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Edible Coatings Formulated with Antifungal GRAS Salts to Control Citrus Anthracnose Caused by *Colletotrichum gloeosporioides* and Preserve Postharvest Fruit Quality

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Abstract: The in vitro antifungal activity of various generally recognized as safe (GRAS) salts against *Colletotrichum gloeosporioides*, the causal agent of citrus postharvest anthracnose, was evaluated as mycelial growth reduction on potato dextrose agar (PDA) dishes amended with salt aqueous solutions at different concentrations. The most effective treatments [0.2% ammonium carbonate (AC), 2% potassium sorbate (PS), 0.2% potassium carbonate (PC), 0.1% sodium methylparaben (SMP), 0.1% sodium ethylparaben (SEP), 2% sodium benzoate (SB) and 2% potassium silicate (PSi)] were selected as antifungal ingredients of composite edible coatings formulated with hydroxypropyl methylcellulose (HPMC)-beeswax (BW) matrixes. Stable coatings containing these salts were applied in in vivo curative experiments to “Nadorcott” mandarins and “Valencia” oranges artificially inoculated with *C. gloeosporioides* and those containing 2% PS, 2% SB and 2% PSi were the most effective to reduce anthracnose severity with respect to control fruit (up to 70% on mandarins). The effect of these selected coatings on the quality of non-inoculated and cold-stored “Valencia” oranges was determined after 28 and 56 days at 5 °C and 90% RH, followed by 7 days of shelf life at 20 °C. None of the coatings significantly reduced weight loss of coated oranges, but they modified their internal atmosphere, increasing the CO₂ content. Overall, the coatings did not adversely affect the physicochemical and sensory attributes of the fruit.

Keywords: food additives; mandarins; oranges; non-polluting postharvest decay control; cold-stored fruit

1. Introduction

Citrus (*Citrus* spp., Rutaceae) are grown in many countries with tropical and subtropical climate and are among the most important crops produced for human consumption in the world. Total worldwide production of fresh fruits exceeded 130 million tons in 2018 and the most important citrus-producing countries are China, Brazil, India, the United States of America (USA), Spain, Mexico, Egypt, Turkey, Iran, Italy, Argentina, South Africa and Morocco, among others. In terms of international trade, Spain is the leading exporter of citrus fruits for fresh consumption and Valencia is the most important citrus growing region in Spain [1].

Postharvest diseases are one of the most important problems affecting both fresh and juice citrus industries and are mainly caused by fungal pathogens. Fungi can infect the fruit before, during or after harvest, but disease develops when the fruit has been picked, causing important economic losses to

the industry in many countries [2–5]. Depending on the climate of the production area where citrus are grown, the importance of the main postharvest diseases varies. In high summer rainfall areas, such as Brazil or Florida, latent infections initiated in the fruit before harvest are the most relevant and are typically caused by the genera *Colletotrichum*, *Lasiodiplodia*, *Phomopsis*, *Alternaria* and *Phytophthora*, among others. In contrast, in areas with low summer rainfall, such as Spain and other Mediterranean countries, California or South Africa, wound pathogens that infect the fruit through injuries inflicted during harvest or after harvest are more prevalent, especially those belonging to the genera *Penicillium*, the cause of green and blue molds, and *Geotrichum*, the cause of sour rot [3,6].

Postharvest anthracnose of citrus fruits, caused by different species of *Colletotrichum*, especially *Colletotrichum gloeosporioides* (Penz.) Penz. & Sacc. (*C. gloeosporioides sensu stricto*), is an important disease in both types of production areas. Citrus anthracnose can also be a field disease, typically caused by *Colletotrichum acutatum* J.H. Simmonds, which can affect leaves and twigs and also cause post bloom fruit drop. *C. gloeosporioides* is a weak pathogen on citrus fruits. Conidia are produced abundantly in acervuli on dead plant parts and are spread over short distances, by rain or overhead irrigation, to the developing fruits. In contrast, ascospores are less numerous but airborne, taking part in long distance dispersal. The spores germinate giving rise to appressoria that generally remain latent on the fruit surface [3,7]. In general, temperatures surrounding 25 °C and relative humidity (RH) higher than 95% are optimal environmental conditions that favor *C. gloeosporioides* germination and appressorium formation [8]. Fruit colonization and decay usually occur after harvest, mostly on tissues weakened due to other factors such as sunburn, overripeness or excessively prolonged cold storage. However, the disease may also develop on early season fruit treated with ethylene for degreening purposes [7]. Symptoms of postharvest anthracnose appear after prolonged wet periods (important in summer-rainfall areas) that favor the production and dispersal of inoculum and the incidence in the field of fruit latent infections. Symptoms associated with weakened fruit are firm, dry, brown to black spots (1.5 mm or more in diameter). Under humid conditions, conidial masses, pink to salmon in color, appear on the lesion surface. Symptoms on ethylene-treated fruits are larger, firm, flat, silver gray lesions with a leathery texture. As the lesion extends, it becomes darker and may affect much of the rind and lead to a brown to black soft rot [3,7].

Postharvest applications of synthetic chemical fungicides have been used for many years as the main tool to control postharvest diseases of citrus fruits, especially green and blue molds. Some of these chemicals, such as thiabendazole (TBZ) and sodium o-phenylphenate (SOPP), have also shown some effect against diseases caused by latent pathogens, particularly against *Diplodia* and *Phomopsis* stem-end rots and anthracnose [3,9]. However, the proliferation of resistant fungal strains and the increasing public concerns about the deleterious effect of chemical residues on human health and the environment are factors limiting this practice. Therefore, the adoption of non-polluting alternatives to control citrus postharvest diseases, including anthracnose, is needed [10,11]. Among them, the use of edible coatings formulated with food-grade antifungal compounds allows coating the fruit directly with a thin layer of edible material in order to extend product shelf life [12]. This type of antifungal coatings could be a cost-effective substitute for the use of citrus commercial waxes containing chemical fungicides [13]. Polysaccharides, proteins and lipids are the main ingredients used to formulate composite edible coatings. These ingredients are mixed to reduce gas and water exchange between the fruit and the environment and to improve fruit mechanical and sensorial properties [14].

Antimicrobial ingredients used for the formulation of edible coatings should be classified as generally recognized as safe (GRAS) and approved for their use as food additives by the United States Food and Drug Administration (US FDA) or the European Food Safety Authority (EFSA) [15]. Food additives are widely used as preservatives for controlling food pH, taste or other qualities. Among them, various organic and inorganic salts have antimicrobial action and may offer a good alternative to the use of synthetic fungicides [10,16]. The main advantages of using GRAS salts include their availability, relatively low cost and high solubility in water [17]. In previous works at the IVIA CTP, we have developed and characterized hydroxypropyl methylcellulose (HPMC)-lipid edible

coatings containing GRAS salts with activity against fungal pathogens causing postharvest diseases of plums [18,19] or cherry tomatoes [20,21]. Furthermore, on citrus fruits, this type of coatings has also been effective against green and blue molds [10,22–25] and Lasiodiplodia stem-end rot [26]. Antifungal edible coatings have been successfully used to reduce postharvest anthracnose caused by *Colletotrichum* spp. on different fresh fruit commodities, such as mango, avocado, papaya and strawberry [27–29]. However, to our knowledge, there is no information available on the development of edible coatings with antifungal food additives to control citrus postharvest anthracnose caused by *C. gloeosporioides*.

The aims of this research were: (1) to evaluate the in vitro activity of various GRAS salts, at different concentrations, against *C. gloeosporioides* and (2) to develop novel stable HPMC-lipid composite coatings containing the most promising salts and concentrations. The ability of the coatings to control citrus anthracnose was assessed in in vivo experiments with mandarins and oranges artificially inoculated with the pathogen. The effects of selected antifungal edible coatings on physico-chemical and sensorial quality was also determined on oranges stored at 5 °C for up to two months.

2. Materials and Methods

2.1. GRAS Salts

The name, acronym, food additive E-number, molecular formula and molecular weight of the antifungal salts used in this work are given in Table 1. Ammonium carbonate (AC) and ammonium bicarbonate (ABC) were purchased from Thermo Fisher Scientific (Leicestershire, UK); potassium bicarbonate (PBC), potassium carbonate (PC) and sodium benzoate (SB) from Carl Roth® GmbH +Co. KG (Karlsruhe, Germany); potassium sorbate (PS), sodium ethylparaben (SEP) and sodium methylparaben (SMP) from Merck® kGaA (Darmstadt, Germany); potassium silicate (PSi) was acquired from Alfa Aesar® GmbH and Co. KG (Karlsruhe, Germany) and sodium propionate (SP) from Merck Life Science S.L.U (Madrid, Spain).

Table 1. Characteristics of antifungal GRAS salts tested in vitro to inhibit *Colletotrichum gloeosporioides* and in vivo as ingredients of edible coatings to control citrus anthracnose.

GRAS Salt	Acronym	Molecular Formula	E-Number ¹	MW ²
Ammonium bicarbonate	ABC	NH ₄ HCO ₃	E-503 (ii)	79.06
Ammonium carbonate	AC	(NH ₄) ₂ CO ₃	E-503 (i)	114.10
Potassium bicarbonate	PBC	KHCO ₃	E-501 (ii)	100.12
Potassium carbonate	PC	K ₂ CO ₃	E-501 (i)	138.21
Potassium silicate	PSi	K ₂ SiO ₃	E-560	154.26
Potassium sorbate	PS	C ₆ H ₇ O ₂ K	E-202	150.22
Sodium benzoate	SB	C ₇ H ₅ O ₂ Na	E-211	144.11
Sodium ethylparaben	SEP	C ₉ H ₉ NaO ₃	E-215	188.16
Sodium methylparaben	SMP	C ₈ H ₇ NaO ₃	E-219	174.13
Sodium propionate	SP	CH ₃ CH ₂ COONa	E-281	96.06

¹ E-number: codes for substances permitted as food additives within the European Union. ² Molecular weight (g/mol).

2.2. Fungal Pathogen

The strain *C. gloeosporioides* NAV-1 was used in the present work. It is an isolate obtained from decayed oranges from a local citrus packinghouse in the Valencia region (Spain). This fungal strain was isolated, purified, molecularly identified and maintained in the culture collection of postharvest pathogens of the IVIA CTP. It was also deposited in the Spanish Type Culture Collection (CECT, University of Valencia, Valencia, Spain) with the accession number CECT 21107. Before the experiments, the fungal isolate was incubated on potato dextrose agar (PDA) (Scharlab S.L., Barcelona, Catalonia, Spain) Petri dishes at 25 °C for 7–14 d.

2.3. In Vitro Antifungal Activity of GRAS Salts

The effect of ABC, PBC, PSi, SEP, SMP and SP on radial mycelial growth of *C. gloeosporioides* was evaluated as previously described by Guimarães et al. [26]. In brief, 90-mm plastic Petri dishes with PDA medium were amended, at 40–50 °C, with sterile aqueous solutions of the salts to achieve final concentrations of 0.2%, 1% and 2% (v/v) for ABC, PBC, PSi and SP and of 0.01%, 0.05% and 0.1% (v/v) for the paraben salts. PDA Petri dishes without salt served as controls. The center of each Petri dish was inoculated with a 5-mm diameter mycelial plug, obtained with a sterilized cork-borer, from 7 to 14-d-old cultures of *C. gloeosporioides*. The plates were incubated in a growth chamber at 25 °C in the dark. Radial mycelial growth was determined in each plate by calculating the mean of two perpendicular fungal colony diameters. Results after 3, 5 and 7 d of incubation are presented. Four replicates, each one corresponding with one plate, were used for each salt and concentration. Results are expressed as percentage of mycelial growth inhibition: $[(dc - dt)/dc] \times 100$, where dc = average diameter of the fungal colony on control plates and dt = average diameter of the fungal colony on Petri dishes amended with the salts.

2.4. Preparation of Antifungal Edible Coatings

HPMC-beeswax (BW) composite edible coatings (ECs) were prepared combining the hydrophilic phase (HPMC) with the hydrophobic phase (BW) suspended in water. Glycerol was used as a plasticizer and stearic acid (Panreac Química SA, Barcelona, Catalonia, Spain) as an emulsifier. HPMC (Methocel E15) was purchased from Dow Europe GmbH (Dow Chemical Co., Stade, Germany), glycerol from VWR International (Leuven, Belgium) and BW and stearic acid were supplied by Guinama S.L.U. (La Pobla de Vallbona, Valencia, Spain). All the formulations contained 1.3% HPMC (w/w, wet basis, wb) and 3% BW (wb). Ratios of HPMC-glycerol (2:1) and BW-stearic acid (3:1) and a total solid concentration of 6% were kept constant for all coatings. GRAS salts and concentrations were selected according to the results of the in vitro tests described above and also from the minimum effective concentration reported for *C. gloeosporioides* in previous literature references [30–36]. Then, selected salts and concentrations (w/v) were tested for compatibility with the HPMC-BW coating matrix and only those forming stable emulsions were eventually selected: AC (0.2%), PS (2%), PC (0.2%), SMP (0.1%), SEP (0.1%), SB (2%) and PSi (2%). The pH and viscosity (cP) values of HPMC-BW composite emulsions formulated with these GRAS salts were the following: 6.83 and 46.2 cP, respectively, for AC coating; 6.27 and 51.2 cP for PS coating; 7.15 and 50.0 cP for PC coating; 7.15 and 46.7 cP for SMP coating; 7.03 and 45.9 cP for SEP coating; 6.07 and 46.7 cP for SB coating and 9.50 and 60.0 cP for PSi coating.

Formulations were prepared as previously described by Guimarães et al. [26]. Briefly, an aqueous solution of HPMC (5%, w/w) was prepared by dispersing the HPMC in hot water at 90 °C and later hydration at 20 °C. Water, BW, glycerol and stearic acid were added to the HPMC solution and heated at 98 °C to melt the lipids. In the case of the coating formulated with SP, Tween® 80 (Panreac-Química S.A., Barcelona, Spain) was used as emulsifier instead of stearic acid. Samples were homogenized with a high-shear probe mixer (Ultra-Turrax IKA® model T25, IKA-Werke, Staufen, Germany) for 1 min at 12,000 rpm and 3 min at 22,000 rpm. After adding the corresponding salts, formulations were cooled under agitation (heating magnetic plate, Falc Instruments, F60, Treviglio, Italy) to a temperature lower than 25 °C by placing them in an ice bath and agitation continued for 25 min to ensure complete hydration of the HPMC.

2.5. Fruit

In vivo disease control experiments were conducted with “Nadorcott” hybrid mandarins (*Citrus reticulata* × *Citrus sinensis*) and “Valencia” oranges [*Citrus sinensis* (L.) Osbeck], whereas quality assessments on coated fruits were performed with cold-stored “Valencia” oranges. Mandarins and oranges were collected from commercial orchards in the Valencia area (Spain) and transported to

the IVIA CTP facilities. No commercial postharvest treatments were applied. Fruits were selected, randomized, surface disinfected (5-min dips in diluted commercial bleach, 0.5% sodium hypochlorite), rinsed with tap water and allowed to air-dry at room temperature to be used the following day in the experiments.

2.6. In Vivo Anthracnose Control of Antifungal Coatings

For inoculation, conidia from 7 to 14-d-old cultures were taken from PDA plates with a sterilized inoculation loop and transferred to a sterile aqueous solution of Tween® 80 (0.05%, w/v). Conidial suspension was filtered through two layers of cheesecloth and the density of the suspension was measured with a hemocytometer. Dilutions with sterile water were done to obtain an exact inoculum density of 2×10^6 spores/mL. Being a weak pathogen on citrus, to prepare the final inoculum of *C. gloeosporioides*, 5 mg/L of cycloheximide (Carl Roth GmbH + Co. KG, Karlsruhe, Germany) were added to the spore suspension in order to inhibit the possible lignification of the inflicted rind wounds.

Each fruit was wounded and inoculated simultaneously, at one point in the equatorial zone, using the tip of a stainless-steel rod (1 mm wide and 2 mm in length) previously immersed in the aforementioned conidial suspension. Inoculated fruits were incubated for 24 h at 25 °C and 95% RH. After this period, fruits were individually coated to assess the curative activity of the coatings. Three hundred microliters of coating material were pipetted onto each fruit and rubbed with gloved hands to simulate the application of coating machinery on roll-conveyors in commercial citrus packinglines [26,37]. Coated fruits were allowed to air-dry at room temperature. Inoculated but uncoated mandarins or oranges served as controls. For each citrus species, four replicates of 10 fruits each were used per treatment. Every trial was repeated once. Treated fruits were arranged on plastic cavity sockets on plastic trays and incubated at 25 °C and 95% RH.

Anthracnose development was assessed as disease severity (lesion diameter) after 7 and 15 d of incubation. Results after 15 d are presented as the percentage of severity reduction with respect to the control treatments.

2.7. Effect of Coatings on Quality of Cold-Stored Fruit

HPMC-BW coatings containing the following GRAS salts and concentrations were selected to evaluate their effect on postharvest quality of non-inoculated and cold-stored oranges: PS, PSi and SB, all at 2% (w/v). These coatings were the three most effective among those previously tested for antifungal activity. "Valencia" oranges were selected, washed, coated and stored at 5 °C for four and eight weeks, followed by a shelf-life period of 7 d at 20 °C. Uncoated oranges were used as controls. The following fruit quality attributes were determined at harvest and after cold storage and shelf life.

2.7.1. Weight Loss

Twenty fruits were used to evaluate orange weight loss during storage. After treatment, each fruit was individually numbered and weighed with a calibrated analytical balance (Alessandrini® P30, Modena, Italy). Measurements were performed at the beginning and at the end of each storage period. Results were expressed as the percentage loss of initial weight by using the formula: % WL = [(Wi – Wf)/Wi] × (100), where % WL = percentage of weight loss, Wi = initial fruit weight (g) and Wf = final fruit weight (g).

2.7.2. Fruit Firmness

Firmness of 20 oranges per treatment was determined as percentage of rind deformation, related to initial diameter, with an Instron Universal testing machine (Model 3343, Instron Corp., Canton, MA, USA), according to Valencia-Chamorro et al. [24].

2.7.3. Juice Quality

Soluble solids concentration (SSC, %), titratable acidity (TA, % of citric acid) and maturity index (MI = SSC/TA) were determined as described by Palou et al. [38] in 5 mL juice samples (three replicates of five oranges each per treatment). TA was determined with an automatic titrator (Titrator T50, Mettler Toledo, Switzerland) and SSC was measured using a digital refractometer (model ATC-1, Atago® Co., LTD, Tokyo, Japan).

2.7.4. Internal Gas Concentration

Concentrations of CO₂ and O₂ (%) in the internal cavity of 10 oranges per treatment were determined using a gas chromatograph (GC) (Thermo Trace, Thermo Fisher Scientific, Inc., Waltham, MA, USA) following the methodology described by Valencia-Chamorro et al. [24].

2.7.5. Ethanol Content (EtC) and Acetaldehyde Content (AcC)

The content of these volatile compounds (mg/L) in the headspace of 10-mL vials filled with 5 mL juice samples (three replicates of five oranges each per treatment) was analyzed by gas chromatography according to Valencia-Chamorro et al. [39].

2.7.6. Sensorial Evaluation

Overall taste (1–9 scale, from 1 = very poor to 9 = optimal), the presence of off-flavours (1–5 scale, from 1 = absence to 5 = very pronounced) and external appearance (1–3 scale: 1 = bad, 2 = acceptable and 3 = good) of four coated oranges per treatment were evaluated by a panel of 10 trained tasters following the procedures described by Valencia-Chamorro et al. [24].

2.8. Statistical Analysis

Data from in vitro tests, in vivo trials and fruit quality assessments were subjected to analyses of variance (ANOVA). Since the experiment was not a significant factor, means of repeated experiments are presented. Data on percent inhibition of mycelial growth was subjected to one-way ANOVA with the concentration of the different GRAS salts as dependent variable. Disease reduction with respect to control fruit was calculated as percentage. When appropriate, means separation was performed by Fisher's protected LEAST SIGNIFICANT DIFFERENCE test (LSD, $P = 0.05$). All statistical analyses were performed with the software Statgraphics Centurion XVII (Statgraphics Technologies Inc., The Plains, VA, USA).

3. Results

3.1. In Vitro Antifungal Activity of GRAS Salts

Table 2 shows the radial growth inhibition of colonies of *C. gloeosporioides* compared to control treatment (fungal growth on PDA not amended with GRAS salts) after 3, 5 and 7 d of incubation at 25 °C. Significant differences were found among treatments and the effect of each salt was dependent on the concentration at which it was applied. ABC and SEP were the most effective salts and completely inhibited fungal growth after 7 d of incubation at the intermediate concentrations (1 and 0.05%, respectively). In a second group, SMP also completely inhibited the growth of *C. gloeosporioides* after 7 d at the highest dose of 0.1% and inhibition with PBC exceeded 90% at the highest concentration of 2%. Growth inhibition with 2% SP after 7 d was about 80%, while the least effective GRAS salt was PSi, with 50% of growth inhibition after 7 d at the highest concentration. None of the salts was effective after 7 d of incubation at the lowest concentration tested. SMP, PBC and SP inhibited fungal growth by more than 60% at the intermediate dose tested.

Table 2. Percentage of radial growth inhibition of *Colletotrichum gloeosporioides* on PDA Petri dishes amended with GRAS salts at different concentrations after 3, 5 and 7 d of incubation at 25 °C.

GRAS Salt ¹	Concentration (%)	Inhibition of <i>C. Gloeosporioides</i> (%) ²		
		Day 3	Day 5	Day 7
ABC	0.2	61.44 de	60.49 d	33.43 g
	1	100 a	100 a	100 a
	2	100 a	100 a	100 a
PBC	0.2	20.92 h	26.80 f	23.53 h
	1	98.04 a	87.14 b	76.46 c
	2	100 a	100 a	92.89 b
PSi	0.2	16.99 h	6.43 g	9.22 i
	1	58.50 e	47.32 e	33.72 g
	2	88.56 b	76.57 c	56.96 e
SEP	0.01	40.33 f	36.04 f	35.22 g
	0.05	100 a	100 a	100 a
	0.1	100 a	100 a	100 a
SMP	0.01	30.8 g	29.38 f	29.58 gh
	0.05	100 a	95.72 ab	87.08 b
	0.1	100 a	100 a	100 a
SP	0.2	68.9 d	51.41 de	44.77 f
	1	79.0 c	71.34 c	64.67 d
	2	93.53 ab	76.5 c	79.96 c

¹ See Table 1 for acronym definitions. ² Colony diameter reduction with respect to control treatments (non-amended PDA dishes). Means in columns with different letters are significantly different by Fisher's protected LSD test ($P < 0.05$) applied after the ANOVA.

3.2. In Vivo Anthracnose Control of Antifungal Coatings

The curative effect of coating application [HPMC-BW coatings containing AC (0.2%), PS (2%), PC (0.2%), SMP (0.1%), SEP (0.1%), SB (2%) or PSi (2%)] to control citrus anthracnose after 15 d of incubation at 25 °C and 90% RH is shown in Figure 1. In a first set of experiments with "Nadorcott" mandarins, average data from two trials showed that all inoculated fruits developed decay and all the tested antifungal coatings reduced the severity of the disease (lesion size) between 45% and 70% with respect to uncoated fruits. Coatings formulated with PSi, SB and PS were the most effective, with severity reductions of 70%, 63% and 61%, respectively (Figure 1A). Similarly, in a second set of experiments with "Valencia" oranges, average data from two trials showed a significant reduction in anthracnose severity on coated oranges compared to control fruits. However, this reduction in severity was lower than in "Nadorcott" mandarins, with percentages between 10% and 35%. The most effective coating was that containing SB, followed by those formulated with PS and PSi (Figure 1B). Hence, among all tested coatings, those containing 2% PSi, SB and PS were the most effective to control citrus anthracnose, both in mandarins and oranges, with no significant differences in severity reduction among them.

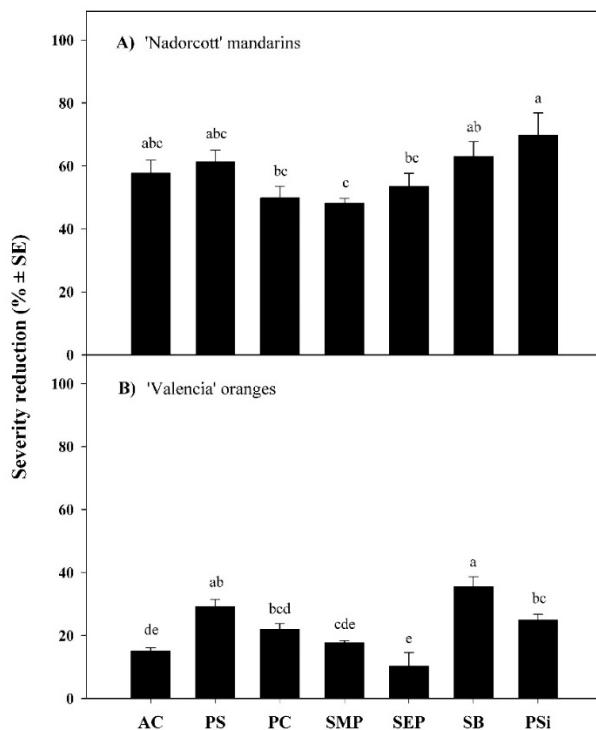


Figure 1. Percentage reduction of anthracnose severity (lesion diameter) with respect to control fruits on “Nadorcott” mandarins (A) and “Valencia” oranges (B) artificially inoculated with *Colletotrichum gloeosporioides* and coated 24 h later with hydroxypropyl methylcellulose (HPMC)-beeswax (BW) composite edible coatings containing GRAS salts and incubated for 15 d at 25 °C and 90% RH. Represented GRAS salts and concentrations are: 0.2% ammonium carbonate (AC), 2% potassium sorbate (PS), 0.2% potassium carbonate (PC), 0.1% sodium methylparaben (SMP), 0.1% sodium ethylparaben (SEP), 2% sodium benzoate and 2% potassium silicate (PSi). Average data from two trials with each citrus species. In every trial, each treatment was applied to four replications of 10 fruits each. Average severity of uncoated controls was: A) mandarins = 47.29 mm, B) oranges = 15.2 mm. Columns with different letters are significantly different according to Fisher’s protected LSD test ($P < 0.05$) applied after the ANOVA.

3.3. Effect of Coatings on the Quality of Cold-Stored Oranges

Composite coatings (HPMC-BW) containing PS, PSi or SB at 2% were selected for fruit quality evaluation due to their higher control of anthracnose severity in the previous in vivo trials. Table 3 shows the quality attributes of uncoated (control) and coated “Valencia” oranges at harvest and after cold storage at 5 °C followed by a shelf-life period of 7 d at 20 °C. Weight loss ranged from 2.1% to 2.6% after 28 d of cold storage and from 3% to 4% after 56 d of cold storage, both periods followed by 7 d of shelf life. None of the coatings significantly reduced weight loss compared to uncoated oranges. In the case of the coating formulated with PS, weight loss was even higher than on control fruit after the 28-d storage period. After 56 d, no significant differences were observed between the different coatings and the controls. Fruit firmness, expressed as percentage of rind deformation, decreased after storage (i.e., higher rind deformation) compared to the value at harvest, but no significant differences were found between 28 and 56 d of cold storage and between coated and uncoated fruits, remaining low for all treatments (in the range of 2.5% to 3%).

Table 3. Quality attributes of “Valencia” oranges coated with hydroxypropyl methylcellulose (HPMC)-beeswax (BW) edible composite coatings containing GRAS salts and stored at 5 °C followed by 7 d of shelf life at 20 °C.

Quality Attributes ¹	At Harvest	Storage Conditions and Treatments ²							
		28 d 5 °C + 7 d 20 °C				56 d 5 °C + 7 d 20 °C			
		Control	HPMC-BW-PS	HPMC-BW-SB	HPMC-BW-PSi	Control	HPMC-BW-PS	HPMC-BW-SB	HPMC-BW-PSi
WL (% ± SE)	—	2.25 ± 0.09 b	2.59 ± 0.10 a	2.24 ± 0.08 b	2.12 ± 0.06 b	3.48 ± 0.12 ab	3.71 ± 0.13 a	3.91 ± 0.22 a	2.97 ± 0.11 b
F (% deformation ± SE)	2.03 ± 0.09	2.58 ± 0.09 a	2.78 ± 0.14 a	2.50 ± 0.07 a	2.56 ± 0.14 a	2.66 ± 0.13 a	2.87 ± 0.15 a	2.47 ± 0.12 a	2.46 ± 0.13 a
SSC (% ± SE)	12.42 ± 0.08	11.70 ± 0.17 a	11.37 ± 0.50 ab	10.28 ± 0.55 bc	10.05 ± 0.22 c	11.60 ± 0.26 a	11.20 ± 0.21 a	10.20 ± 0.15 b	10.02 ± 0.23 b
TA (% citric acid ± SE)	1.34 ± 0.01	1.08 ± 0.06 a	1.23 ± 0.10 a	0.97 ± 0.07 a	0.98 ± 0.09 a	0.90 ± 0.04 a	0.84 ± 0.04 a	0.83 ± 0.04 a	0.98 ± 0.04 a
MI (average ± SE)	9.24 ± 0.08	10.85 ± 0.47 a	9.31 ± 0.38 a	10.59 ± 0.24 a	10.36 ± 0.70 a	12.95 ± 0.63 a	13.28 ± 0.07 a	12.28 ± 0.49 a	10.29 ± 0.52 b
EtC (mg/L ± SE)	221.05 ± 10.42	412.35 ± 40.5 c	533.65 ± 16.21 ab	439.51 ± 23.70 bc	628.01 ± 50.27 a	441.49 ± 24.62 c	606.90 ± 29.78 ab	543.17 ± 80.63 bc	699.63 ± 48.35 a
AcC (mg/L ± SE)	3.16 ± 0.21	5.78 ± 0.26 b	6.51 ± 0.22 b	6.17 ± 0.07 b	7.39 ± 0.39 a	5.72 ± 0.23 c	6.88 ± 0.16 b	7.47 ± 0.31 ab	7.72 ± 0.33 a

¹ WL: weight loss, F: firmness, SSC: soluble solids content, TA: titratable acidity, MI: maturity index, EtC: ethanol content, AcC: acetaldehyde content. Means in rows with different letters are significantly different according to Fisher’s protected LSD test ($P < 0.05$) applied after the ANOVA. ² Control: uncoated fruits; HPMC-BW coatings containing: PS: potassium sorbate, SB: sodium benzoate, PSi: potassium silicate.

Regarding juice quality, SSC and TA decreased and MI increased after cold storage and shelf life compared to values at harvest. In general, oranges coated with HPMC-BW-SB and HPMC-BW-PSi coatings had lower SSC than control fruit after both storage periods and no significant differences in TA and MI were observed between control and coated fruits (Table 3). On the other hand, EtC and AcC in “Valencia” oranges increased during storage compared to the values at harvest and reached values after 56 d that ranged from 400 to 700 mg/L of ethanol and from 5 to 8 mg/L of acetaldehyde. Uncoated samples and samples coated with HPMC-BW-PSi had the lower and higher volatile contents, respectively (Table 3).

Figure 2 shows the internal CO₂ and O₂ concentrations of uncoated and coated oranges after storage. At the end of the 28-d and 56-d storage periods, all tested coatings modified the internal atmosphere of “Valencia” oranges with an increase of internal CO₂ and a decrease of internal O₂ compared to uncoated fruit, and the concentrations of internal CO₂ and O₂ on coated oranges reached values around 4–6 and 15–17 kPa, respectively.

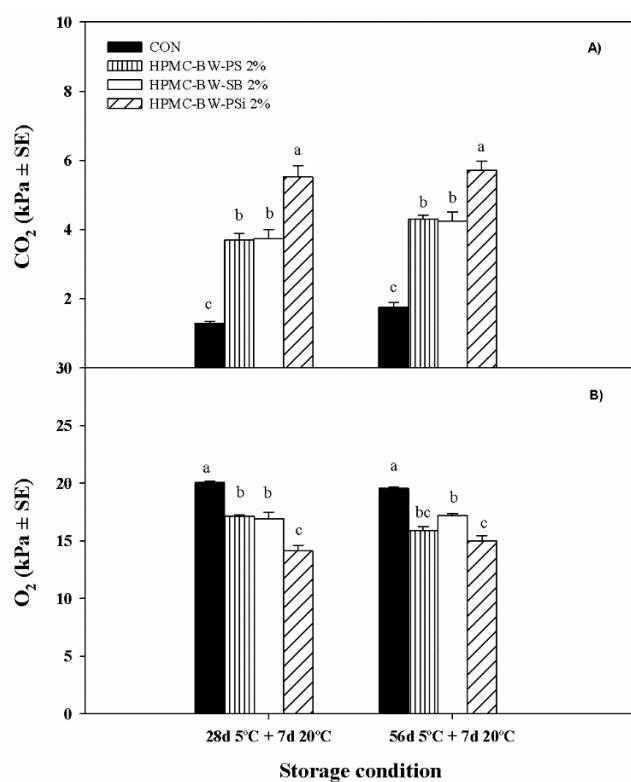


Figure 2. Internal CO₂ (A) and O₂ (B) concentrations of “Valencia” oranges uncoated (CON) or coated with antifungal hydroxypropyl methylcellulose (HPMC)-beeswax (BW) composite edible coatings and stored at 5 °C followed by 7 d at 20 °C. Coatings contained 2% potassium sorbate (PS), 2% potassium silicate (PSi) or 2% sodium benzoate (SB). For each storage period, columns with different letters are significantly different according to Fisher's protected LSD test ($P < 0.05$) applied after the ANOVA.

The HPMC-BW coatings containing GRAS salts did not modify the flavor of “Valencia” oranges during cold storage, compared to uncoated samples, as determined by the trained judges of the sensory panel (Table 4). Off-flavors ranged between 1.0 (absence) and 1.8 (very slight), with the coating containing 2% SB showing the poorest overall taste and the highest presence of off-flavors after the 56-d storage period, although no significant differences were observed with the control samples ($P > 0.05$). Coating appearance in a 1–3 scale was evaluated according to the presence or absence of cracks, blemishes, stains, and homogeneity of the coating. In general, the appearance of all coated oranges ranged between acceptable and good (1.6–2.7) after both periods of cold storage. However, the incorporation of 2% PS and 2% SB to the HPMC-BW coating matrixes negatively affected the

appearance of coated oranges, with the SB-based coating the worst evaluated. The coating containing 2% PSi was the best evaluated in terms of external appearance, without significant differences with the uncoated samples (Table 4).

Table 4. Sensory quality attributes of “Valencia” oranges coated with hydroxypropyl methylcellulose (HPMC)-beeswax (BW) composite edible coatings containing GRAS salts and stored at 5 °C followed by 7 d of shelf life at 20 °C.

Treatments ²	Storage Conditions and Sensory Attributes ¹					
	28 d 5 °C + 7 d 20 °C			56 d 5 °C + 7 d 20 °C		
	Overall Taste (1–9 Scale) ³	Off-Flavours (1–5 Scale) ⁴	Appearance (1–3 Scale) ⁵	Overall Taste (1–9 Scale) ³	Off-Flavours (1–5 Scale) ⁴	Appearance (1–3 Scale) ⁵
Control	6.43 ± 0.43 a	1.00 ± 0.00 a	2.43 ± 0.20 a	5.70 ± 0.63 a	1.38 ± 0.16 a	2.38 ± 0.24 a
HPMC-BW-PS	5.71 ± 0.56 a	1.71 ± 0.19 a	1.58 ± 0.20 b	5.50 ± 0.54 a	1.33 ± 0.22 a	1.63 ± 0.16 b
HPMC-BW-SB	6.43 ± 0.20 a	1.00 ± 0.00 a	2.00 ± 0.22 b	5.30 ± 0.58 a	1.80 ± 0.36 a	1.88 ± 0.26 b
HPMC-BW-PSi	5.83 ± 0.28 a	1.57 ± 0.20 a	2.71 ± 0.18 a	5.61 ± 0.41 a	1.33 ± 0.16 a	2.63 ± 0.24 a

¹ Means in columns with different letters are significantly different according to Fisher’s protected LSD test applied after the ANOVA ($P < 0.05$). ² Control: uncoated fruits; HPMC-BW coatings containing: PS: potassium sorbate, SB: sodium benzoate, PSi: potassium silicate. ³ Flavour ranked from 1 (very poor) to 9 (optimum). ⁴ Off-flavours ranked from 1 (absence) to 5 (presence). ⁵ Coating/fruit appearance ranked from 1 (bad) to 3 (good).

4. Discussion

The present work highlights the antifungal activity of different GRAS salts or food preservatives against *C. gloeosporioides* and their potential use as ingredients of antifungal composite edible coatings for the control of postharvest anthracnose of citrus fruits. Our in vitro results showed that, among all GRAS salts tested, ABC and SEP were the most effective to inhibit the mycelial growth of *C. gloeosporioides*. Previous works have reported the potential of carbonate salts to reduce the in vitro mycelial development of different *Colletotrichum* spp. Aqueous solutions of the salts AC at 3% [36] and sodium bicarbonate (SBC) at 2% [34] completely inhibited the mycelial growth of *C. gloeosporioides* isolated from papaya, while SBC significantly reduced the mycelial development of the species *Colletotrichum musae* (Berk. & Curtis) Arx. isolated from banana. Similarly, other researchers have also identified ABC as the most effective salt, at all concentrations tested (0.2, 1.0 and 2.0%), to inhibit the growth on PDA dishes of other important postharvest pathogens such as *Monilinia fructicola* (G. Wint.) Honey [19] and *Lasiodiplodia theobromae* (Pat.) Griffon & Maubl. [26]. In addition, various salts, mainly carbonates, were effective to inhibit the in vitro radial growth of *Botrytis cinerea* Pers. [40,41], *Geotrichum citri-aurantii* (Ferraris) Butler [42] and *Penicillium expansum* L. [43]. Likewise, the salt SEP effectively inhibited the growth of different fungi causing major postharvest diseases on fresh horticultural produce, such as *B. cinerea*, *Alternaria alternata* (Fr.) Keiss. [20] and *L. theobromae* [26].

The present results and former research clearly show that some GRAS salts have a broad spectrum of antifungal activity since they are able to inhibit the in vitro growth of a variety of fungal pathogens. The in vitro toxicity of a GRAS salt is influenced by many factors, such as the pathogen species and strain, the salt components (ions) and concentration, the pH, the culture medium and the incubation conditions [15,44,45]. General antifungal mechanisms of action of GRAS salts include the alteration of the integrity and permeability of the fungal cell membranes, interferences in the transport of nutrients and energy metabolism and collapse of hyphae or spores [42,44,46]. It is known that the addition of inorganic or organic salts to the medium modifies its pH and, in general, the antifungal activity of the salt is higher as the pH increases [19,47]. However, the pH alone cannot explain the toxicity of these compounds as different salts with the same pH can affect the same fungal strain differently [26,45]. Moreover, the salt cations and anions also play an important and complex role. In fact, sodium, potassium or ammonium forms of the same salt can show large differences in their toxicity to a particular fungal strain [19,36,48].

Salts and concentrations to be used as ingredients of HPMC-BW edible coatings were selected according to previous in vitro results and their capability to form stable emulsions with appropriate characteristics. For this reason, effective salts, such as ABC, PBC and SP, had to be discarded due to incompatibility with the coating matrix leading to phase separation or undesirable properties of coated fruit. Excessive viscosity, bad surface coverage or appearance of salt residues, blemishes or pitting on the surface of oranges and mandarins were major causes for rejection of some experimental coatings.

Among the salts and concentrations selected to be tested in in vivo trials with "Nadorcott" mandarins and "Valencia" oranges, coatings containing 2% PS, SB and PSi were the most effective in reducing anthracnose severity (up to 70% and 35% on mandarins and oranges, respectively). It is worthy to note that the antifungal effect of salt-containing coatings was higher on the citrus species more susceptible to anthracnose, namely mandarins. As pointed out in the Figure 1 caption, the average anthracnose severity (lesion size) on artificially inoculated and uncoated control fruits was 47.29 mm on mandarins, while it was only 15.2 mm on oranges. Since the inoculum density and the methodology used for artificial inoculation with *C. gloeosporioides* was exactly the same for both types of fruit, these values clearly point out that susceptibility to anthracnose was much lower on oranges than on mandarins. This is a feature that has been previously reported for other citrus postharvest diseases, such as green and blue molds caused by *Penicillium* spp., and can be related with the physical and biochemical properties of the fruit rind [3,6,10]. Previous information on the use of GRAS salts, such as AC, SC, SB or SBC, to control postharvest anthracnose on different fruit crops is available, but in most cases the salts were applied by dipping the fruit in aqueous solutions [30,32–34,36]. On the other hand, although the number of studies is considerably lower, some reports are available on the postharvest use of coatings and waxes to control anthracnose caused by *Colletotrichum* spp. on fresh produce. In general, the most studied coatings are applications of chitosan or other edible matrixes containing essential oils as antifungal ingredients [29,49–51]. Nevertheless, some waxes or coatings formulated with GRAS salts or food additives have also been evaluated for anthracnose reduction on different fresh fruits. For example, AC (3%) and SB (2%) in paraffin wax-based formulations significantly reduced anthracnose in papaya caused by *C. gloeosporioides* [36,52]; the same disease was effectively reduced in papaya by chitosan alone or in combination with 3% AC or 2% SBC during storage at 13.5 °C and 95% RH [35]; the combination of fruit-coating polymers with PS or SB significantly decreased the size of the lesions caused by *C. musae* in wound-inoculated bananas after 7 d of incubation at 25 °C and 90% RH [31]. To our knowledge, this is the first work in which edible coatings formulated with antifungal GRAS salts are applied to control citrus postharvest anthracnose. Within this context, the general antifungal activity of different coating matrices containing food additives reported by these authors working with other fresh commodities is in agreement with the results obtained with citrus fruit in the present study.

HPMC-BW edible coatings formulated with GRAS salts, with similar characteristics to those tested here, have been also evaluated to control other important postharvest diseases of citrus fruits. Among a large variety of HPMC-BW edible films containing GRAS salts, those with PS, SB, SP or their mixtures exhibited a noteworthy in vitro antifungal activity against the citrus pathogens *Penicillium digitatum* (Pers.:Fr.) Sacc. and *Penicillium italicum* Wehmer, and coatings containing these salts were effective in reducing green and blue molds on "Valencia" oranges and "Ortanique" and "Clemenules" mandarins artificially inoculated with these pathogens and incubated at 20 °C for 7 d [22–24]. In another study [26], a large amount of GRAS salts and concentrations were evaluated in in vitro tests against *L. theobromae* and the selected salts were assessed as ingredients of HPMC-BW coatings to control Diplodia stem-end rot caused by this fungus in in vivo experiments. Coatings containing 2% PS, 0.1% SEP, 2% SB and 2% PSi were the most effective, with reductions of disease severity of up to 50% on "Barnfield" oranges and "Ortanique" mandarins artificially inoculated with the pathogen and incubated for 10 d at 28 °C and 90% RH. Moreover, the curative activity of similar composite coatings has also been proved in other fresh fruit pathosystems. Significant reductions of black spot on cherry tomatoes artificially inoculated with *A. alternata* were observed by Fagundes et al. after treatment with

HPMC-based coatings containing SB, SEP or SMP [20,21]. In the same way, Karaca et al. [19] reported that HPMC-BW coatings containing PS, SEP, SMP or PSi effectively reduced the incidence and severity of brown rot caused by *M. fructicola* on artificially inoculated plums. Therefore, our present results with citrus anthracnose confirm that HPMC coatings containing PS, SB or PSi are broad-spectrum alternatives for the control of postharvest decay of fresh fruits.

This work and previous research show that the selection of the most appropriate antifungal GRAS salt to confer disease control ability to a coating is greatly dependent on the characteristics of each particular pathosystem, such as the type and properties of the fruit (species and even cultivar) and the particular activity of the salt against the target pathogen. However, other factors are also relevant and often the *in vitro* antifungal activity cannot anticipate the actual *in vivo* disease control ability. Tests in Petri dishes allow fully exposure of the fungal structures to the salt, while in *in vivo* assays, with the salt incorporated into the coating and the coating applied to fruit, the contact between the salt and the pathogen can be limited depending on factors such as the emulsion properties (pH and viscosity), the interaction of the salts with the coating matrix and other components (e.g., emulsifiers and plastisizers), the characteristics of the fruit peel and the environmental storage conditions, among others [19,20,26,39,53,54]. Moreover, in some cases, negative results lead to think that some salts presumably provide additional nutrients or enhanced environmental conditions for the development of the fungal pathogen [20,55]. The mentioned factors may explain why some GRAS salts assayed in this work, such as SMP and SEP, were not as effective in *in vivo* trials as ingredients of the coatings as they were in the *in vitro* tests. Therefore, it is very important to adapt the formulations and develop appropriate coatings for each particular fruit species and cultivar and for specific target pathogens and postharvest applications. Postharvest use of coatings containing GRAS salts as ingredients may facilitate a slow diffusion of the active ingredient from the matrix compared to the application of aqueous solutions, which could contribute to extend the antifungal effect on the fruit surface and may also reduce phytotoxicity risks [13,15,56]. Hence, the packingline application in citrus packinghouses of these antifungal edible coatings can be a good alternative for commercial anthracnose control to the application of salt aqueous solutions by drencher, dipping or spraying systems.

After both cold storage periods and shelf life, none of the coatings significantly reduced weight loss with respect to uncoated oranges. Among the different coatings tested, those with SB or PS induced higher weight loss than those with PSi. In general, cellulose-lipid composite coatings are reported to reduce fruit weight loss due to the moisture barrier created by the lipid ingredients (BW, shellac, etc.) of the coating formulation [57]. However, several works have confirmed that the addition of food additives such as GRAS salts to HPMC-based coatings greatly affects the moisture barrier properties of stand-alone films or coatings when applied to different fruits such as cherry tomatoes, citrus or table grapes [22,23,58,59]. Thus, the application of HPMC-BW coatings containing SB or PS did not reduce weight loss of coated “Barnfield” and “Valencia” oranges compared to control samples after cold storage at 5 °C, and in both cases PS was less effective than SB for weight retention [24,26]. However, similar coatings significantly reduced weight loss and maintained firmness of “Clemenules” mandarins without adverse effects on the overall quality of coated fruit [39]. Similar results have been reported in research work with other crops. For example, a HPMC-BW coating containing 2% SB showed potential for postharvest industrial application to cherry tomatoes as it reduced weight loss and controlled black spot during prolonged cold storage [21]. Since the antifungal HPMC-BW coatings developed in this work have not satisfactorily reduced weight loss of cold-stored oranges, probably due to changes originated in the permeability of the cuticle, an aspect to consider for further research might be the modification of their physical characteristics in order to improve water loss control while maintaining their antifungal activity.

In the present work, fruit firmness was not affected by the application of HPMC-BW coatings amended with PS, SB or PSi. Polysaccharides present in the cell wall are responsible for the maintenance of fruit firmness and the degradation of these compounds by hydrolyzing enzymes is the cause of fruit softening during ripening and storage. In addition, the effect of coatings on the maintenance of

fruit firmness is usually related to their control of weight loss. According to previous results with HPMC-BW coatings, it seems that the influence of coating on fruit firmness is not only dependent on the coating characteristics but also on the citrus cultivar. For instance, in accordance with our results, Valencia-Chamorro et al. [24] and Guimarães et al. [26] reported that HPMC-BW coatings amended with SB or PS did not affect significantly the firmness of coated “Valencia” and “Barnfield” oranges, respectively. However, “Clemenules” mandarins treated with the same type of coatings were significantly firmer after cold storage and shelf life than uncoated control fruits [39]. This could be related to particular properties of the rind of each citrus species or cultivar by which the effects of coating might be modified. Nevertheless, contradictory results have been reported on the relationship between weight loss and firmness on coated citrus fruits. For instance, while a positive correlation was found by Navarro-Tarazaga et al. [60] for “Ortanique” mandarins, no correlation was observed in studies with “Fortune” mandarins [61], indicating the intervention of multiple factors.

Edible coatings can have the capacity to modify the internal gas composition of fresh fruit in terms of O₂ and CO₂ concentrations [18]. The effect of edible coatings on the delay of changes related to fruit ripening (softening, color change, decrease in acidity, appearance of some physiological disorders, etc.) has been related to the gas barrier exerted on the fruit surface, leading to reductions in the respiration rate and/or weight loss [21,62]. The capacity of an edible coating to create an effective gas barrier depends not only on the coating composition and properties (including the addition of GRAS salts), but also on the fruit, cultivar and storage conditions. In a work conducted by Gunaydin et al. [18], the application of HPMC-BW coatings containing paraben salts resulted in the lowest CO₂ production rates, showing the potential of these coatings as gas barriers on plums. However, Fagundes et al. [21] reported the highest respiration rates in cherry tomatoes coated with HPMC-BW emulsions containing SEP. In the present study, the three selected coatings modified the fruit internal atmosphere, and the internal CO₂ and O₂ levels were significantly higher and lower, respectively, in coated fruit than in control samples, which indicates that the coatings were effective as gas barrier. The CO₂ values (3.5–4.5 kPa) in oranges treated with coatings containing PS or SB were equivalent to those observed in coated “Barnfield” oranges [26] and “Clemenules” mandarins [39], but lower than those observed in “Ortanique” mandarins [25] or “Valencia” oranges [63] coated with similar HPMC-lipid coatings containing GRAS salts. To our knowledge, this is the first report on the potential of HPMC-BW coatings amended with PSi as effective gas barriers for citrus fruits. This coating modified the internal atmosphere of “Valencia” oranges in a greater extend than the rest of tested coatings, to reach internal CO₂ and O₂ values of 5.7 and 15.0 kPa, respectively, which could be due to the interaction of PSi with the coating matrix to form a tider structure with less O₂ permeability.

In general, the creation of a modified atmosphere in coated citrus fruits is accompanied by an increase in the volatiles associated with anaerobic respiration, such as ethanol and acetaldehyde [24,39,61]. This was confirmed in this work, and the coating amended with PSi induced the highest volatile content in accordance with the higher internal CO₂ concentration in the fruit. It is assumable that the specific composition and characteristics of the coatings (i.e., total solid content, viscosity, surface tension, barrier and mechanical properties) may explain the different behavior among coating formulations.

Overall, fruit taste and off-flavors were slightly modified during cold storage. However, there were not significant differences between coated and uncoated “Valencia” oranges after both storage periods and shelf life (Table 4). It is known that citrus off-flavor during storage is due to the accumulation of volatiles, with ethanol the most relevant. Moreover, the application of fruit coatings may enhance this process as they can restrict gas exchange through the peel surface [64,65]. However, in citrus, the level of ethanol in the juice that marks the threshold associated with off-flavor appearance depends on the cultivar and, in general, mandarins are more sensitive to anaerobic conditions and develop off-flavors easier than other citrus fruits [66]. For instance, minimum EtC associated with off-flavors has been reported to be 2000 mg/L in “Valencia” oranges [65], 1000 mg/L in “Clemenules” mandarins [67] and 500–600 mg/L in “Murcott” mandarins [68]. In the present work, EtC levels were much lower

(400–700 mg/L) than those reported by other authors, which may explain why the tested coatings did not induce off-flavors.

Regarding appearance, coatings containing 2% SB and 2% PB were the worst evaluated in terms of external aspect (acceptable). In general, HPMC-BW coatings are not characterized for providing significant gloss to coated fruit, generally due to the macro emulsion character of the coating formulation [21,23,24,39]. Moreover, some studies have also reported the presence of white spots on the surface of coated mandarins or oranges that reduced the general good appearance of the fruit when using HPMC-based coatings amended with some GRAS salts, including SB and PS [25,26]. On the other hand, the aspect of oranges treated with coatings amended with PSi was quite good and similar to that of uncoated fruits.

In summary, this research allowed the development of HPMC-BW edible coatings effective to reduce citrus postharvest anthracnose through the addition of antifungal GRAS salts such as PS, SB and PSi to the coating matrix. These coatings significantly reduced anthracnose severity on “Nadorcott” mandarins and “Valencia” oranges artificially inoculated with *C. gloeosporioides* and, although they did not reduce weight loss of coated “Valencia” oranges in comparison with uncoated fruits during cold storage, they modified the internal atmosphere of the fruit without adversely affecting the physicochemical and sensorial attributes of the fruit. Further research should focus on the improvement of physical characteristics of the coatings to enhance water loss control and the external aspect of coated citrus fruit. Information gathered from this study provides a basis for further research into the application of these antifungal coatings and their possible combination with other alternative nonpolluting methods to improve the control of postharvest anthracnose in citrus packinghouses. This is especially important in the case of early-season cultivars of mandarins and oranges that are artificially degreened with exogenous ethylene to obtain the appropriate orange color in the rind before commercialization. Exposure to this gas at typical degreening environmental conditions (20–22 °C and RH > 90%) stimulates the germination of conidia and the formation and germination of appressoria of *C. gloeosporioides* and, thus, exacerbates the development of latent infections and the incidence of citrus postharvest anthracnose [3]. Since citrus degreening are typically performed before fruit handling in the packingline, the application of these antifungal edible coatings in the packingline can be a suitable curative treatment against anthracnose and effectively substitute the use of conventional waxes amended with synthetic chemical fungicides.

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