average data are presented.

Statistical analyses

Data from all experiments were subjected to analysis of variance (ANOVA). Since experiment was not a statistically significant factor, means of repeated experiments are presented. Disease incidence proportions were arcsine square root transformed to improve the homogeneity of variances. Where appropriate, Fisher’s Protected Least Significant Difference (LSD) test, at the 95% level of confidence ($P = 0.05$), was used for means separation. Non-transformed means are presented. All statistical analyses were carried out using Statgraphics Centurion XVII software (Statgraphics Technologies Inc.).

RESULTS

Curative activity of fruit coatings

The ECs formulated without SB (F6 and F10) did not exhibit activity against GM and BM on ‘Orri’ mandarins incubated for 4 and 7 d at 20°C, with similar or greater mean incidence and severity values in all cases than those for uncoated control fruit (Figure 1). In contrast, the ECs formulated with SB (F6/SB and F10/SB) significantly reduced the incidence and

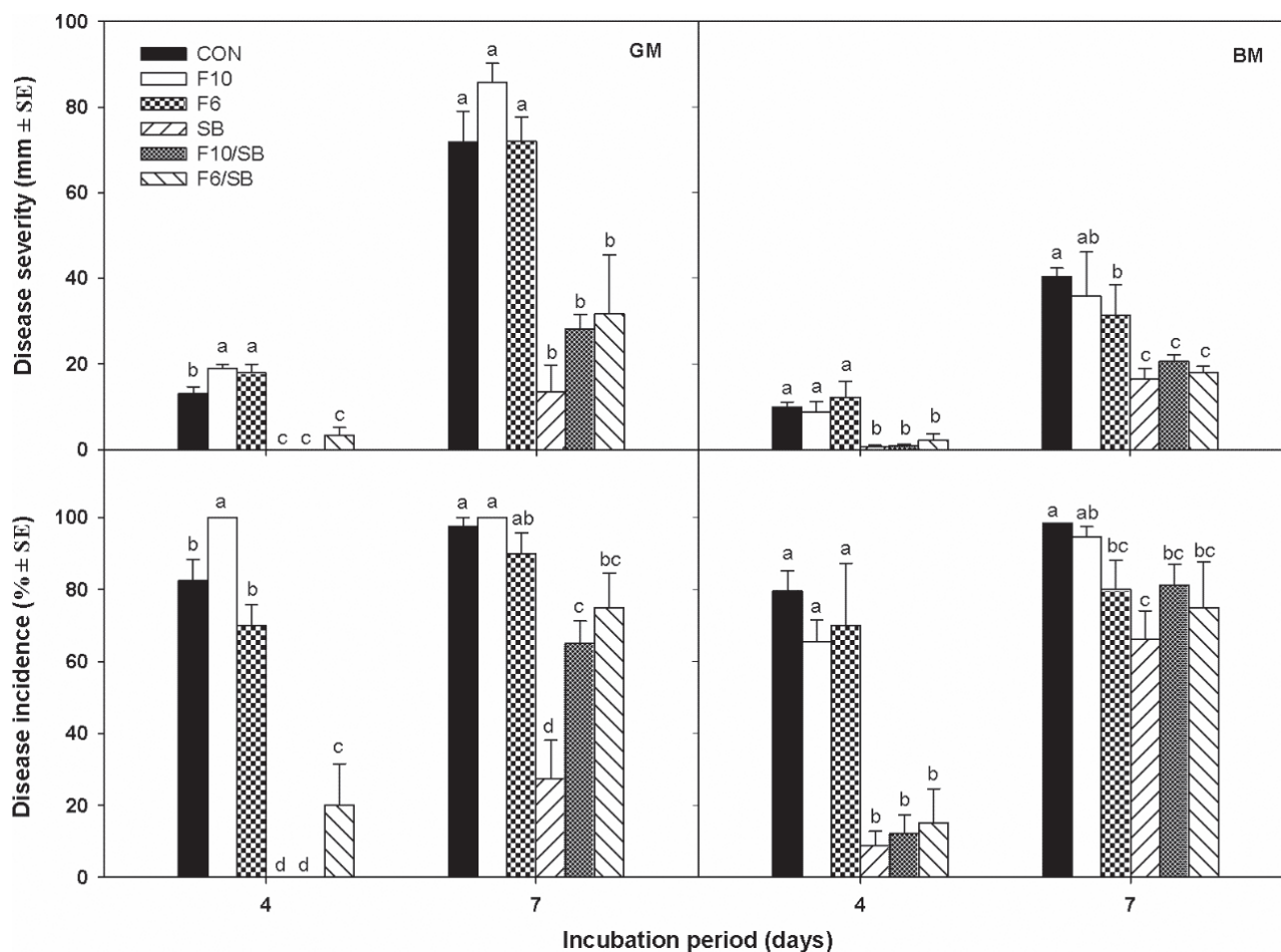


Figure 1. Mean severity and incidence of green mould (GM) or blue mould (BM) on 'Orri' mandarins artificially inoculated, respectively, with *Penicillium digitatum* or *P. italicum*, then coated 24 h later and incubated for 4 and 7 d at 20°C and 90% RH. Treatments applied were: control (CON) = uncoated (immersion in water), F10 coating, F6 coating, 2% sodium benzoate (SB) aqueous solution (w/v), F10/SB coating, or F6/SB coating (see Table 2 for coating composition). For each disease and incubation period, columns accompanied by different letters are significantly different (Fisher's protected LSD test; $P < 0.05$). Vertical lines above columns indicate standard errors. Incidence values were arcsine-transformed before statistical analyses. Non-transformed means are shown.

severity of GM and BM compared to uncoated control fruit after 4 d of incubation. Effectiveness of these antifungal ECs to reduce incidence and severity of GM and BM was similar to that obtained with the SB aqueous treatment. After 7 d, incidence and severity increased significantly in all cases. The antifungal ECs, F6/SB and F10/SB, reduced the severity of GM and BM, with severity reductions of 50–80% with respect to uncoated control fruit, and no significant differences were observed with the SB treatment. However, although both antifungal ECs reduced the incidence of GM, they were less effective than SB in aqueous solution, with reductions of approx. 30% for coated fruit in comparison to 70% for SB-treated fruit. Similar reductions of 20–30% were observed for BM incidence

on coated 'Orri' mandarins after 7 d of storage, but in this case without statistically significant differences from the SB treatment.

On 'Fino' lemons incubated up to 7 d at 20°C, F6 and F10 (without SB) did not reduce the incidence and severity of GM and BM compared to uncoated control fruit, with few exceptions (Figure 2). For example, incidence reductions of approx. 20–30% were obtained with F6 against BM. However, these reductions were always less than those obtained with the antifungal ECs containing SB or the SB aqueous solution, showing that the PPS-based matrices without GRAS salt exhibited no relevant antifungal activity. After 7 d of incubation, the incidence and severity of GM was nil on fruit coated with F10/SB or treated with 2% SB aqueous solution,

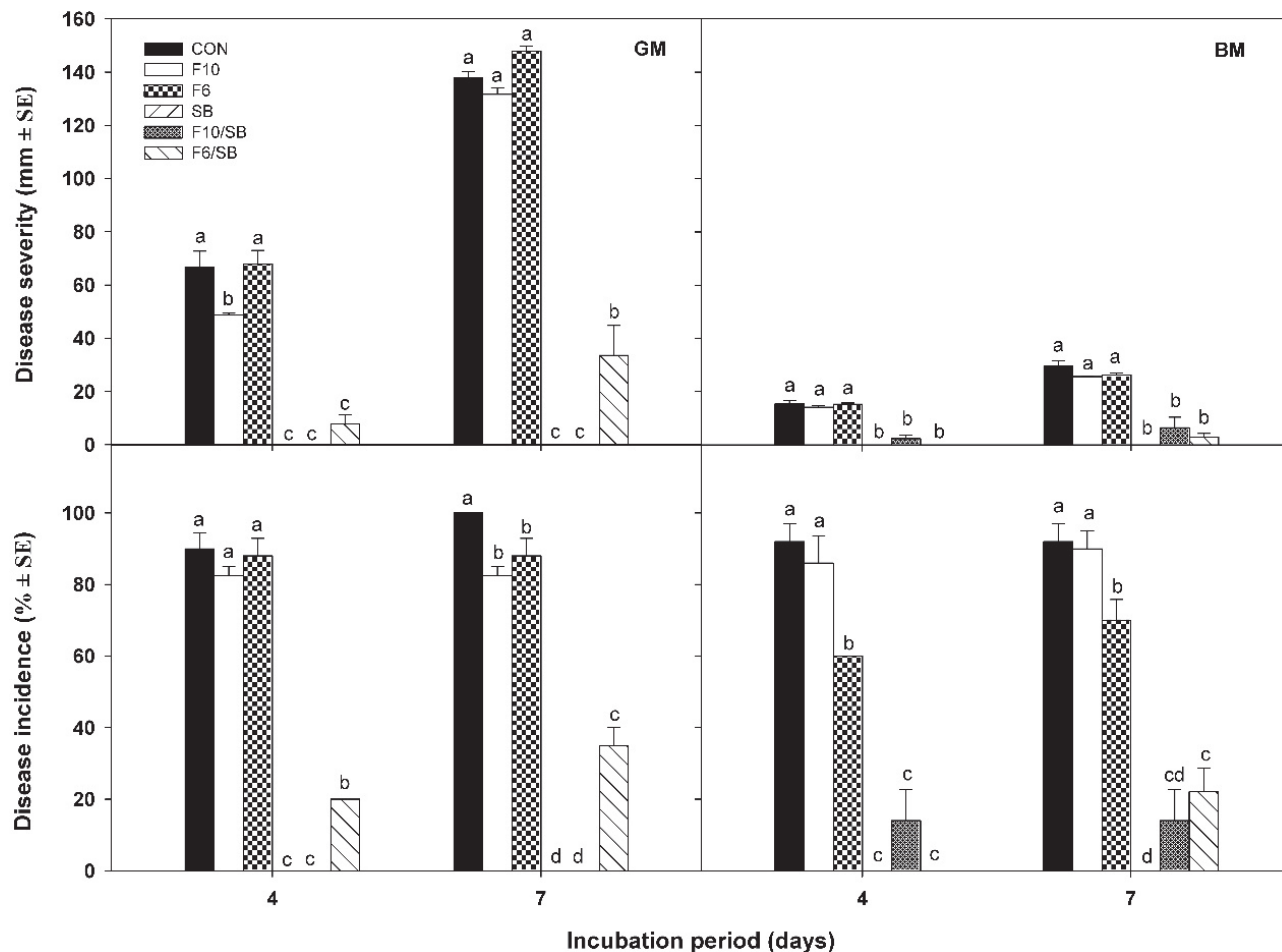


Figure 2. Mean severity and incidence of green mould (GM) or blue mould (BM) on 'Fino' lemons artificially inoculated, respectively, with *Penicillium digitatum* or *P. italicum*, then coated 24 h later and incubated for 4 and 7 d at 20°C and 90% RH. Treatments applied were: control (CON) = uncoated (immersion in water), F10 coating, F6 coating, 2% sodium benzoate (SB) aqueous solution (w/v), F10/SB coating, or F6/SB coating (see Table 2 for coating composition). For each disease and incubation period, columns accompanied by different letters are significantly different (Fisher's protected LSD test; $P < 0.05$). Vertical lines above columns indicate standard errors. Incidence values were arcsine-transformed before statistical analyses. Non-transformed means are shown.

whereas the F6/SB coating reduced incidence of GM by 65% and severity by 75%, compared to control fruit. For BM, the reductions after 4 d of both incidence and severity were 85–100% on coated lemons and lemons immersed in 2% SB solution, without statistically significant differences among these treatments. These reductions were maintained after 7 d of incubation, although the reduction in BM incidence for the lemons coated with F6/SB and F10/SB was less than for the fruit treated with aqueous SB, which completely inhibited GM development. Nevertheless, these antifungal coatings reduced the incidence of BM by approx. 85% compared to control fruit.

In every test, no phytotoxicity was observed on the rind of treated fruit.

Effectiveness of fruit coatings during cold storage

The effectiveness of treatments applied for control of GM and BM on 'Orri' mandarins cold-stored at 5°C for up to 5 weeks is illustrated in Figure 3. The ECs without SB (F6 and F10) did not control either GM or BM. In contrast, the applications of SB, alone as aqueous solution or incorporated into ECs, reduced GM severity, achieving reductions of 80–100% after 2 weeks, 90–100% after 3 weeks, 75–95% after 4 weeks, and 70–90% after 5 weeks, compared to the uncoated control. On the other hand, F10/SB and SB treatments reduced GM incidence, with reductions of 95–100% after 2 weeks, 60–80% after 4 weeks, and 55–65% after 5 weeks, compared to control fruit, without statistically significant differences between

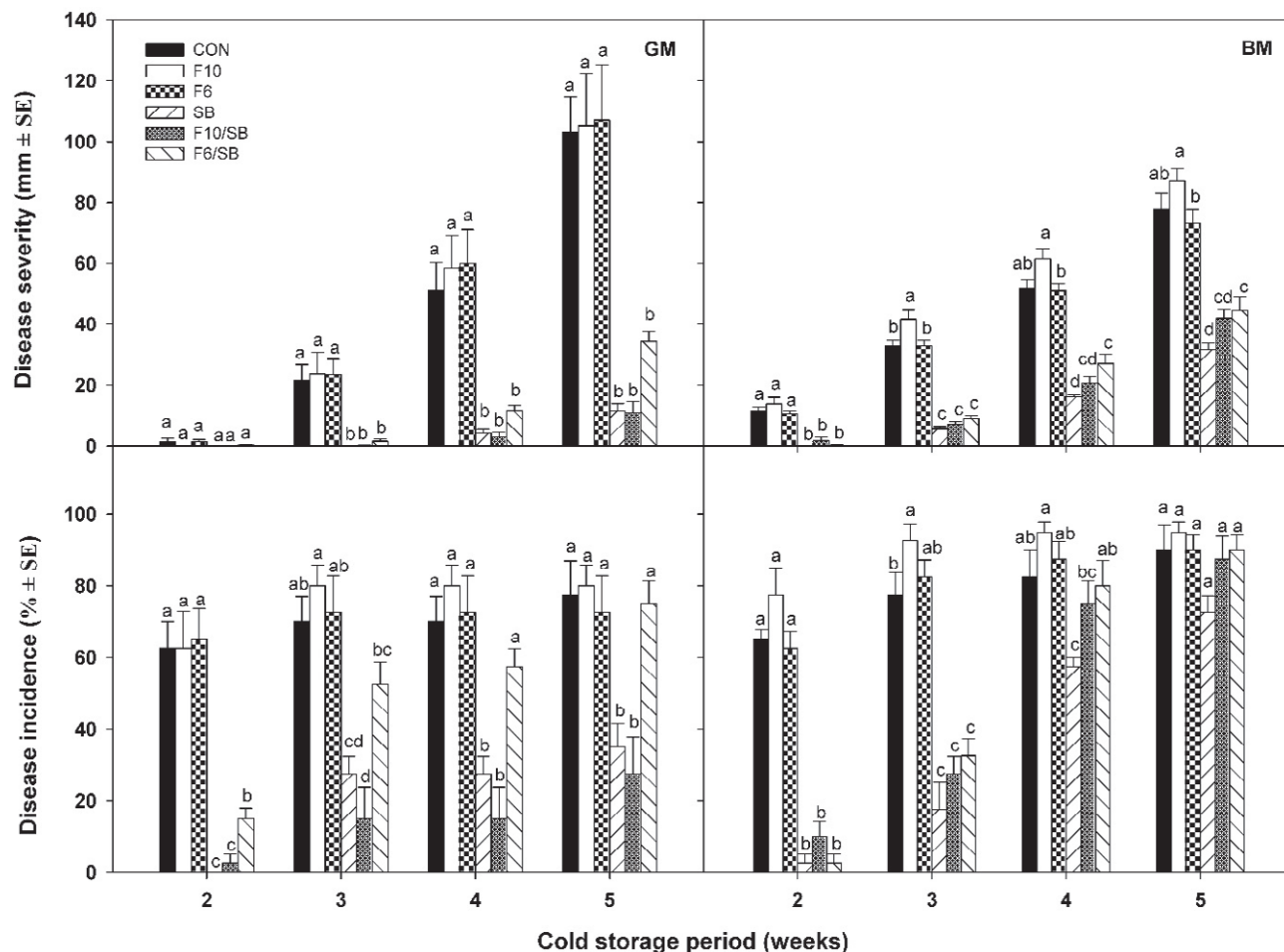


Figure 3. Mean severity and incidence of green mould (GM) or blue mould (BM) on 'Orri' mandarins artificially inoculated, respectively, with *Penicillium digitatum* or *P. italicum*, then coated 24 h later and cold-stored for 2, 3, 4 and 5 weeks at 5°C and 90% RH. Treatments applied were: control (CON) = uncoated (immersion in water), F10 coating, F6 coating, 2% sodium benzoate (SB) aqueous solution (w/v), F10/SB coating, or F6/SB coating (see Table 2 for coating composition). For each disease and incubation period, columns accompanied by different letters are significantly different (Fisher's protected LSD test; $P < 0.05$). Vertical lines above columns indicate standard errors. Incidence values were arcsine-transformed before statistical analyses. Non-transformed means are shown.

these treatments. Effectiveness of F6/SB in reducing GM incidence was less than that of F10/SB or SB, and after 4 weeks of cold storage there were no statistically significant differences between these treatments and the experimental controls. Similar results were observed for BM severity. Only the ECs or the SB aqueous solution reduced BM severity on 'Orri' mandarins during cold storage, and these reductions at the end of the storage period were greater on SB-treated fruit (60% reduction) than on coated fruit (40% reduction). For BM, the ECs reduced disease incidence during the first 3 weeks of cold storage, without statistically significant differences between coated- and SB-treated fruit, whereas, after 4 weeks, only the SB aqueous solution reduced BM incidence (up to 30%), and this effectiveness disappeared after 5 weeks of storage.

Data of severity and incidence of GM and BM on coated and uncoated 'Valencia' oranges stored for up to 10 weeks at 5°C are summarized in Figure 4. Similar to results for mandarins, only the treatments containing SB, alone or incorporated to the PPS-based ECs, reduced GM and BM on 'Valencia' oranges. In general, in all cases, the effectiveness of the fruit coatings was similar to that from the SB aqueous treatment. Thus, for example, after 10 weeks of storage, reductions in severity were 85-90% for GM and 65-75% for BM, and reductions of incidence were 30-50% for GM and 20-40% for BM.

On 'Fino' lemons, incidence of GM and BM on uncoated control samples and fruit coated with F10 and F6 (without SB) were 100% after 1 week of storage at 12°C (Figure 5). The treatments F6/SB, F10/SB and

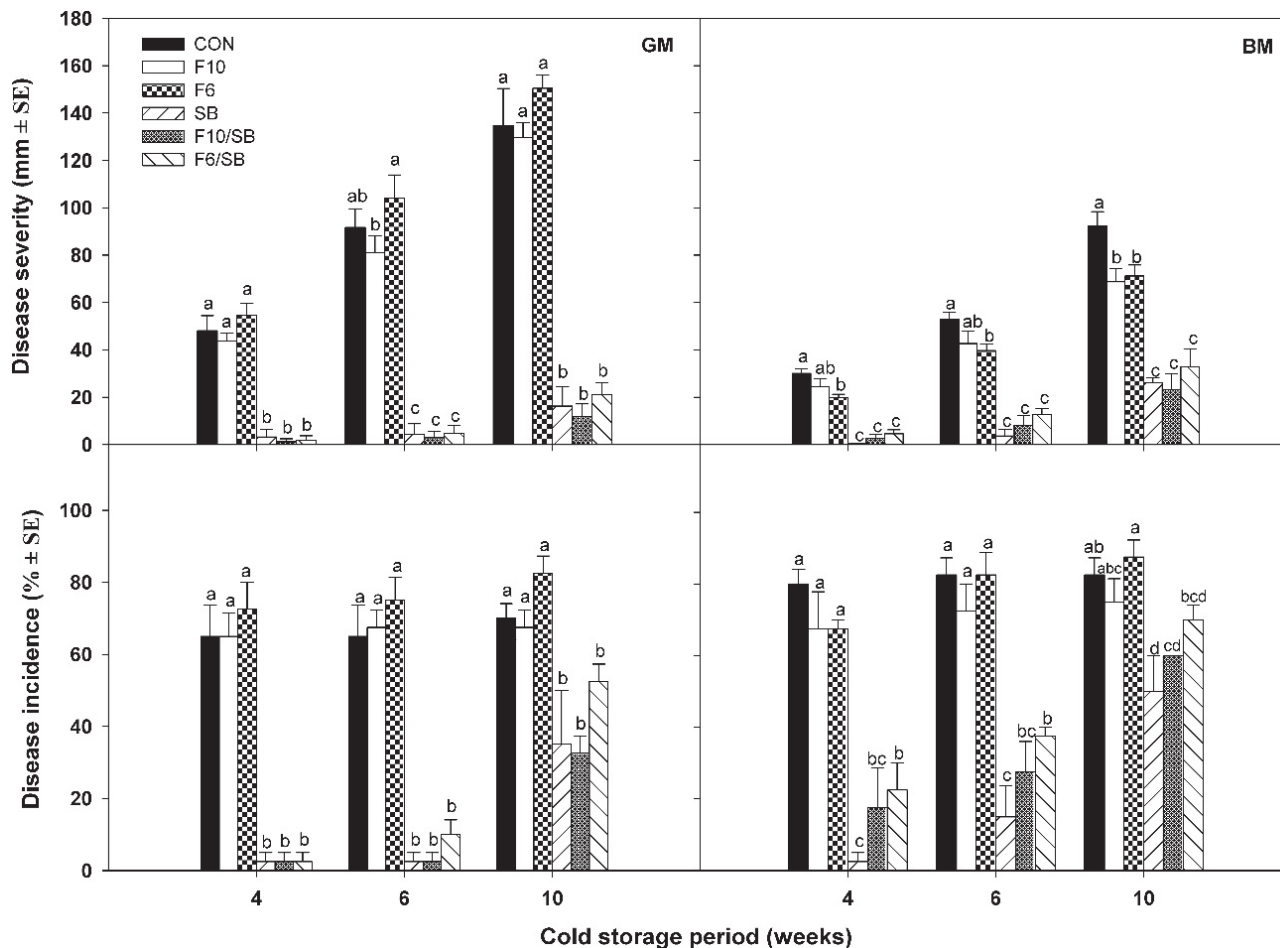


Figure 4. Mean severity and incidence of green mould (GM) or blue mould (BM) on 'Valencia' oranges artificially inoculated, respectively, with *Penicillium digitatum* or *P. italicum*, then coated 24 h later and cold-stored for 4, 6 and 10 weeks at 5°C and 90% RH. Treatments applied were: control (CON) = uncoated (immersion in water), F10 coating, F6 coating, 2% sodium benzoate (SB) aqueous solution (w/v), F10/SB coating, or F6/SB coating (see Table 2 for coating composition). For each disease and incubation period, columns accompanied by different letters are significantly different (Fisher's protected LSD test; $P < 0.05$). Vertical lines above columns indicate standard errors. Incidence values were arcsine-transformed before statistical analyses. Non-transformed means are shown.

SB reduced incidence and severity of GM and BM during the 3-week storage period compared to control fruit and fruit treated with ECs without GRAS salt. Among the treatments, F6/SB was the least effective coating against GM, with reductions of 35% in incidence and 40% in severity after 3 weeks. In contrast, the coating F10/SB and SB aqueous solution reduced GM incidence by 60–65% and severity by 75–80%. Reduction of BM severity ranged from 90–100% after 1 week of storage, and from 50–70% at the end of the 3-week storage period on lemons treated with F6/SB, F10/SB or SB, without statistically significant differences among these treatments. These treatments also reduced BM incidence during storage, and the SB treatment was more effective than F6/SB and F10/SB treatments after 1 and 2 weeks.

However, after 3 weeks, all three treatments were equally effective against BM, reducing incidence of the disease by 30–40% compared to control fruit.

Irrespective of the citrus species and the storage conditions, none of the treatments was visibly phytotoxic.

DISCUSSION

SB is regarded as a GRAS salt by regulations in many countries, and this compound is widely used as a food preservative with broad spectrum activity against yeasts and moulds (Chiple, 2005). Furthermore, the compound is effective for controlling postharvest *Penicillium* decay of citrus fruit (Montesinos-Herrero *et*

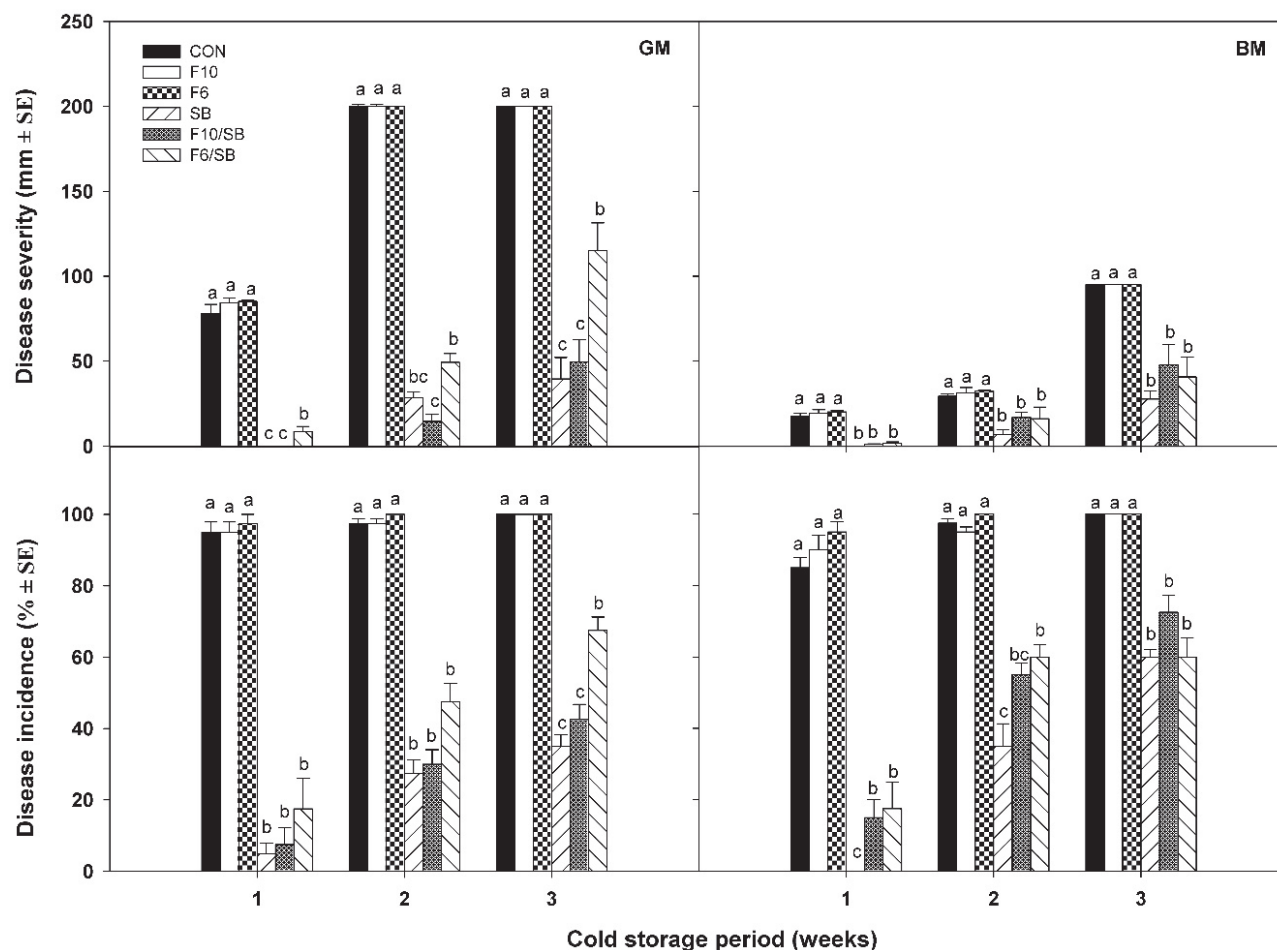


Figure 5. Mean severity and incidence of green mould (GM) or blue mould (BM) on 'Fino' lemons artificially inoculated, respectively, with *Penicillium digitatum* or *P. italicum*, then coated 24 h later and cold-stored for 7, 14 and 21 d at 12°C and 90% RH. Treatments applied were: control (CON) = uncoated (immersion in water), F10 coating, F6 coating, 2% sodium benzoate (SB) aqueous solution (w/v), F10/SB coating, or F6/SB coating (see Table 2 for coating composition). For each disease and incubation period, columns accompanied by different letters are significantly different (Fisher's protected LSD test; $P < 0.05$). Vertical lines above columns indicate standard errors. Incidence values were arcsine-transformed before statistical analyses. Non-transformed means are shown.

al., 2016; Palou, 2018). The present study has evaluated incorporation of SB at a concentration of 2% as an ingredient of novel PPS-GMS-based ECs, after optimization in previous research by response surface methodology of fruit coating ingredients for improving the postharvest quality of 'Orri' mandarins (Soto-Muñoz *et al.*, 2021). The results presented here demonstrate the effectiveness of these antifungal PPS-GMS-based ECs for control of GM and BM on fruits of three citrus species. This is the first report on the effectiveness of these types of ECs for control of major citrus postharvest diseases.

In general, the functionality of ECs based on polysaccharide matrices that do not exert direct inhibitory effects against spoilage microorganisms, in contrast to other ECs such as chitosan or *Aloe vera* gels, can be

improved by incorporation of additional antifungal ingredients such as GRAS salts or food-grade preservatives. In these amended coatings, incorporation of the antifungal ingredients may facilitate slow diffusion of active ingredients from the matrices, regulating temporal and spatial release and facilitating continuous and effective contact with target pathogens, thus enhancing their effectiveness (Mehyar *et al.*, 2011). These incorporations may also reduce possible phytotoxicity risks or adverse sensory properties derived from the direct application of the antifungal ingredient (Vargas *et al.*, 2008; Palou *et al.*, 2015; Palou, 2018; Sapper and Chiralt, 2018).

Overall, we observed that the coatings without SB did not exhibit activity in any of the conditions tested, confirming that the SB salt was responsible for the effec-

tiveness of the emulsions F6 and F10 for control of GM and BM on all three citrus species studied. These results are similar to those from previous studies, which showed that starch-based coatings and films can control pathogenic fungi and bacteria only if the coating matrices are amended with antifungal ingredients. Durango *et al.* (2006) developed antimicrobial ECs based on yam starch combined with chitosan to control microbial growth on minimally processed carrots, and their results showed that only the ECs containing chitosan reduced growth of pathogenic bacteria. Similarly, Ratnawati and Afifah (2019) reported that arrowroot starch-based films alone did not inhibit foodborne pathogenic bacteria, whereas films amended with the GRAS salts SB, potassium sorbate or calcium propionate did inhibit these organisms. SB was the most effective antibacterial salt, and its antimicrobial effect was related to decreased external pH, alteration of the integrity and permeability of bacteria cell membranes, as well as disturbance of nutrient transport (Lucera *et al.*, 2012). A similar mechanism is likely to be associated with the behaviour of the ECs F6/SB and F10/SB applied to control GM and BM in citrus fruit. The pH of the albedo tissue of the citrus fruit rind, the site initially colonized by pathogenic *Penicillium* spp., is between 5 and 6, and is influenced by fruit maturity (Widodo *et al.*, 1996; Smilanick *et al.*, 2005). Since the ionization constant of benzoic acid is 4.1, a substantial portion would be protonated and active within wounds of citrus rind. This reduction in intracellular pH caused by the accumulation of benzoic acid at low external pH inhibits glycolysis at the stage of phosphofructokinase, causing a fall in ATP and consequent inhibition of cell growth (Krebs *et al.*, 1983; Chipley, 2005; Montesinos-Herrero *et al.*, 2016).

In general, both antifungal ECs equally reduced BM incidence and severity in all three citrus fruit and in the studied storage conditions. However, the emulsion F10/SB was more effective than F6/SB for control of GM on 'Fino' lemons after incubation at 20°C and cold storage, and on 'Orri' mandarins during cold storage. On the other hand, overall, no significant differences were found between the antifungal ECs and the application of SB as aqueous solution. When a GRAS salt is incorporated into an EC and the coating is applied to fruit, the contact between the salt and the pathogen may be limited, enhanced or unaltered depending on intrinsic and extrinsic factors. These include the emulsion properties (pH and viscosity), interaction of the salt with the coating matrix and other components (e.g., emulsifiers and plasticizers), release of the salt from the coating, characteristics of the fruit outer structures, and the storage conditions (Chung *et al.*, 2001; Valencia-Chamorro *et al.*,

2011b; Fagundes *et al.*, 2013; Karaca *et al.*, 2014; Valdés *et al.*, 2017; Guimarães *et al.*, 2019; Martínez-Blay *et al.*, 2020a). Although F6/SB and F10/SB are coating matrices containing the same ingredients, their ingredient proportions are different, which confers different physical properties to the resulting coatings. The emulsion F10/SB has greater viscosity than F6/SB, which may lead to the formation of a thicker coating for F10/SB than F6/SB (i.e., greater surface solid content), ensuring greater concentration of the GRAS salt per unit fruit surface area. However, these differences may not completely explain why F10/SB was superior to F6/SB for control GM in some experiments, and further research is required to fully define their roles in disease control, particularly regarding the proportions of components used in the F10/SB and F6/SB matrices. The similar effects of the ECs and the SB in aqueous solution for control of GM and BM suggest that the coating matrix did not limit salt activity, allowing it to act within the infected fruit rind wounds. However, since similar effectiveness of ECs and aqueous SB was also observed on long-term cold-stored fruit, it can also be concluded that the coating matrix played no role in improving the persistence of the aqueous treatment during storage. In contrast, for instance, the study of López *et al.* (2013) showed that corn starch matrices containing potassium sorbate retained this salt for long periods in polymeric matrices, and actively released the salt to product surfaces, where its action was required during product storage. However, if this salt was applied by immersion or spray methods, its surface antimicrobial action decreased rapidly, so highly concentrated solution was necessary to ensure satisfactory antimicrobial activity.

The present results show that both ECs containing SB and aqueous SB controlled both GM and BM more effectively on lemons than oranges and more effectively on oranges than mandarins. Considering that disease on control fruit was similar for all citrus species tested, these results indicate that variations in the efficacy of the treatments were not only caused by differences in fruit species susceptibility. Disease development is affected by complex interactions between the fruit host, the pathogen and the environment. In the case of diseases caused by wound pathogens, efficacy of an antifungal GRAS salt depends on the amount of salt residue present within wound infection sites occupied by fungi, and on interactions between this residue and rind constituents (Palou *et al.*, 2002; Montesinos-Herrero *et al.*, 2016; Palou, 2018). As previously reported, and depending on the citrus species, such interactions may alter the original toxicity of the salt to the pathogen as a consequence of different rind characteristics, composition or pH (Valencia-

Chamorro *et al.*, 2009a; Montesinos-Herrero *et al.*, 2016; Palou *et al.*, 2016). In the present case, the fact that rind pH is lower in lemons than in oranges and mandarins, and that effectiveness of SB is pH-dependent, increasing the effectiveness of the salt as the pH decreases within the rind wounds (Palou *et al.*, 2002; Chipley, 2005), may explain why the effectiveness of the treatments was greater on lemons than on the other citrus species. In addition, release of SB from the polymer matrix to the rind wounds in each type of fruit may vary according to the degree of rind resistance to the diffusion of the salt. Therefore, the same ECs may considerably differ in suitability for management of fungal diseases on different fruit species and cultivars (Park, 1999; Palou *et al.*, 2015). Further research may clarify if histological and/or ultrastructural differences between the rinds of lemons, oranges and mandarins can account for different degrees of SB diffusion.

The ECs F6/SB and F10/SB and the SB treatment reduced GM more than BM, during incubation at 20°C and cold storage at 5°C. Comparing these results with those obtained in previous studies by Valencia-Chamorro *et al.* (2009a; 2009b; 2011), HPMC-beeswax-based ECs containing SB also controlled GM more effectively than BM on 'Clemenules' and 'Ortanique' mandarins and on 'Valencia' oranges incubated for up to 7 d at 20°C or long-term stored at 5°C. However, the present results show greater reductions in disease incidence and severity during incubation at 20°C, which may be due to greater release of SB in the PPS-GMS-based matrix than in the HPMC-beeswax-based matrix. During cold storage, the present results were very similar to those reported for HPMC-based coatings with SB applied to 'Valencia' oranges in equivalent experimental conditions (Valencia-Chamorro *et al.*, 2009a). Moreover, it is well known that at storage at temperatures below 10°C, *P. italicum* is well adapted, and grows more rapidly than *P. digitatum* (Smilanick *et al.*, 2020). In general on cold-stored citrus, therefore, the efficacy of postharvest antifungal treatments such as GRAS salts and antifungal ECs as alternatives to synthetic fungicides is less for control BM than GM. Furthermore, the effectiveness of the PPS-GMS-based ECs formulated with SB and the SB aqueous treatment to control BM and GM decreased during the cold storage period, confirming that the effect of the SB salt, either in aqueous solution or incorporated in the ECs, is probably fungistatic rather than fungicidal, in agreement with previous studies (Valencia-Chamorro *et al.*, 2009a, 2011b; Montesinos-Herrero *et al.*, 2016). In this sense, and considering the importance of the pH, the salt diffusion properties and the fruit host characteristics, further research on the

modes of action of SB and/or ECs containing SB against the pathogens causing citrus GM and BM should focus on the evaluation of actual SB residue levels on fruit, after treatment and during storage. These studies should also consider the role of commercial storage conditions, particularly temperature, on the stability and diffusion of SB on coated and stored fruit, and elucidate the influence of EC emulsion pH on SB persistence and effectiveness. Theoretically, an EC emulsion with low pH would have increased efficacy since a greater portion of the SB would be protonated. Another aspect that deserves further research is the ability of antimicrobial ECs in general and ECs containing SB in particular to kill or inactivate microorganisms of food safety concern, such as *Salmonella* spp., *Listeria* spp. and *Escherichia coli* (Aloui and Khwaldia, 2016). Currently, this is particularly important in citrus packhouses in the United States of America, where sanitation programmes are required to satisfy food safety audits under the Food Safety Modernization Act (FSMA) established by the Food and Drug Administration.

In summary, there is little information available on the addition of antifungal ingredients to starch-based ECs for management of postharvest fruit diseases. Nevertheless, some studies have reported significant antifungal activity when starch-based matrices were amended with antimicrobial ingredients, such as essential oils (Sapper *et al.*, 2019), biocontrol agents (Marín *et al.*, 2016, 2019), natamycin (Yang *et al.*, 2019) and the GRAS salt potassium sorbate (Mehyar *et al.*, 2011). Within this context, the general antifungal activity of starch-based ECs containing antimicrobial compounds outlined in these studies involving a variety of fresh fruit pathosystems is in agreement with the results outlined in the present paper.

The main objective of this work was to assess the antifungal curative activity of PPS-based ECs amended with the GRAS salt SB for control of major postharvest citrus diseases. We have found that PPS-GMS-based ECs reduced GM and BM on 'Orri' mandarins, 'Valencia' oranges and 'Fino' lemons artificially inoculated with *P. digitatum* and *P. italicum*, showing curative activity during fruit incubation in room conditions and postharvest storage at low temperatures. Hence, these new PPS-based ECs containing the GRAS salt SB as antifungal ingredient showed potential as promising treatments to reduce *Penicillium* citrus decay. Although both ECs gave curative antifungal activity, F10/SB was superior to F6/SB for control of GM and BM on three citrus species incubated at room temperature and also for control of GM on citrus stored at low temperatures. Therefore, PPS-GMS-based ECs, and particularly the coating F10/

SB, could be promising means for reducing decay and maintaining fruit quality during long-term cold storage, and thus be effective substitutes for conventional waxes amended with synthetic fungicides.

The information generated in this study provides a basis for further research into the application of antifungal PPS-based ECs on other commercially important citrus cultivars, and their possible combination with other alternative non-polluting methods. This research will assist the establishment of cost-effective multi-strategies to improve the control of *Penicillium* postharvest decay in citrus packhouses while preserving the overall fruit quality.

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