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Curative and Preventive Activity of Hydroxypropyl Methylcellulose-Lipid Edible Composite Coatings Containing Antifungal Food Additives to Control Citrus Postharvest Green and Blue Molds

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Edible composite coatings based on hydroxypropyl methylcellulose (HPMC), lipid components (beeswax and shellac), and food preservatives with antifungal properties were evaluated in vivo on clementine mandarins cv. Clemenules, hybrid mandarins cv. Ortanique, and oranges cv. Valencia. Their curative and preventive activity against citrus postharvest green (GM) and blue molds (BM), caused by *Penicillium digitatum* (PD) or *Penicillium italicum* (PI), respectively, were determined. Fruits were artificially inoculated before or after the application of the coatings and incubated up to 7 days at 20 °C. Selected food preservatives included mineral salts, organic acid salts, parabens, and 2-deoxy-D-glucose. Inoculated but uncoated fruits were used as controls. For curative activity, HPMC-lipid edible composite coatings containing sodium benzoate (SB) were most effective in reducing the incidence and severity of GM on clementine mandarins cv. Clemenules (86 and 90%, respectively). On this cultivar, the reduction in GM incidence by the SB-based coating was twice that of potassium sorbate (PS)-based coating. On mandarins cv. Ortanique, PS- and SB-based coatings reduced the incidence of GM and BM by more than 40 and 21%, respectively. However, the HPMC-lipid coating containing a mixture of PS and sodium propionate (PS + SP) exhibited a synergistic effect in the reduction of the incidence of GM (78%) and BM (67%). Coatings with parabens modestly reduced disease incidence and severity. On oranges cv. Valencia, coatings with food preservatives better controlled BM than GM. Coatings containing SB + PS and SB + SP reduced the incidence and severity of BM by 85% and 95%, respectively. PS- and SB- based coatings controlled GM more effectively than coatings formulated with other food preservatives. In every cultivar, fruit coated before inoculation did not show any incidence or severity reduction of both GM and BM (preventive activity). In every test, the antifungal action of the coatings was fungistatic rather than fungicidal.

KEYWORDS: Edible coatings; food preservatives; mandarins; oranges; postharvest

INTRODUCTION

Main postharvest diseases of citrus fruit are caused by *Penicillium digitatum* (Pers.:Fr.) Sacc. (PD) and *Penicillium italicum* Wehmer (PI), which causes green (GM) and blue molds (BM), respectively. For many years, these diseases have been traditionally controlled by synthetic fungicides such as thiabendazole, imazalil, or sodium ortho-phenyl phenate (*1*). New synthetic fungicides such as azoxystrobin, fludioxonil, Pyrimethanil, or trifloxystrobin, have been largely tested in Europe and in the USA (*2*−*4*), and some of them are already in use in the USA. These more recent compounds have been classified as reduced-risk fungicides by the United Sates Environmental Protection Agency (US EPA). However, extensive and prolonged use of these fungicides has elicited consumer concerns about human health risks and environmental contamination. In general, decay control methods that are alternatives to conventional synthetic fungicides can be classified as physical, chemical, or biological (*5*). Physical control methods include application of heat (curing and hot water) or irradiation (UV−C and ionizing radiation) treatments (*6*−*8*) and cold storage in controlled environments such as ozonated atmospheres (*9*). Chemical control methods comprise the use of natural or

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synthetic chemicals such as food additives or low toxicity compounds, classified as generally recognized as safe (GRAS) by the Unites States Food and Drug Administration (US FDA) (*10*−*12*). Biological control methods use yeasts, bacteria, or filamentous fungi as microbial antagonists against postharvest pathogens (*13*, *14*). In an effort to control postharvest diseases of citrus fruit, some studies have combined physical, chemical, and biological methods (*5*, *15*, *16*).

In the food industry, the use of edible films and coatings is a potential method to increase the shelf life of many food products including fruits and vegetables (*17*, *18*). Consumer interest toward natural products has led researchers to develop new edible films and coatings, which could be recognized as safe. Several advantages have been observed from the use of edible films and coatings. In fresh fruit, the creation of a semipermeable barrier to gas exchange and water vapor between the fruit and the surrounding atmosphere reduces respiration rate and moisture loss, which delay produce senescence (*19*, *20*). Moreover, edible films and coatings can add gloss and improve the fruit's visual quality (*21*, *22*). The main components of edible coatings are polysaccharides, proteins, lipids, and natural resins (*23*−*25*). Several other compounds such as plasticizers and emulsifiers may be added to edible coatings to improve their mechanical properties and form stable emulsions when lipids and hydrocolloids are combined (*23*, *26*). In addition, edible coatings and films can also act as carriers of food additives, including antioxidants, colorants, flavoring agents, and antimicrobial compounds (*25*, *27*−*29*). Edible films and coatings containing antimicrobials, such as organic acids and their salts (*30*−*32*), parabens (*33*), chitosan (*34*, *35*), essential oils, or natural antimicrobials (*36*, *37*), have been effective in delaying the growth of contaminating microorganisms during storage or distribution of fresh or minimally processed horticultural products.

In a previous study, hydroxypropyl methylcellulose (HPMC)-lipid stand-alone edible composite films containing food additives with antifungal properties were tested in vitro for inhibitory activity against PD and PI on dichloran rose-bengal chloramphenicol agar (DRBC) using a disk diameter test (*38*). Among a wide variety of tested films, those containing either some organic acid salts and their mixtures or parabens and their mixtures resulted in a significant inhibitory activity against PD and PI. It was concluded that selected HPMC-lipid films containing an appropriate food additive may hold promise as commercial antifungal edible coatings for fresh citrus fruit (*38*).

The objective of this work was to evaluate the curative and preventive activity of selected HPMC-lipid edible composite coatings containing food preservatives to control GM and BM on artificially inoculated fruit of commercially important cultivars of mandarin and orange.

MATERIALS AND METHODS

Materials. HPMC (Methocel E15) was purchased from Dow Chemical Co. (Midland, MI, USA). Shellac and beeswax (BW) (grade 1) were supplied by Fomesa Fruitech, S.L. (Valencia, Spain). Stearic acid and glycerol were from Panreac Química, S.A (Barcelona, Spain). Silicone antifoam (FG-1510) and ammonia (25%) were from Dow Corning (Belgium) and Scharlau (Sentmenat, Spain), respectively. Food preservatives were purchased from Sigma (Sigma-Aldrich Chemie, Steinheim, Germany) and included mineral salts, salts of organic acids, sodium salts of parabens, and 2-deoxy-D-glucose. Most of these chemicals are classified as food additives or GRAS compounds by the European Union or US regulations. **Table 1** shows the characteristics of the selected food preservatives applied in this study to each citrus cultivar.

Emulsions/Coating Preparation. HPMC-lipid edible composite emulsions were prepared combining the hydrophilic phase (HPMC) and the hydrophobic phase (BW and shellac) suspended in water. Glycerol and stearic acid were used as plasticizer and emulsifier, respectively. Ratios of HPMC-glycerol (2:1) (dry basis, db) and lipid components (BW/shellac)-stearic acid (5:1) (db) were kept constant throughout the study. Emulsions were made as described previously by Valencia-Chamorro et al. (*38*). Briefly, an aqueous solution of HPMC (5% w/w) was prepared. The corresponding food preservative, BW, glycerol, stearic acid, water, and two drops of antifoam were added to the HPMC solution and heated at 90 °C to melt the lipids. Shellac was previously dispersed in water at 40 °C, and ammonia (15% w/w shellac/ammonia) was added to dissolve the resin. Shellac solution was heated separately at 90 °C and added to the HPMC dispersion. Samples were homogenized with a high-shear probe mixer (Ultra-Turrax model T25, IKA-Werke, Steufen, Germany) for 4 min at 22,000 rpm. Emulsions were cooled to less than 25 °C and further agitated for 25 min. Emulsions were kept 2−3 days at 5 °C before use. These formulations were stable, and no phase separation was observed. **Table 1** shows the total solid content, the concentration of the food preservative (% wet basis, wb), the BW-shellac content (%, db), and the pH and viscosity of the formulations.

Fungal Inoculum and Fruit Inoculation. PD isolate NAV-7 and PI isolate MAV-1, obtained from decayed oranges from Valencia packinghouses, were isolated, identified, and maintained in the IVIA culture collection of postharvest pathogens. These strains were selected for their aggressiveness on the most commercially important mandarin and orange cultivars. Prior to each experiment, the isolates were grown on potato dextrose agar (PDA) (Sigma) in Petri dishes at 25 ± 1 °C for 7−10 days. A high-density conidial suspension was prepared in Tween 80 (0.05%, w/v; Panreac Química S.A.) in sterile water, passed through two layers of cheesecloth, measured with a hemeacytometer, and diluted with sterile water to achieve the desired inoculum density. Oranges (*Citrus sinensis* [L] Osbeck) cv. Valencia, clementine mandarins (*Citrus reticulata* Blanco) cv. Clemenules, and hybrid mandarins (*Citrus reticulata* × [*C. sinensis* × *C. reticulata*]) cv. Ortanique from commercial orchards in the Valencia area (Spain) were selected by hand and used in the experiments before any postharvest treatment was applied. The fruits were stored up to one week at 5 °C and 90% relative humidity (RH) before use. Before each experiment, the fruits were randomized, washed with fresh water, and allowed to dry at room temperature. The fruits were artificially inoculated with PD and/or PI (inoculum density of 10^5 spores/mL) by immersing a stainless steel rod with a probe tip 1 mm wide and 2 mm in length into the spore suspension and wounding each fruit on the equator.

Evaluation of the Curative and Preventive Activity of the Coatings. In order to determine curative activity, the fruits were inoculated with the pathogens, incubated at 20 °C for 24 h, coated by immersion (15 s at 20 °C) with the selected HPMC-lipid edible composite coatings, drained, and allowed to dry at 20 °C. To test preventive activity, the fruits were coated with the selected HPMC-lipid edible composite coatings (15 s at 20 °C), drained, allowed to dry, kept at 20 °C for 24 h, and then inoculated with the pathogens. Inoculated but uncoated fruits were used as controls. In every experiment, each treatment was applied to three replicates of 20 fruit each. All fruits were placed on plastic trays on corrugated cartons and then incubated up to 7 days at 20 °C and 90% RH to resemble the typical shelf life of citrus fruit. On clementine cv. Clemenules, different fruits were used to inoculate each pathogen. On oranges and mandarins cv. Ortanique, each fruit was inoculated with both fungus, each one on the opposite side of the equator.

Disease incidence of GM and BM was assessed as the percentage of decayed fruit after 7 days at 20 °C. For each treatment, the percentage of incidence reduction with respect to control fruit was calculated. Disease severity was determined as the diameter of the lesion (mm), and the results were reported as severity reduction (%) with respect to control fruits.

An additional study to test the performance of related coatings during longer periods of storage was conducted with oranges. Fruit inoculated with both PD and PI were coated by immersion (15 s at 20 °C) with PS- and SB-based coatings, drained, and allowed to dry at 20 °C.

Table 1. Composition and Characterization of HPMC-Lipid Edible Composite Coatings Containing Antifungal Food Additives Applied to Clementine Mandarins cv. Clemenules, Hybrid Mandarins cv. Ortanique, and Oranges cv. Valencia

food preservatives added to HPMC-lipid edible composite coatings	molecular formula	E-code[a]	food preservative (%, wb)	SC[b] (%, wb)	BW-shellac[c] (%, db)	viscosity[d] (cp)	pH[e]
Mandarins cv. Clemenules							
Mineral Salts							
sodium bicarbonate	$NaHCO_3$	E-500(I)	2.0	6	45−5	10.50	9.15
ammonium bicarbonate	NH_4HCO_3	E-237	2.0	6	25−25	5.33	9.63
Organic Acid Salts							
potassium sorbate	$C_6H_7O_2K$	E-202	2.0	6	25−25	12.50	6.83
sodium benzoate	$C_7H_5O_2Na$	E-211	2.5	8	25−25	20.67	7.44
Parabens							
sodium salt of methyl paraben	$C_8H_7NaO_3$	E-219	1.5	6	50−0	12.50	8.98
sodium salt of ethyl paraben	$C_9H_9NaO_3$	E-215	1.0	6	50−0	12.67	9.39
sodium salt of propyl paraben	$C_{10}H_{11}NaO_3$	E-217	1.0	6	50−0	13.17	9.57
Mandarins cv. Ortanique							
Mineral Salts							
sodium bicarbonate			2.0	6	45−5	11.92	8.86
Organic Acid Salts							
potassium sorbate			2.0	6	25−25	23.50	7.29
sodium benzoate			2.5	8	25−25	17.25	7.39
sodium acetate	CH_3COONa	E-262-(i)	1.0	6	50−0	11.83	7.09
sodium diacetate	$(CH_3COO)_2HNa$	E-262-(II)	1.0	6	50−0	11.17	4.61
sodium propionate	CH_3CH_2COONa	E-281	2.0	6	25−25	19.08	7.09
sodium formate	HCOONa	E-237	1.0	6	50−0	8.57	6.55
Organic Acid Salts (Mixtures)							
potassium sorbate + sodium propionate			1.5 + 0.5	6	25−25	32.50	6.95
sodium benzoate + potassium sorbate			2.0 + 0.5	8	25−25	12.08	8.23
sodium benzoate + sodium propionate			2.0 + 0.5	8	25−25	8.23	8.19
Paraben							
sodium salt of methyl paraben			1.5	6	50−0	13.92	9.06
Paraben (Mixture)							
sodium salt of methyl paraben + sodium salt of propyl paraben			1.0 + 0.5	6	50−0	36.00	9.36
Other Compounds							
EDTA	$C_{10}H_{12}N_2O_8CaNa_2$	E-385	1.5	6	45−5	13.92	6.79
2-deoxy-D-glucose	$C_6H_{12}O_5$	−	0.3	6	25−25	27.50	6.94
Oranges cv. Valencia							
Organic Acid Salts							
potassium sorbate			2.0	6	25−25	14.33	7.48
sodium benzoate			2.5	8	25−25	21.83	7.47
calcium propionate	$Ca(CH_3CH_2COO)_2$	E-282	1.0	6	50−0	13.17	5.45
calcium formate	$Ca(HCOO)_2$	E-238	1.0	6	50−0	15.00	4.93
Organic Acid Salts (Mixtures)							
potassium sorbate + sodium propionate			1.5 + 0.5	6	25−25	22.33	7.38
sodium benzoate + potassium sorbate			2.5 + 0.5	8	25−25	12.17	7.09
sodium benzoate + sodium propionate			2.5 + 0.5	8	25−25	31.67	7.12
Paraben							
sodium salt of methyl paraben			1.5		50−0	17.25	8.83
Other Compounds							
2-deoxy-D-glucose			0.5	6	25−25	24.33	7.57

[a] E-Code = number codes for food additives approved by the European Union. [b] SC = solid concentration of HPMC-lipid edible composite emulsions with food preservatives. wb = wet basis. [c] BW-shellac = concentration of Beeswax-shellac. db = dry basis. [d] Viscosity (centipoise, cp) of HPMC-lipid edible composite emulsions with food preservatives. [e] pH of HPMC-lipid edible composite emulsions with food preservatives.

Inoculated but uncoated fruits were used as controls. Each treatment was applied to three replicates of 20 fruits each. Treated fruits were stored up to 21 days at 20 °C and 90% RH, and disease incidence and severity were determined after 3, 7, 11, 18, and 21 days of storage.

Statistical Analysis. Statistical analyses of data were performed using the software Statgraphics Plus 2.1 (Manugistics, Inc., Rockville, MD). For each disease, mean differences were determined by Fisher's protected least significant difference test (LSD, $P < 0.05$) applied after an analysis of variance (ANOVA). For incidence and incidence reduction data, the ANOVA was applied to the arcsine of the square root of the percentage of decayed fruit. Nontransformed means are shown.

RESULTS

Curative Activity. Clementines cv. Clemenules, mandarins cv. Ortanique, and oranges cv. Valencia were coated with 8, 14, and 9 HPMC-lipid composite coatings containing food preservatives, respectively (**Table 1**). HPMC-based coatings reduced to some extent the incidence and severity of both GM and BM on clementines cv. Clemenules coated 24 h after artificial inoculation with PD or PI showing, therefore, variable curative activity against PD and PI (**Figure 1**). In general, the coatings more effectively controlled GM than BM. Coatings containing minerals salts such as sodium bicarbonate (SBC) and

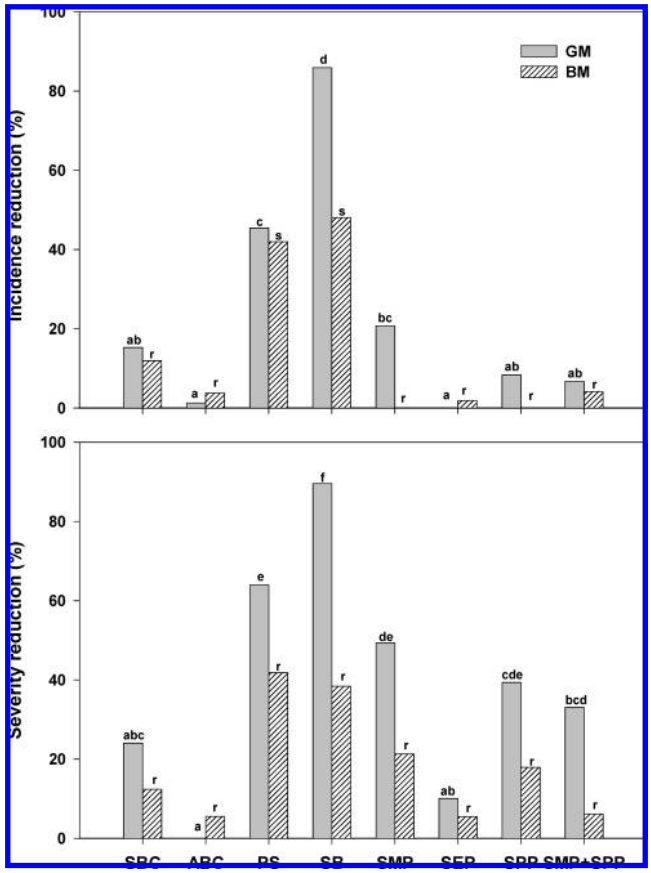

Figure 1. Curative activity of HPMC-lipid edible composite coatings against green (GM) and blue (BM) molds on mandarins cv. Clemenules artificially inoculated with *Penicillium digitatum* and *Penicillium italicum*, coated 24 h later, and incubated for 7 days at 20 °C and 90% RH. Coatings contained the following preservatives: SBC = sodium bicarbonate, ABC = ammonium bicarbonate, PS = potassium sorbate, SB = sodium benzoate, SMP = sodium salt of methyl paraben, SEP = sodium salt of ethyl paraben, SPP = sodium salt of propyl paraben, or SMP + SPP = mixture of sodium salts of methyl and propyl parabens. For each mold, disease incidence and severity reductions were determined with respect to control fruits (inoculated but uncoated fruits). Disease incidence in control treatments was 85−98% and 80−98% for *Penicillium digitatum* and *Penicillium italicum*, respectively. Disease severity in control treatments was 53−68 mm and 24−29 mm for *Penicillium digitatum* and *Penicillium italicum*, respectively. For disease incidence reduction, ANOVA was applied to the arcsine-transformed values. Nontransformed means are shown. For each mold, columns with different letters are significantly different according to Fisher's protected LSD test ($P < 0.05$) applied after an ANOVA.

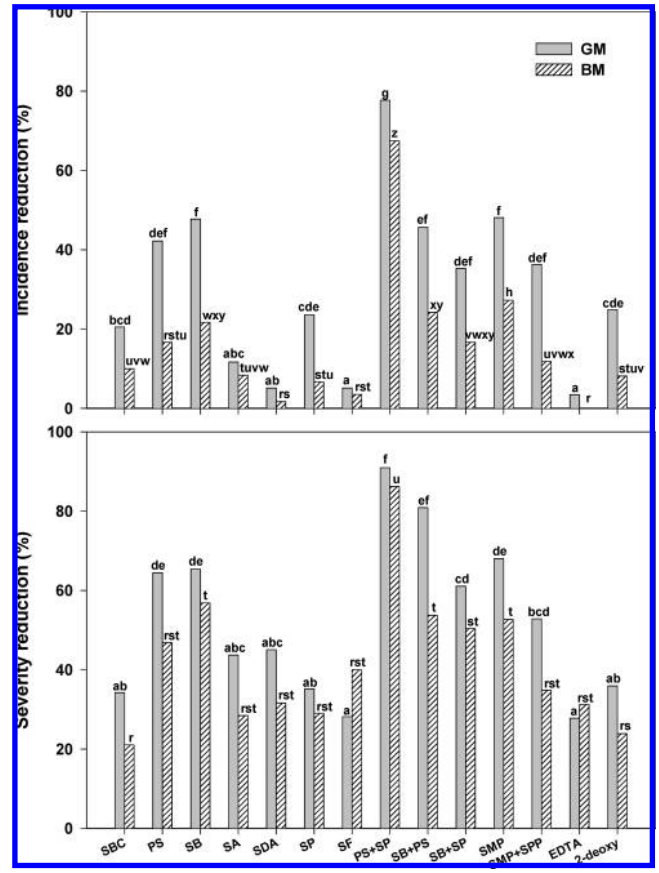

Figure 2. Curative activity of HPMC-lipid edible composite coatings against green (GM) and blue (BM) molds on hybrid mandarins cv. Ortanique artificially inoculated with *Penicillium digitatum* and *Penicillium italicum* coated 24 h later and incubated for 7 days at 20 °C and 90% RH. Coatings contained the following preservatives: SBC = sodium bicarbonate, PS = potassium sorbate, SB = sodium benzoate, SA = sodium acetate, SDA = sodium diacetate, SP = sodium propionate, SF = sodium formate, PS + SP = mixture of potassium sorbate and sodium propionate, SB + PS = mixture of sodium benzoate and potassium sorbate, SB + SP = mixture of sodium benzoate and sodium propionate, SMP = sodium salt of methyl paraben, SMP + SPP = mixture of sodium salts of methyl and propyl parabens, EDTA = ethylendiamine-tetraacetic acid, or 2-deoxy = 2-deoxy-D-glucose. For each mold, disease incidence and severity reductions were determined with respect to control fruits (inoculated but uncoated fruits). Disease incidence in control treatments was 95−100% and 85−100% for *Penicillium digitatum* and *Penicillium italicum*, respectively. Disease severity in control treatments was 50−80 mm and 30−40 mm for *Penicillium digitatum* and *Penicillium italicum*, respectively. For disease incidence reduction, ANOVA was applied to the arcsine-transformed values. Nontransformed means are shown. For each mold, columns with different letters are significantly different according to Fisher's protected LSD test ($P < 0.05$) applied after an ANOVA.

ammonium bicarbonate (ABC) very slightly reduced disease incidence. In contrast, HPMC-lipid coatings containing sodium benzoate (SB) greatly reduced disease incidence (86%) and severity (90%). GM was reduced twice as effectively on mandarins by the SB-based coating than by potassium sorbate (PS)-based coating ($P < 0.05$). The rest of food preservatives tested, including parabens or their mixtures, caused an incidence reduction of both GM and BM lower than 20%. The combination of sodium salt of methyl paraben and sodium salt of propyl paraben (SMP + SPP) did not cause any synergistic effect on the control of GM or BM (**Figure 1**).

The curative activity of HPMC-based coatings against GM and BM on mandarins cv. Ortanique is shown in **Figure 2**. In general, the coatings controlled GM more effectively than BM. Coatings with SBC reduced the incidence of BM and GM by approximately 20%. Among the six organic acid salts tested, only coatings with PS or SB reduced the incidence of GM by more than 40%. However, these coatings only reduced the incidence of BM by 21%. When mixtures of two organic acid salts were used, only the coating containing a mixture of PS and sodium propionate (PS + SP) showed a synergistic effect for the incidence reduction of GM (78%) and BM (67%) ($P < 0.05$). Moreover, this coating was overall the most effective, and reduced GM and BM by 91 and 86%, respectively ($P < 0.05$). When HPMC-lipid coatings containing SB with PS (SB + PS) or SP (SB + SP) were applied to Ortanique mandarins, the incidence reduction of GM and BM were not significantly

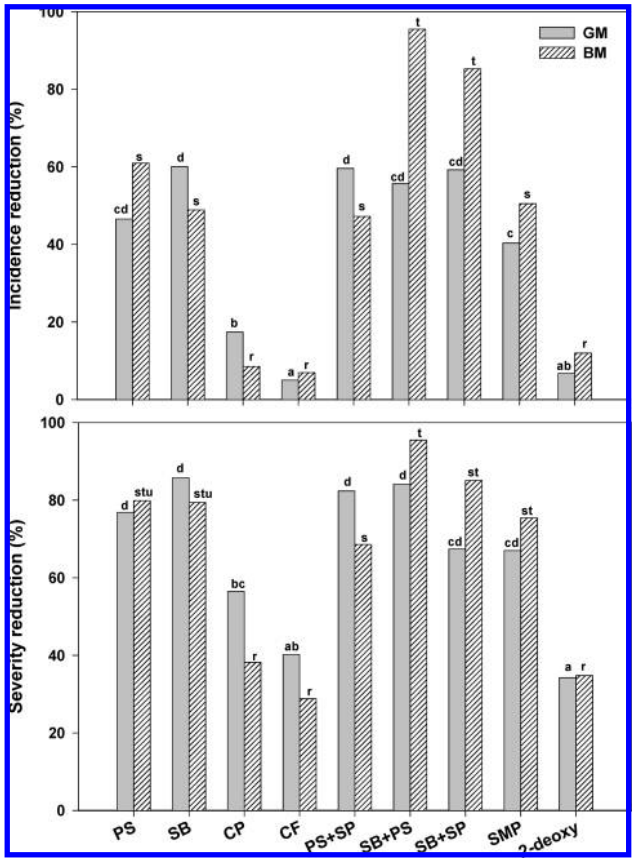

Figure 3. Curative activity of HPMC-lipid edible composite coatings against green (GM) and blue (BM) molds on hybrid oranges cv. Valencia artificially inoculated with *Penicillium digitatum* and *Penicillium italicum* coated 24 h later and incubated for 7 days at 20 °C and 90% RH. Coatings contained the following preservatives: PS = potassium sorbate, SB = sodium benzoate, CP = calcium propionate, CF = calcium formate, PS + SP = mixture of potassium sorbate and sodium propionate, SB + PS = mixture of sodium benzoate and potassium sorbate, SB + SP = mixture of sodium benzoate and sodium propionate, SMP = sodium salt of methyl paraben, or 2-deoxy = 2-deoxy-D-glucose. For each mold, disease incidence and severity reductions were determined with respect to control fruits (inoculated but uncoated fruits). Disease incidence in control treatments was 65−95% and 45−95% for *Penicillium digitatum* and *Penicillium italicum*, respectively. Disease severity in control treatments was 45−115 mm and 6−45 mm for *Penicillium digitatum* and *Penicillium italicum*, respectively. For disease incidence reduction, ANOVA was applied to the arcsine-transformed values. Nontransformed means are shown. For each mold, columns with different letters are significantly different according to Fisher's protected LSD test ($P < 0.05$) applied after an ANOVA.

different from that in mandarins coated with HPMC-lipid coatings formulated with SB alone. The rest of the coatings containing parabens, alone or in combination, modestly reduced the incidence of GM and BM. The incidence reduction of both GM and BM after the application of coatings containing 2-deoxy-D-glucose or EDTA was very low (**Figure 2**).

HPMC-lipid edible composite coatings containing food preservatives had effective curative activity on Valencia oranges. Both the incidence and severity of both GM and BM were markedly reduced (**Figure 3**). Coatings with PS, SB, or their mixtures controlled GM more effectively than coatings with the rest of the organic acid salts or 2-deoxy-D-glucose ($P < 0.05$). Among all coatings tested, those containing mixtures of SB + PS or SB + SP reduced the incidence and severity of BM by 85 and 95%, respectively ($P < 0.05$). The SMP-based coating reduced the incidence of GM and BM by 40% and 50%, respectively, and the severity of these pathogens by 67 and 75%, respectively. These reduction values were similar to those obtained with coatings formulated with organic acid salts. Low incidence reduction of GM and BM was observed on fruits coated with the rest of the food preservatives tested (**Figure 3**).

PS- and SB-based coatings effectively controlled GM and BM on Valencia oranges. Thus, an additional assay was conducted to study the performance of these coatings on Valencia oranges stored up to 21 days at 20 °C. The coatings greatly reduced disease incidence and severity when compared to those of uncoated fruit (**Figure 4**). After 7 days, GM and BM were effectively controlled by these coatings (**Figure 4**). The incidence of GM on oranges coated with PS- and SB-based coatings was around 4 and 7%, respectively, while incidence on control fruit was as high as 78% ($P < 0.05$). In addition, disease severity was approximately 3 and 11 mm, respectively, whereas disease severity on control fruit was 65 mm ($P < 0.05$) (**Figure 4**). After 14 and 21 days of incubation, GM incidence on oranges treated with PS- and SB-based coatings was less than 40 and 60%, respectively, while it was of 100% on control fruit. GM was better controlled than BM by HPMC-lipid coatings containing PS and SB. After 7 days of incubation, BM incidence was 14 and 17% for PS- and SB-based coatings, respectively. After prolonged a incubation time of 14 and 21 days, the incidence of BM was as high as 74 and 96%, respectively (**Figure 4**).

Preventive Activity. On clementines cv. Clemenules, mandarins cv. Ortanique, or oranges cv. Valencia, coating the fruit before inoculation did not significantly reduce the incidence and severity of either GM or BM (data not shown). Therefore, none of the tested coatings showed any preventive activity against the pathogens on these citrus cultivars.

DISCUSSION

In a previous study, stand-alone HPMC-lipid edible composite films containing 23 food preservatives, including mineral salts, organic acid salts and their mixtures, parabens and their mixtures, and other compounds, were tested in vitro against PD and PI using the disk diameter test (*38*). Among 14 films containing organic acid salts, only those containing PS and SB were consistently effective for the control of both PD and PI, PS showing the highest inhibitory activity (*38*). In the present in vivo study, it was found on Clemenules mandarins and Valencia oranges that HPMC-lipid composite coatings containing PS, SB, and their mixtures were, among all organic acid salts tested, the most effective to reduce the development of GM and BM. PS and SB are generally considered as safe compounds by regulations all over the world, and they are therefore widely used as food preservatives with a broad-spectrum activity against a variety of yeasts and molds (*39*, *40*). To control molds, these salts have been usually applied as aqueous solutions, but they have also been incorporated into films. Palou et al. (*11*) reported that among more than 40 food additives and low-toxicity chemicals, aqueous solutions of PS or SB were the most promising compounds to control postharvest penicillium decay on citrus fruit. In the same study, it was pointed out that GM was better controlled on lemons than on oranges. The inhibitory ability of the chemical was attributed to interactions between salt residues present in the wound infection courts, the fungus, and constituents of the rind (*39*, *40*). In other work, PS or SB was added to methylcellulose/chitosan films to study the antimycotic properties of films containing 2,

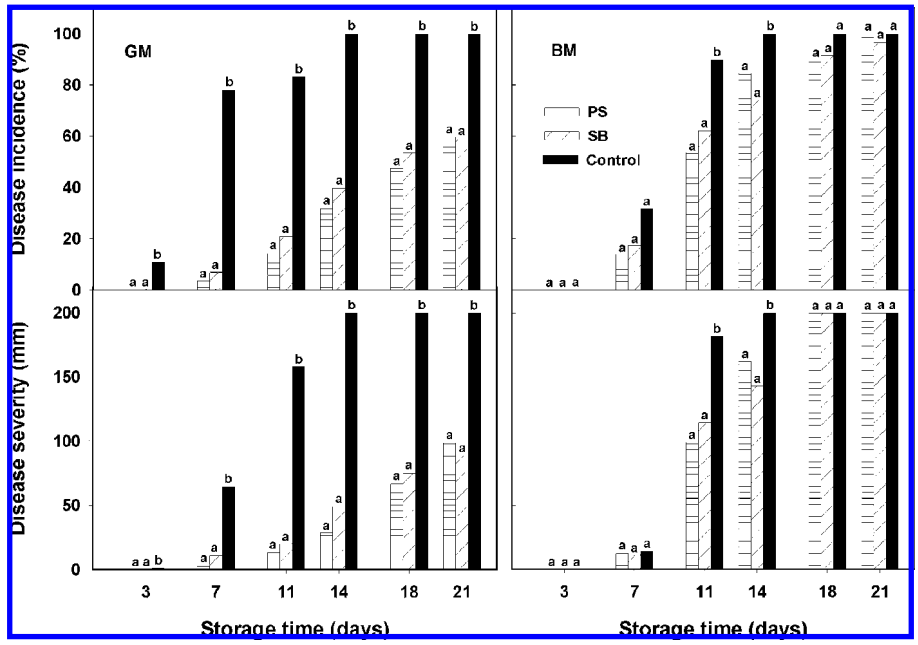

Figure 4. Curative activity of HPMC-lipid edible composite coatings against green (GM) and blue (BM) molds on oranges cv. Valencia artificially inoculated with *Penicillium digitatum* and *Penicillium italicum*, coated 24 h later, and incubated for 7 days at 20 °C and 90% RH. Treatments were as follows: Control = inoculated but uncoated, PS = potassium sorbate, and SB = sodium benzoate. For each mold, disease incidence and severity were analyzed. For disease incidence, ANOVA was applied to the arcsine-transformed values. Nontransformed means are shown. For each mold, columns with different letters are significantly different according to Fisher's protected LSD test ($P < 0.05$) applied after an ANOVA.

4, or 5% of these food preservatives (*31*). Clear inhibition zones for *Penicillium notatum* and *Rhodotorula rubra* on PDA were reported for methylcellulose films containing 2% of PS or SB. In contrast, chitosan films with the same concentration of food preservatives did not produce inhibition zones. This behavior was attributed to interactions between chitosan and the food preservatives, which prohibited their release from the films. When food preservatives were added at a concentration up to 5% to chitosan, clear inhibitory zones were reported.

Some mixtures of food preservatives were added to the coatings with the aim of obtaining a synergistic effect on antigungal activity against target molds. On Ortanique mandarins, PS + SP-based coating caused an incidence reduction of GM and BM almost two-times and three−four times higher than coatings with PS alone, respectively. SB + PS- and SB + SP-based coatings caused the highest incidence and severity reductions of BM on Valencia oranges. Specifically, the incidence reduction was almost two-times higher than that observed with coatings containing SB or PS alone. These results obtained with coated citrus fruit contrasted with those obtained with stand-alone films in previous work (*38*). Stand-alone films containing the mixtures of the food preservatives PS + SP, SB + PS, and SB + SP had similar or lower antimicrobial activity against PD and PI than films with PS or SB alone. These dissimilar results are probably due to the complex interactions among the host, pathogen, and environment that occur during in vivo disease development. Likewise, it is probable that notable variations on the growth of PD and PI in vitro and in vivo resulted from differences on the release rate of food preservatives from films located on agar medium and from coatings located on the rind of citrus fruits. Any of the three steps involved in the release of antimicrobial agents from polymer matrices, diffusion within the polymer matrices, mass transfer across the interface, and dispersion into the bulk food (*41*), can greatly affect the release of antimicrobial agents. Ponce et al. (*42*) reported some differences on the antimicrobial activity of natural extracts against *Listeria monocytogenes* when they were applied alone and when they were incorporated into coatings on squash slices. Film-forming solutions included chitosan, casein, and carboxymethyl cellulose alone as well as films enriched with oleoresins. Lower antibacterial activity was reported when olive and rosemary extracts were applied to chitosan coatings than when the extracts were applied alone. They concluded that those results were probably due to the dispersion effect of the active compounds and the interactions among them.

When coatings containing parabens were applied to citrus fruits, the incidence and severity reduction of GM and BM on mandarins were very low (**Figures 1** and **2**). In addition, the use of SMP + SPP-based coatings did not cause any synergistic effect for the control of GM and BM, resulting in lower incidence reductions than those obtained with coatings containing parabens alone. These results also contrasted with those observed with similar stand-alone films (*38*). Among all additives tested, HPMC-lipid films containing parabens with SMP, sodium salt of ethyl paraben (SEP), and the mixture SMP + SPP exhibited the highest inhibition zone for PD and PI. The poor inhibitory activity of HPMC-lipid edible composite coatings containing parabens and their mixtures when applied in vivo to citrus fruit could be likely attributed to a limited chemical release from the coating matrix to the fruit surface. In a study simulating the release of propyl paraben from a polymer coating (styrene−acrylate copolimer), Chung et al. (*33*) found that the release of the chemical from the coating into water and food-simulating solvents depended on the interactions among propyl paraben, the polymer coating, and the solvents. In addition to the release ability of the antimicrobial from the polymer matrix, each type of fruit may considerably differ in skin resistance to the diffusion of the antimicrobial agent, gas diffusion, and fruit respiration rate, among other attributes. Therefore, coatings developed for one fruit cultivar may not be suitable for another (*43*). These potential differences were confirmed in this work, in which coatings such as those containing SB or PS were generally more effective on oranges than on mandarins.

On Valencia oranges, SB- and PS-based coatings greatly reduced both disease incidence and severity after 7 days of storage at 20 °C (reductions even higher than 90%). Since these reductions were lower after longer storage periods (**Figure 4**), it can be concluded that, in general, the effects on citrus fruit of HPMC-lipid edible composite coatings containing food preservatives were fungistatic rather than fungicidal. Similarly, some aqueous solutions containing food preservatives applied to citrus fruit such as organic acid salt aqueous solutions (*11*), hot water, sodium carbonate, or sodium bicarbonate treatments (*44*, *45*), have been reported to be primarily fungistatic rather than fungicidal.

The application of HPMC-lipid edible composite coatings containing food preservatives are a simple and environmentally friendly method to reduce the losses caused by citrus postharvest diseases. Thus, these coatings could be used as an alternative to synthetic chemical fungicides for decay control in citrus packinghouses. The lack of preventive activity could be compensated for with their integration with other postharvest treatments such as the application of biological control antagonists.

Further research with HPMC-lipid coatings containing food preservatives, at a semicommercial or commercial scale, is needed to fully define the impact of these edible composite coatings on citrus fruit quality.

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