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Antifungal activity of GRAS salts against *Lasiodiplodia theobromae* in vitro and as ingredients of hydroxypropyl methylcellulose-lipid composite edible coatings to control Diplodia stem-end rot and maintain postharvest quality of citrus fruit

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ABSTRACT

A large amount of GRAS (generally recognized as safe) salts and concentrations were evaluated in in vitro tests (inhibition of mycelial growth on PDA dishes) against *Lasiodiplodia theobromae*, the causal agent of citrus Diplodia stem-end rot. Ammonium carbonate (AC, 0.2%), potassium sorbate (PS, 2.0%), potassium carbonate (PC, 0.2%), sodium methylparaben (SMP, 0.1%), sodium ethylparaben (SEP, 0.1%), sodium benzoate (SB, 2.0%), and potassium silicate (PSi, 2.0%) were selected as the most effective. Disease control ability of edible composite coatings formulated with hydroxypropyl methylcellulose (HPMC), beeswax (BW), and these selected antifungal GRAS salts was assessed in in vivo experiments with 'Ortanique' mandarins and 'Barnfield' oranges artificially inoculated with L. *theobromae*. Coatings containing 2% PS, 0.1% SEP, or 2% SB were the most effective reducing disease severity (up to 50% reduction) and were also applied to non-inoculated and cold-stored 'Barnfield' oranges to determine their effect on postharvest fruit quality. After periods of 21 and 42 d at 5 °C followed by 7 d of shelf life at 20 °C, coatings containing SEP and SB significantly reduced weight loss and did not adversely affect the physicochemical quality attributes (firmness, soluble solid content, titratable acidity, and ethanol and acetaldehyde content) and sensory flavor with respect to uncoated control fruit. Although the internal gas concentration (CO_2 level) of coated fruit increased, the coatings did not induce off-flavors.

1. Introduction

Citrus fruit are grown in > 100 countries with tropical and subtropical climate. Spain, producing > 6 million tons in 2016, ranked sixth in the world, preceded by Brazil with a production higher than 22 million tons, China, USA, India, and Mexico. While 80% or more of the Brazilian production is devoted to the juice industry, in Spain this percentage is devoted to the fresh fruit market. Spain, in fact, is the first worldwide exporter of citrus fruit for fresh consumption (FAOSTAT, 2018).

One of the most important problems affecting both the fresh and juice citrus industries are postharvest losses caused by fungal pathogens that infect the fruit before, during or after harvest, but develop disease after harvest. Depending on many factors, these losses are estimated to reach 20–50% on developing countries and up to 25% in developed countries, even though the sector in these countries has availability of major postharvest technologies for fruit preservation and quality maintenance. Depending on the climatic areas where citrus are grown, the main pathogenic fungi that cause the highest incidence of fruit postharvest disease differ. In high rainfall areas such as Brazil and Florida the most relevant belong to genera that typically produce latent infection in the orchard, e.g., *Lasiodiplodia*, *Phomopsis*, *Colletotrichum*, *Phytophthora*, *Alternaria*, and *Botrytis*, among others. Nevertheless, in Mediterranean-type climate areas with lower summer rainfall such as Spain, California, and South Africa, the most prevalent are wound pathogens such as *Penicillium*, *Geotrichum*, and *Rhizopus* (Eckert and Eaks, 1989; Palou, 2014; Smilanick et al., 2006).

Lasiodiplodia theobromae (Pat.) Griffon & Maubl. (Botryosphaeriaceae) [synonyms: *Botryodiplodia theobromae* Pat., *Diplodia theobromae* (Patouillard) W. Nowell, *Diplodia natalensis* Pole-

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Evans; teleomorph *Botryosphaeria rhodina* (Berk. & M.A. Curtis) Arx] is a polyphagous and opportunistic fungus with reduced pathogenic specialization, which infects many species of plants and can cause serious damage on fruit commodities both before and after harvest. Important postharvest diseases caused by L. *theobromae* are stem-end rots of subtropical and tropical fruits such citrus, avocado, mango, banana, papaya, pineapple, and persimmon, among others (Palou et al., 2013; Ploetz et al., 1994). On citrus fruit, it causes the postharvest disease commonly known as Diplodia stem-end rot (Zhang, 2014). Typically, the attack of the fungus occurs at the stem end of the fruit before harvest, the infection remains latent in the calyx and disk (button) and disease symptoms and fruit deterioration are only manifested when the fruit ripens after harvest, the button senesces and abscises from the fruit, and the pathogen begins to actively develop through both the central axis and the external rind surface of the fruit, where it causes characteristic finger-like projections of brown tissue. The progress of the infection depends on the growth and the enzymatic capability of the microorganism and the physiological and biochemical status of the fruit (Brown and Wilson, 1968; Zhang, 2014). Disease incidence can be especially high on early-season degreened citrus fruit because the presence of exogenous ethylene favors the activity of the button abscission enzymes polygalacturonase (PG) and cellulase (CX) (Brown and Burns, 1998). Decayed fruit tissue is initially firm, but later becomes wet and mushy (Brown and Eckert, 2000). On artificial medium such as potato dextrose agar (PDA), the colonies of L. *theobromae* are grayish to black, with abundant aerial cottony mycelia and globular pycnidia. Conidia are ovoid to ellipsoidal, thick walled, initially hyaline, aseptate, but turn dark brown and uniseptate as the colony ages (Luo et al., 2011; Pereira et al., 2006). Another fungal pathogen that causes citrus postharvest stem-end rot, but not with the tear-staining symptoms of L. *theobroame*, is *Phomopsis citri* H.S. Fawc. (teleomorph *Diaporthe citri* F.A. Wolf).

Chemical fungicides are commonly used as the main tool to control both preharvest and postharvest diseases of citrus fruit. While postharvest fungicide application is mainly devoted to reduce green mold, caused by *Penicillium digitatum* (Pers.) Sacc., and blue mold, caused by *P. italicum* Wehmer, some common active ingredients such as thiabendazole, imazalil, fludioxonil, and sodium *o*-phenylphenate have also shown effect against stem-end rots (Zhang, 2014). However, their use is increasingly restricted due to public concerns about possible toxicological risks for people and the environment associated with excessive chemical residues. In addition, continuous fungicide usage can lead to the proliferation of resistant pathogenic fungal strains that make the treatments ineffective. Therefore, alternatives to control citrus postharvest diseases including Diplodia stem-end rot are of great interest to ensure safe agricultural production and reduce environmental pollution (Palou et al., 2016; Wisniewski et al., 2016). One of such alternatives is the use of edible coatings formulated with food-grade antifungal compounds. Fruit can be directly coated with a thin layer of edible material in order to improve gas and moisture barriers, mechanical and sensory properties, convenience, and microbial protection and, as a consequence, prolong product shelf life (Janjarasskul and Krochta, 2010). Polysaccharides, proteins, and lipids are the main ingredients used to formulate edible coatings. In many cases, two or more of these ingredients can be mixed to produce composite edible coatings in order to reduce both water and gas exchange between the fruit and the surrounding environment (Hernández-Izquierdo and Krochta, 2008). In postharvest applications, different polysaccharides such as chitosan, methylcellulose, hydroxypropyl methylcellulose (HPMC), and carboxy methylcellulose (CMC) have been found to affect positively weight loss, firmness, brightness, taste, and other physicochemical and sensory quality attributes of citrus fruit (Arnon et al., 2015; Navarro-Tarazaga et al., 2007, 2008).

To wide and enhance the functionalities of these coatings and further extend the shelf life of coated fresh produce, additional antimicrobial ingredients can be added to the emulsions to provide activity against pathogens and contaminating microorganisms (Valencia-Chamorro et al., 2011a). Antimicrobial agents used for the formulation of edible coatings should be classified as food-grade additives or compounds generally recognized as safe (GRAS) by the competent authorities. International regulators are responsible for approving antimicrobials for use in food. In the European Union (EU), these compounds are regulated by the EU Framework Directive 89/107 (EU, 1989), while in the United States (USA) by the title 21CFR172 promulgated by the US Food and Drug Administration (US FDA, 2009). According to Palou et al. (2016), the antimicrobial ingredients used for formulation of antifungal synthetic biopolymer-based coatings can belong to three different categories, depending on their nature: i) synthetic food preservatives or GRAS compounds such as various inorganic and organic salts, ii) natural compounds such us essential oils and other natural plant extracts, and iii) microbial antagonists as biocontrol agents (bacteria, yeast, yeast-like fungi, and even some filamentous fungi). General advantages of GRAS salts are their high solubility in water, availability, feasibility of use as postharvest treatments for fresh fruit, and good prize. Intensive previous work at the IVIA CTP has resulted in the development and characterization of HPMC-lipid edible coatings containing GRAS salts with antifungal activity against citrus postharvest green and blue molds (Palou et al., 2015; Valencia-Chamorro et al., 2008, 2009a). This type of coatings were also effective for the control of important postharvest diseases of other fresh commodities such as plums (Karaca et al., 2014) and cherry tomatoes (Fagundes et al., 2013, 2015). However, to our knowledge, no studies are available on the development of edible coatings with antifungal activity against citrus postharvest stem-end rot caused by L. *theobromae*.

The aim of this research work was to evaluate the in vitro activity of GRAS salts against L. *theobromae* and to develop novel stable HPMC-lipid coatings containing selected GRAS salts. The ability of these coatings to effectively control Diplodia stem-end rot was assessed in in vivo experiments with mandarins and oranges artificially inoculated with the pathogen. Furthermore, the effects of selected antifungal edible coatings on fruit physico-chemical and sensory quality were determined on oranges stored at 5 °C for up to 42 d.

2. Materials and methods

2.1. Pathogen and fungal inoculum

In this work, the strain *L. theobromae* NEU-1 from the IVIA CTP culture collection of postharvest pathogens was used. It is an isolate obtained from a decayed orange found in a citrus packinghouse in Valencia province (Spain). Before each experiment, the isolate was grown on PDA medium (Sigma-Aldrich Chemie, Steinheim, Germany) in Petri dishes in the dark in an incubation cabinet at 25 °C for 7–14 d. Five-mm diameter mycelial plugs were cut from these cultures with a sterilized cork borer and used as described below for plate and fruit inoculations in in vitro and in vivo tests, respectively.

2.2. GRAS salts

The compounds, acronyms, molecular formulas, and molecular weights of the antimicrobial agents used in this work are given in Table 1. They are inorganic and organic salts classified as GRAS or as food additives by the USA or EU competent legislation. Laboratory reagent grade preservatives (99% minimum purity) were purchased from Sigma-Aldrich Chemie, Fluka Chemie AG (Buchs, Switzerland), Panreac Química S.L.U. (Castellar del Vallés, Catalonia, Spain), and Merck KGaA (Darmstadt, Germany). Potassium silicate (PSi), as the commercial product Sil-Matrix® (29% PSi) was purchased from PQ Corporation (Valley Forge, PA, USA).

Table 1
Characteristics of antifungal GRAS salts tested in vitro for inhibition of *Lasiodiplodia theobromae* and in vivo (as coating ingredients) for control of Diplodia stem-end rot.

GRAS salt	Acronym	Molecular formula	E-code[a]	MW[b]
Ammonium phosphate	APh	$NH_4H_2PO_4$	E-342 (i)	149.09
Ammonium bicarbonate	ABC	NH_4HCO_3	E-503 (ii)	79.06
Ammonium carbonate	AC	$(NH_4)_2CO_3$	E-503 (i)	114.10
Potassium bicarbonate	PBC	$KHCO_3$	E-501 (ii)	100.12
Potassium carbonate	PC	K_2CO_3	E-501 (i)	138.21
Potassium silicate	PSi	K_2SiO_3	E-560	154.26
Potassium sorbate	PS	$C_6H_7O_2K$	E-202	150.22
Sodium bicarbonate	SBC	$NaHCO_3$	E-500 (ii)	84.01
Sodium benzoate	SB	$C_7H_5O_2Na$	E-211	144.11
Sodium carbonate	SC	Na_2CO_3	E-500 (i)	105.99
Sodium ethylparaben	SEP	$C_9H_9Na\ O_3$	E-215	188.16
Sodium methylparaben	SMP	$C_8H_7Na\ O_3$	E-219	174.13
Sodium propionate	SP	CH_3CH_2COONa	E-281	96.06

[a] Code number for food additives approved by the European Union.
[b] Molecular weight (g/mol).

2.3. In vitro antifungal activity of GRAS salts

The effect of GRAS salts on radial mycelial growth of *L. theobromae* was determined in Petri dishes with PDA medium as described by Karaca et al. (2014) for evaluation of the activity against the stone fruit pathogen *Monilinia fructicola* (G. Winter) Honey. Briefly, PDA was amended at 40–50 °C with sterile aqueous solutions of each salt at concentrations of 0.2, 1.0, and 2.0% (*v*/v) (0.01, 0.05, and 0.1% in the case of paraben salts). PDA plates without salt were used as control dishes. One plug of a 7–14 d-old culture of *L. theobromae* was inoculated in the center of each PDA dish and incubated for up to 14 d at 25 °C. Radial fungal growth was measured every 2–3 d and results expressed as percentage of growth inhibition with respect to control dishes. For each salt and salt concentration, 4 replicates (4 PDA dishes) were used and each combination was tested twice.

2.4. Preparation of antifungal coatings

The hydrocolloid of the composite coating matrixes, HPMC (Methocel E15), was purchased from Dow Chemical Co. (Midland, MI, USA) and the lipid of the matrixes, beeswax (BW) (grade 1), was supplied by Fomesa Fruitech S.L. (Beniparrell, Valencia, Spain). Stearic acid and glycerol were purchased from Panreac Química S.L.U. HPMC-BW composite edible emulsions were prepared combining the hydrophilic phase (HPMC) with the hydrophobic phase (BW) suspended in water. Glycerol and stearic acid were used as plasticizer and emulsifier, respectively. All the emulsions contained 1.3% HPMC (*w*/w, wet basis, wb) and 3% BW (wb). HPMC-glycerol (2:1) and BW-stearic acid (3:1) ratios were kept constant for all coatings. The concentrations of GRAS salts in the formulations varied between 0.01 and 2.0% (wb) and were determined according to the effective doses previously obtained in the in vitro tests. For emulsion preparation, 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 (Tween 80 in the case of emulsions with sodium propionate) were added to the HPMC solution and heated at 98 °C to melt the lipids. Samples were homogenized with a high-shear probe mixer (Ultra-Turrax model T25, IKA-Werke, Steufen, Germany) for 1 min at 12,000 rpm and 3 min at 22,000 rpm. After adding the corresponding salts at the indicated amounts, emulsions were cooled under agitation to a temperature lower than 25 °C by placing them in an ice bath and agitation was continued for 25 min to ensure complete hydration of the HPMC. Viscosity of the emulsions was determined with a viscosimeter (Visco Star Plus R, Fungilab, S.A., Barcelona, Spain) and pH values using a pH-meter (Consort C830 multi-parameter analyzer, Turnhout, Belgium). The formulations were tested for stability according to the method described by Valencia-Chamorro et al. (2008). In brief, the emulsions were placed in volumetric tubes and phase separation was assessed after 24 h at 25 °C. Emulsions were kept overnight at 10 °C before use in the experiments.

2.5. Fruit

Hybrid mandarins [*C. reticulata* × (*C. sinensis* × *C. reticulata*) cv. 'Ortanique'; synonym: 'Topaz'] and oranges (*Citrus sinensis* L. cv. 'Barnfield'; Navel group) were used for in vivo experiments and fruit quality assessments. Fruit were harvested in commercial orchards in the Valencia area and no postharvest treatments were applied. Fruit with no wounds, bruises, or any external damage were selected, washed, disinfected by immersion in diluted commercial bleach (0.5% NaClO), rinsed thoroughly with tap water, allowed to dry at room temperature, and placed in plastic trays in cartoon boxes to be used in the experiments the next day.

2.6. In vivo curative activity of antifungal coatings

For fruit inoculation, a circular wound (1–2 mm deep, 5 mm in diameter) was inflicted with a sterile cork borer in the stem-end of each fruit and a PDA plug of *L. theobromae* culture (5 mm in diameter) was deposited on the wound (the mycelium growing side in the inner part) and ensured with transparent tape to avoid desiccation. To favor infection, inoculated fruit were placed in humid chambers and incubated at 28 °C and high RH (> 90%) for 48 h. After this period, the tape and the mycelium discs were removed using a small sterile spatula, and the fruit were individually coated. For coating application, 400 μL of the desired emulsion were pipetted onto each fruit and rubbed with gloved hands to simulate the industrial application of waxes and coatings in rotating brushes in the packinglines in citrus packinghouses (Bai et al., 2002). After draining and air-drying at room temperature, coated fruit were incubated for up to 15 d at 28 °C and 90% RH in a climatic walk-in room. Inoculated but uncoated mandarins or oranges served as controls. Each treatment was applied to 4 replicates of 10 fruit each and each trial was conducted twice.

The incidence (percentage of infected fruit) and severity (lesion diameter in mm) of Diplodia stem-end rot were assessed, depending on the experiment, after 3, 7, and 10 d of incubation at 28 °C in the case of mandarins and after 4, 8, 12, and 15 d of incubation in the case of oranges. Results were reported as disease reduction (%) with respect to the control treatments. The area under the disease progress stairs (AUDPS) was also calculated.

2.7. Effect of coating application on fruit quality

Among the edible coatings tested for antifungal activity, the three most effective were selected to determine their effect on postharvest quality of non-inoculated and cold-stored oranges. HPMC-BW coatings containing the following GRAS salts and concentrations were selected: potassium sorbate (PS) at 2%, sodium ethylparaben (SEP) at 0.1%, and sodium benzoate (SB) at 2%. Selected healthy 'Barnfield' oranges were washed, coated, and stored at 5 °C and 90% RH for 21 or 42 d, followed by a shelf-life period of 7 d at 20 °C. Uncoated oranges served as controls. The following quality attributes were determined at harvest and after cold storage and shelf life.

2.7.1. Weight loss

Thirty oranges per treatment were individually marked and weighted. After storage and shelf life, they were weighted again and the percentage weight loss was expressed with respect to the initial weight.

2.7.2. Fruit firmness

Firmness of 20 oranges per treatment was determined as percentage of rind deformation with an Instron Universal testing machine according to Valencia-Chamorro et al. (2009b).

2.7.3. Internal quality

Soluble solids concentration (SSC, %), titratable acidity (TA, %), and maturity index (MI, SSC/TA) of the juice from three previously weighed samples of 10 oranges each per treatment were determined as described by Palou et al. (2007).

2.7.4. Internal CO_2 concentration

CO_2 concentration (%) in the internal cavity of 10 oranges per treatment was determined by gas chromatography using the methodology described by Valencia-Chamorro et al. (2009b).

2.7.5. Ethanol content (EC) and acetaldehyde content (AC)

The content of these volatile compounds (mg/L) in the headspace of juice from three replicates of 10 oranges per treatment was analyzed by gas chromatography according to Valencia-Chamorro et al. (2011b).

2.7.6. Sensory evaluation

Taste (1–9 scale; 1 = very poor and 9 = optimal) and external appearance (1–3 scale; 1 = bad, 2 = acceptable, and 3 = good) of four coated oranges per treatment were evaluated by 8–10 trained judges following the procedures described by Valencia-Chamorro et al. (2009b).

2.8. Statistical analysis

Data from both in vitro and in vivo efficacy tests and fruit quality assessment were subjected to analyses of variance (ANOVAs; Statgraphics 5.1, Manugistics, Inc., Rockville, MD, USA). 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, $P = 0.05$).

3. Results and discussion

3.1. In vitro antifungal activity of GRAS salts

The determination of antifungal activity in this study was based on the inhibition of the radial growth of fungal colonies of *L. theobromae* compared to growth on control plates (PDA with no salt addition) after 3, 5, and 7 d of incubation at 25 °C (Table 2). Further readings are not reported in this table because 7 d was the incubation period after which the pathogen entirely covered the control plates; however, after 14 d of incubation, it was observed that the fungal growth remained completely inhibited in those plates with 100% inhibition after 7 d.

Significant differences between treatments were found and the effect of each salt was clearly dependent on the concentration at which it was applied (Table 2). Ammonium bicarbonate (ABC), ammonium carbonate (AC), sodium carbonate (SC), and potassium carbonate (PC) were the most effective antifungal salts against the pathogen, with complete inhibition of fungal growth after 7 d of incubation at all the tested concentrations of 0.2, 1.0, and 2.0% and with no significant differences among these concentrations (Table 2). In research work with banana postharvest pathogens, SC and sodium bicarbonate (SBC) also effectively reduced the in vitro growth of *L. theobromae* (Alvindia, 2013). In a work similar to the present research, Karaca et al. (2014) also identified some of these salts, particularly AC and ABC, as the most effective at all tested concentrations (0.2, 1.0, and 2.0%) to inhibit the growth of *M. fructicola* on PDA dishes. These salts, but also SEP, sodium methylparaben (SMP), and potassium silicate (PSi) at similar

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

GRAS salt	Inhibition of *L. theobromae* (%)[a]			
	Concentration (%)	Day 3	Day 5	Day 7
Ammonium phosphate	0.2	1.27h	0j	3.15j
	1	5.93h	0j	0j
	2	73.73de	56.08gh	48.06gh
Ammonium bicarbonate	0.2	100a	100a	100a
	1	100a	100a	100a
	2	100a	100a	100a
Ammonium carbonate	0.2	100a	100a	100a
	1	100a	100a	100a
	2	100a	100a	100a
Sodium bicarbonate	0.2	85.59bcd	79.47cde	72.6de
	1	100a	100a	100a
	2	100a	100a	100a
Sodium benzoate	0.2	73.73de	69.21ef	59.49f
	1	100a	100a	100a
	2	100a	100a	100a
Sodium carbonate	0.2	100a	100a	100a
	1	100a	100a	100a
	2	100a	100a	100a
Sodium propionate	0.2	52.54fg	54.89gh	47.22h
	1	77.11cd	74.22def	71.76e
	2	89.83ab	89.02abc	83.53bc
Sodium methylparaben	0.01	51.69fg	49.16hi	44,2h
	0.05	97.03ab	85.91bc	82.52bcd
	0.1	100a	100a	100a
Sodium ethylparaben	0.01	41.95g	38.9i	29.07i
	0.05	100a	100a	100a
	0.1	100a	100a	100a
Potassium sorbate	0.2	88.56abc	78.76cde	72.77de
	1	100a	94.03ab	90.75ab
	2	100a	100a	100a
Potassium carbonate	0.2	100a	100a	100a
	1	100a	100a	100a
	2	100a	100a	100a
Potassium silicate	0.2	61.44ef	62.77fg	57.48fg
	1	100a	100a	100a
	2	100a	100a	100a
Potassium bicarbonate	0.2	86.44bc	81.62cd	75.63cde
	1	100a	100a	100a
	2	100a	100a	100a

Means in columns with different letters are significantly different by Fisher's protected LSD test ($P < 0.05$) applied after an ANOVA.
[a] Colony diameter reduction with respect to control treatments (non-amended PDA plates).

concentrations greatly inhibited the growth of the fungi *Botrytis cinerea* Pers. and *Alternaria alternata* (Fr.) Keiss. in in vitro experiments (Fagundes et al., 2013). These pathogens have a very wide range of fruit hosts, including citrus fruit. Olivier et al. (1998) reported that ABC, SC, PC, and potassium bicarbonate (PBC) substantially reduced the in vitro growth of *Helminthosporium solani* Durieu & Mont., the causal agent of potato silver scurf. In the present study, a second group of preservative salts including sodium bicarbonate (SBC), PBC, SB, and PSi were also completely effective against *L. theobromae*, but only at the concentrations of 1.0 and 2.0%. SEP also completely inhibited the fungus at the two highest concentrations tested of 0.05 and 0.1%. Sodium propionate (SP), potassium sorbate (PS), and SMP were significantly more effective only at the highest concentration tested (2.0 or 0.1%). The least effective GRAS salt was ammonium phosphate (APh), with which growth inhibition after 7 d was lower than 50% at the highest concentration.

It is clear from this and previous research that some of these GRAS salts show a broad spectrum antimicrobial activity since they are able to inhibit in vitro a large number of fungal pathogens. For instance, besides the examples just mentioned, different carbonate salts have been found effective in other reports to inhibit the radial growth of strains of *B. cinerea* (Alaoui et al., 2017; Youssef and Roberto, 2014), *Geotrichum* *citri-aurantii* (Talibi et al., 2011), *Colletotrichum gloeosporioides* (Penz.) Penz. & Sacc. (Sivakumar et al., 2002), and *Penicillium expansum* L. (Lai et al., 2015). Nevertheless, the toxicity in vitro of a particular salt to different fungal pathogens greatly differs and it is influenced by different factors such as the pathogen species and strain, the salt composition (the nature of anions and cations) and concentration (typically the toxicity is concentration-dependent), the pH, the culture medium, and the incubation conditions. The addition of inorganic or organic salts to the medium largely modifies its pH and, in general, the toxicity is also pH-dependent (Xu and Hang, 1989). However, the pH alone cannot account for the strong antifungal activity of these compounds since different salts with the same pH show different toxicity to the same fungal strain. Therefore, the salt cation also plays an important role and, in fact, sodium, potassium, and ammonium forms of the same salt can show large differences in toxicity to a particular fungal strain (Karaca et al., 2014; Palmer et al., 1997). General antifungal mechanisms of action of GRAS salts include the alteration of the integrity and permeability of the fungal cell membranes, disturbances in the transport of nutrients that eventually cause cell inactivation and death (Lucera et al., 2012), and reduction of cellular turgor pressure with collapse and shrinkage of conidia and/or hyphae (Talibi et al., 2011).

3.2. In vivo curative activity of antifungal coatings

GRAS salts and concentrations to be used as ingredients of HPMC-BW edible coatings were selected according to the previous in vitro results and results from preliminary experiments conducted in the laboratory to evaluate their compatibility with the rest of coating ingredients. APh and SP were discarded due to their low toxicity to *L. theobromae* (Table 2). Effective salts such as ABC, SBC, SC, and PBC had to be discarded due to incompatibility with the coating matrix that led to the occurrence of phase separation, instability of the emulsions, or undesirable characteristics such as too high viscosity or presence of apparent salt residues on the surface of treated mandarins or oranges. Effective salts that formed stable emulsions with appropriate characteristics were used at their minimum effective concentration. Therefore, the selected salts and concentrations were the following: AC (0.2%), PS (2%), PC (0.2%), SMP (0.1%), SEP (0.1%), SB (2%), and PSi (2%). Composite coatings formulated with these GRAS salts all contained similar HPMC and BW amounts and a total solid content in the range of 6.1–8% depending on salt concentration. The most important properties of the emulsion formulations, pH and viscosity, are presented in Table 3.

In a first set of in vivo experiments with ‘Ortanique’ mandarins, fruit artificially inoculated with *L. theobromae* were coated 24 h later with these HPMC-lipid composite coatings containing the selected GRAS salts and incubated for 10 d at 28 °C and 90% RH. The effect of coating application on the inhibition of Diplodia stem-end rot is shown in Fig. 1. Due to the use of mycelium plugs as artificial inoculation method, disease incidence (percentage of infected fruit) was 100% on all inoculated fruit (data not shown) and the variables percent reduction of disease severity (reduction of lesion size with respect to control fruit)

Table 3
pH and viscosity of edible coatings formulated with HPMC-BW and GRAS salts.

Coating with GRAS salt	pH	Viscosity (cp)
No salt (HPMC-BW)	5.76	45.2
Ammonium carbonate 0.2%	6.83	46.2
Potassium sorbate 2%	6.27	51.2
Potassium carbonate 0.2%	7.15	50.0
Sodium methylparaben 0.1%	7.15	46.7
Sodium ethylparaben 0.1%	7.03	45.9
Sodium benzoate 2%	6.07	46.7
Potassium silicate 2%	9.50	60.0

HPMC, hydroxypropyl methylcellulose; BW, beeswax.

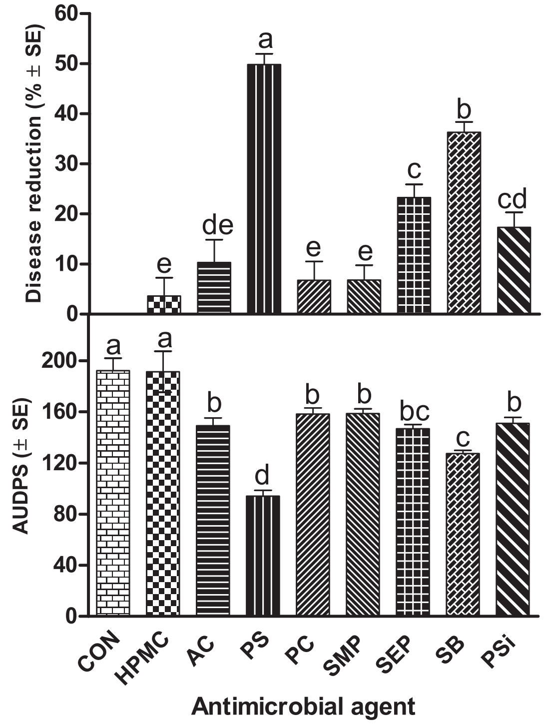

Fig. 1. Reduction of the severity with respect to control fruit (inoculated but uncoated) and area under the disease progress stairs (AUDPS) of stem-end rot on mandarins ‘Ortanique’ artificially inoculated with *Lasiodiplodia theobromae*, coated 24 h later with HPMC-BW composite edible coatings containing GRAS salts, and incubated for 10 d at 28 °C and 90% RH. GRAS salts and concentrations are those indicated in Table 3. AUDPS was determined with readings of lesion diameter after 3, 7, and 10 d of incubation. Columns with different letters are significantly different according to Fisher's protected LSD test ($P < 0.05$) applied after an ANOVA.

and AUDPS were used as means to assess the disease control ability of the coatings. Although all the tested antifungal coatings reduced somewhat the severity of stem-end rot in comparison with uncoated controls, disease reduction was only important with coatings containing PS (about 50% reduction) and SB (about 40% reduction). Coatings formulated with SEP and PSi reduced disease severity about 20–25% and the rest only 10% or less (Fig. 1).

AUDPS on artificially inoculated and coated ‘Ortanique’ mandarins was determined with readings of lesion diameter after 3, 7, and 10 d of incubation at 28 °C and 90% RH. Data showed that the emulsion of HPMC-BW had no antifungal activity by itself since AUDPS was the same (about 190) on fruit treated with this coating (without salt addition) than on uncoated control fruit. In contrast, all the coatings formulated with GRAS salts significantly reduced AUDPS if compared with the control ($P < 0.05$), although important reductions were only obtained with coatings containing PS at 2% (from 190 to 100) and SB at 2% (from 190 to 120). Coatings with SEP at 0.1% reduced AUDPS from 190 to about 150 (Fig. 1).

These three coatings at these concentrations were therefore selected to be tested again in a confirmation experiment with ‘Barnfield’ oranges artificially inoculated with *L. theobromae*. In this case, treated fruit were incubated for 15 d at 28 °C and 90% RH and readings of lesion size were performed after 4, 8, 12, and 15 d of incubation. Similarly to the previous results with mandarins, all coatings significantly reduced Diplodia stem-end rot severity on oranges, being the coatings containing 2% PS or 2% SB significantly more effective (about 60% reduction) than the coating containing 0.1% SEP (about 30% reduction) ($P < 0.05$) (Fig. 2). AUDPS results were in accordance with these observations, although in this case the AUDPS value on oranges treated with PS-coatings was significantly lower than on fruit treated with SB-

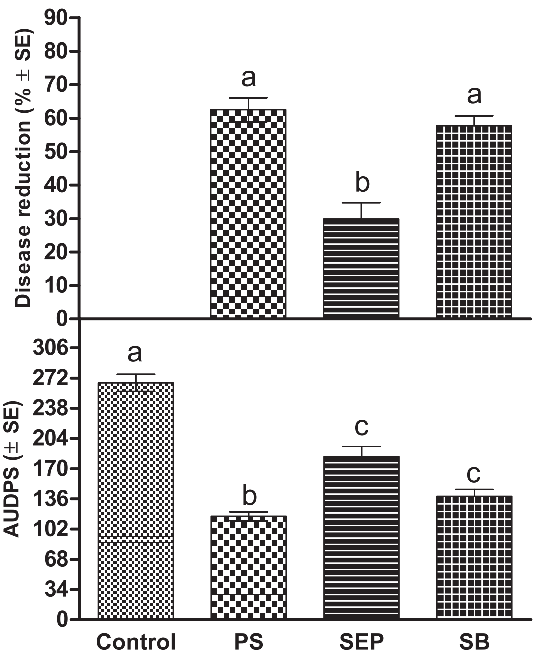

Fig. 2. Reduction of the severity with respect to control fruit (inoculated but uncoated) and area under the disease progress stairs (AUDPS) of stem-end rot on 'Barnfield' oranges artificially inoculated with *Lasiodiplodia theobromae*, coated 24 h later with HPMC-BW composite edible coatings containing GRAS salts, and incubated for 15 d at 28 °C and 90% RH. Coatings were formulated with 2% potassium sorbate (PS), 0.1% sodium ethylparaben (SEP), or 2% sodium benzoate (SB). AUDPS was determined with readings of lesion diameter after 4, 8, 12, and 15 d of incubation. Columns with different letters are significantly different according to Fisher's protected LSD test ($P < 0.05$) applied after an ANOVA.

or SEP-coatings.

To our knowledge, this is the first research work in which edible coatings with GRAS salts are applied to citrus fruit for the control of Diplodia stem-end rot. Likewise, previous references to the use for this purpose of coatings or waxes non-amended with conventional fungicides are really scarce. Early work by Waks et al. (1985) showed that while the application of citrus commercial waxes reduced the incidence of green and blue molds during cold storage, it increased that of stem-end rots caused by *L. theobromae* or *Alternaria citri* Ellis & N. Pierce. They discussed that the cause could be changes in the internal atmosphere of the fruit induced by waxing, particularly an increment of EC. A coating comprised of glycolchitosan and the biocontrol yeast *Candida saitoana* reduced the incidence of stem-end rot on semi-commercial trials with 'Valencia' oranges (El-Ghaouth et al., 2000). Recently, Xu et al. (2018) reported that chitosan and chitosan/montmorillonite coatings significantly reduced the accumulated decay rate of cold-stored tangerines, but they did not identify the actual pathogens causing decay. Nevertheless, some information is available on the postharvest use of coatings to control Diplodia stem-end rot on other fruit commodities. For instance, decay caused by *L. theobromae* on avocado was significantly reduced by a commercial coating amended with essential oil from the plant *Lipia scaberrima* (Regnier et al., 2010) or by a pectin-based edible coating (Maftoonazad et al., 2007). The disease was also reduced on banana by coatings comprised of chitosan and cinnamon extract (Win et al., 2007), on mango by chitosan coatings formulated with the active antimicrobial lactoperoxidase system (Cissé et al., 2015), and on longan by chitosan coatings formulated with the GRAS salt PS (Apai et al., 2008). The noteworthy activity of PS is in agreement with the present results obtained with citrus fruit. The compound ethylparaben produced by the biocontrol agent *Brevibacillus brevis* was identified in recent work as effective against *L. theobromae* and mutants of the antagonistic bacteria producing more ethylparaben were promising for the biocontrol of Diplodia stem-end rot on apple fruit (Che et al., 2018).

The type of HPMC-BW antifungal edible coatings with GRAS salts tested here have been already evaluated for the control of other important postharvest diseases of citrus fruit. Particularly, Valencia-Chamorro et al. (2008, 2009a) found that, among a large variety of GRAS salts tested, HPMC-BW coatings containing the salts PS, SB, SP, and their mixtures exhibited antifungal activity in vitro against the citrus pathogens *P. digitatum* and *P. italicum* and were the most effective for green and blue mold reduction on 'Valencia' oranges and 'Ortanique' and 'Clemenules' mandarins artificially inoculated with these pathogens, coated 24 h later, and incubated at 20 °C for 7 d to simulate fruit shelf life. The curative activity of similar composite coatings has also been observed in other fresh fruit pathosystems. Fagundes et al. (2013, 2015) found significant reductions of black spot on cherry tomatoes artificially inoculated with *A. alternata* and coated 24 h later with HPMC-BW coatings formulated with SB, SEP, or SMP. Karaca et al. (2014) reported that HPMC-BW coatings containing PS, SEP, or SMP, among other salts, effectively reduced the incidence and severity of brown rot caused by *M. fructicola* on artificially inoculated plums. It seems clear from this research that the selection of the most appropriate antimicrobial agent in general and GRAS salt in particular to confer disease control ability to a coating is greatly dependent on each particular pathosystem (type and properties of the stored fruit and activity of the agent against the target pathogen), but also on issues such as the good interaction between the composite matrix and the antifungal salt, the matrix capacity to gradually release the salt during storage, the presence of other additives in the emulsion, and the environmental conditions during storage and shelf life (Valdés et al., 2017; Valencia-Chamorro et al., 2011a, 2011b). Emulsion attributes such as pH and viscosity (Table 3) clearly influence these properties and are dependent not only on the coating matrix (HPMC-BW) and the GRAS salt and their respective proportions, but also on the nature and amount of the other components of the coating (i.e., stearic acid and glycerol). The importance of the role of the coated fruit host (species and even cultivar) and the environmental conditions on the disease control ability of antifungal coatings may explain why results from in vivo experiments cannot often be anticipated by the in vitro antifungal activity of the GRAS salt (Fagundes et al., 2013; Karaca et al., 2014). Tests in Petri dishes allow fully exposure of the fungal structures to the antifungal salt, while in in vivo tests with coatings the contact can be limited depending on the emulsion properties, the characteristics of the fruit peel, and the storage conditions. Therefore, it is important to tailor the formulation of appropriate coatings for particular fruit species and cultivars and even specific postharvest applications. On the other hand, the application of antifungal compounds such as GRAS salts as coating ingredients can be advantageous if compared to their postharvest application in citrus packinghouses as aqueous solutions in drench, dip or spray systems. Coatings could be designed to slow down the diffusion of the active ingredient from the matrix to the commodity, which could contribute to maintain for longer periods of time the effective residue of the antifungal on the fruit surface (Vargas et al., 2008). In addition, coating application could reduce in some cases phytotoxicity risks or alleviate potential adverse effects on fruit quality associated with aqueous applications (Palou et al., 2015).

3.3. Effect of coating application on fruit quality

HPMC-BW composite coatings containing PS at 2%, SB at 2%, and SEP at 0.1% were selected for fruit quality evaluation because they showed the highest control ability in in vivo trials. Weight loss of non-inoculated 'Barnfield' oranges uncoated (control) or coated and cold-stored at 5 °C and 90% RH followed by shelf life at 20 °C is shown in Fig. 3. As expected, weight loss was higher after 42 d (about 3–3.5%) than after 21 d (2–2.5%) of cold storage followed by 7 d of shelf life.

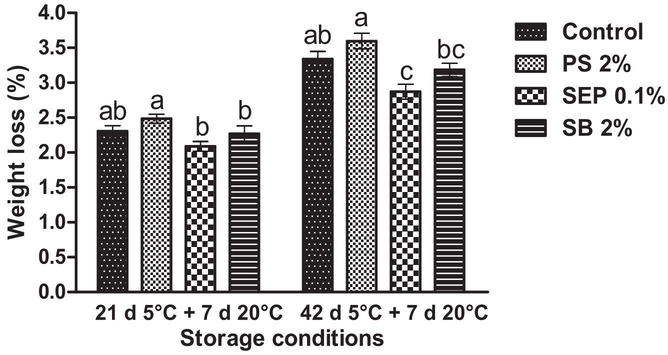

Fig. 3. Weight loss of 'Barnfield' oranges uncoated (control) or coated with HPMC-BW composite edible coatings containing GRAS salts at the indicated concentrations and stored for the indicated periods: Potassium sorbate (PS), sodium ethylparaben (SEP), sodium benzoate (SB). For each storage period, columns with different letters are significantly different according to Fisher's protected LSD test ($P < 0.05$) applied after an ANOVA.

After both storage periods, weight loss reduction on coated oranges was not high, but coatings containing SEP significantly reduced weight loss with respect to uncoated control fruit ($P < 0.05$). Coatings containing SB and PS slightly influenced weight loss (positively and negatively), but not significantly. Working with 'Valencia' oranges, Valencia-Chamorro et al. (2009b) also found that HPMC-BW-shellac coatings containing SB were superior to coatings containing PS for weight loss reduction during cold storage at 5 °C. They discussed that PS-coatings exhibited higher water vapor permeability than SB-coatings. Similarly, SB-coatings also reduced weight loss and maintained firmness of 'Clemenules' clementine mandarins without adverse effects on the overall quality of coated fruit (Valencia-Chamorro et al., 2011a, 2011b). Gunaydin et al. (2017) reported similar results working with plums, where HPMC-BW coatings, with or without antifungal agents, significantly reduced weight loss compared to uncoated control samples, and the coatings containing sodium paraben salts such as SEP were more effective than those containing PS. In general, cellulose-lipid composite coatings are reported to reduce fruit weight loss due to the moisture barrier exerted by the lipid ingredients (BW, shellac, etc.) of the coating formulation (Contreras-Oliva et al., 2011). Similarly to postharvest decay control, their effectiveness to reduce fruit weight loss depends not only on the lipid composition and concentration, but also on the species and cultivar of the fruit. Thus, similar HPMC-lipid coatings effectively reduced weight loss of 'Ortanique' and 'Chemenules' mandarins (Valencia-Chamorro et al., 2010, 2011b) but not of 'Valencia' oranges (Valencia-Chamorro et al., 2009b).

Table 4 shows the external and internal physicochemical quality attributes of 'Barnfield' oranges at harvest and after treatment with HPMC-BW coatings containing the selected antifungal GRAS salts. Firmness, SSC, TA, EC, and AC were evaluated in fruit stored at 5 °C for 21 or 42 d followed by a shelf-life period of 7 d at 20 °C. After 21 d, rind deformation (expressing fruit firmness) of all coated samples was in the range 1.0–1.5% and fruit coated with emulsions formulated with 2% SB were less firm (higher percentage of deformation) than fruit treated with the other coatings. After 42 d, oranges treated with SB- and PS-coatings were less firm than control fruit and fruit treated with SEP-coatings ($P < 0.05$), although in all cases the percent deformation was low, in the range of 2–3% (Table 4). According to previous results with HPMC-BW coatings, it seems that the citrus cultivar plays an important role on the effect of coating on fruit firmness. For instance, while HPMC-BW coatings amended with SB, PS, or mixtures did not affect the firmness of coated 'Valencia' oranges (Valencia-Chamorro et al., 2009b), they significantly increased the firmness of coated 'Clemenules' mandarins (Valencia-Chamorro et al., 2011a, 2011b), showing that the response of coated fruit is not only dependent on the coating characteristics but also on the fruit inherent attributes. Polysaccharides such as pectin, starch, and hemicellulose, present in the cell wall, are important for the maintenance of fruit firmness and the degradation of these compounds by hydrolyzing enzymes, such as pectin methylesterase and polygalacturonase, causes softening of the fruit during ripening and storage. Edible coatings can have the capacity to modify the internal gas composition of the fruit in terms of O_2 and CO_2 concentrations, which might influence the activities of the cell wall degrading enzymes, reducing fruit softening (Gunaydin et al., 2017). Furthermore, there is usually a correlation between the effect of coatings on fruit firmness and weight loss, although this aspect is often dependent on the citrus cultivar. For instance, while, in accordance with the present results, no correlation was observed in the studies by Pérez-Gago et al. (2002) with 'Fortune' mandarins, a positive correlation was reported by Navarro-Tarazaga et al. (2008) for 'Ortanique' mandarins.

Regarding juice quality, coated oranges, irrespective of the coating used and the storage period, were less sweet than control fruit ($P < 0.05$ for SSC parameter) and no significant differences in TA were observed between control and coated fruit (Table 4). The lower SCC of coated oranges could be related to changes in fruit respiration and gas exchange patterns induced by coating application. The concentration of internal CO_2 in 'Barnfield' oranges uncoated or coated with HPMC-BW coatings containing the selected salts is showed in Fig. 4. All three coatings modified the internal atmosphere of coated oranges, especially after 42 d at 5 °C, and internal CO_2 levels were significantly higher in coated fruit, which indicates that the coatings were effective as gas barriers ($P < 0.05$). After 21 d of cold storage, but not after 42 d, CO_2 levels were lower in oranges treated with the coating containing SB than in those coated with the coating formulated with PS ($P < 0.05$). In this study, internal CO_2 values in coated oranges (4–6 kPa) were equivalent to those observed in coated 'Valencia' oranges (Valencia-Chamorro et al., 2009b) and 'Clemenules' clementines (Valencia-

Table 4
Quality attributes of 'Barnfield' oranges coated with HPMC-BW composite edible coatings containing antifungal GRAS salts, stored at 5 °C followed by 7 d of shelf life at 20 °C.

Coating with GRAS salt	21 d 5 °C + 7 d 20 °C					42 d 5 °C + 7 d 20 °C				
	Firmness (% deformation)	SSC (%)	TA (% citric acid)	EC (mg/L)	AC (mg/L)	Firmness (% deformation)	SSC (%)	TA (% citric acid)	EC (mg/L)	AC (mg/L)
At harvest	1.12	12.32	0.694	106.0	5.43					
Control	1.39ab	13.91a	0.636ab	434.2a	7.951ab	2.16c	13.9a	0.680ab	458.3a	8.852a
PS 2%	1.25b	10.38d	0.724a	382.5ab	8.838a	2.74ab	12.45b	0.706a	384.1a	10.813a
SEP 0.1%	1.22b	10.93c	0.721a	388.1ab	9.155a	2.33bc	12.35b	0.479b	448.2a	9.990a
SB 2%	1.49a	13.15b	0.574b	271.9b	6.013b	2.80a	11.91b	0.581ab	609.1a	10.532a

HPMC, hydroxypropyl methylcellulose; BW, beeswax; PS, potassium sorbate; SEP, sodium ethylparaben; SB, sodium benzoate.
SSC, soluble solids content; TA, titratable acidity; EC, ethanol content; AC, acetaldehyde content.
Columns with different letters are significantly different according to Fisher's protected LSD test ($P < 0.05$) applied after an ANOVA.

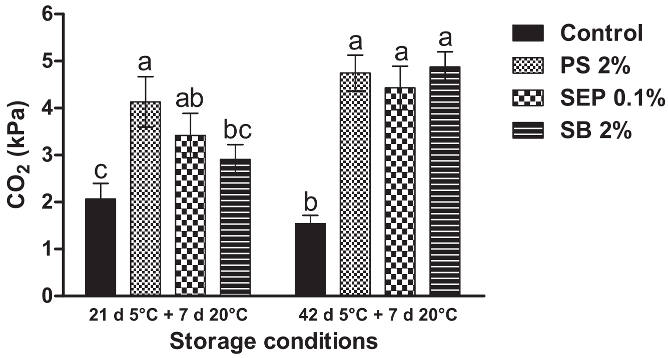

Fig. 4. Concentration of internal CO_2 in 'Barnfield' oranges uncoated (control) or coated with HPMC-BW composite edible coatings containing GRAS salts at the indicated concentrations and stored for the indicated periods: Potassium sorbate (PS), sodium ethylparaben (SEP), sodium benzoate (SB). Columns with different letters are significantly different according to Fisher's protected LSD test ($P < 0.05$) applied after an ANOVA.

Chamorro et al., 2011b), but lower than those observed in 'Ortanique' mandarins coated with similar HPMC-lipid coatings containing GRAS salts (6–8 kPa) (Valencia-Chamorro et al., 2010), and in 'Valencia' oranges (Navarro-Tarazaga et al., 2007) and 'Fortune' mandarins (Pérez-Gago et al., 2002) coated with HPMC-lipid coatings without GRAS salts. Factors such as fruit peel morphology and physical properties of the coating, including the addition to the emulsion of GRAS salts or other food additives, should be considered as factors influencing fruit respiration when coatings are applied to citrus fruit. These factors could influence the coating flexibility or its capacity of adaptation to the fruit surface, affecting the gas barrier of the coating. The effect of edible coatings on delaying changes related to fruit ripening, such as softening, color change, decrease in acidity, and some physiological disorders has been associated with the gas barrier exerted on the fruit surface leading to reductions in respiration rate and/or weight loss (Fagundes et al., 2015; Valero et al., 2013). In the work conducted by Gunaydin et al. (2017), application of HPMC-BW matrixes containing paraben salts resulted in the lowest CO_2 production rates, showing the potential of these coatings as gas barriers on plums. In contrast, Valencia-Chamorro et al. (2008) reported an increase in O_2 permeability of HPMC-BW-shellac edible films amended with SEP and Fagundes et al. (2015), working with similar coatings amended with a variety of antifungal agents, observed the highest respiration rates in cherry tomatoes coated with emulsions containing SEP. This confirms that the capacity of an edible coating to create an effective gas barrier depends not only on the coating composition and properties, but also on the commodity, cultivar, and storage conditions (Gunaydin et al., 2017).

In general, after both storage periods of 21 and 42 d, volatile content in 'Barnfield' oranges was not affected by coating application and ranged 200–600 mg/L of ethanol and 6–11 mg/L of acetaldehyde, in spite of the higher internal CO_2 concentration in coated than in uncoated fruit (Table 4, Fig. 4). This was not an anticipated result since in previous studies fruit coating increased EC in the juice of coated oranges and mandarins cold-stored for long periods, indicating the creation of a modified atmosphere within the fruit (Pérez-Gago et al., 2002; Valencia-Chamorro et al., 2009b, 2011a, 2011b). However, differences in the citrus cultivar, in the composition of the HPMC-BW coatings used in these studies (presence of shellac or different antifungal ingredients and concentrations), and in the total solid content and viscosity may explain this different behavior. In contrast to ethanol, it is more frequent that the levels of acetaldehyde on coated and cold-stored citrus fruit do not differ significantly from those in uncoated control fruit (Valencia-Chamorro et al., 2009b, 2010).

Results from the sensory evaluation of 'Barnfield' oranges treated with HPMC-BW edible coatings containing antifungal GRAS salts and stored at 5 °C followed by 7 d of shelf life at 20 °C are showed in Table 5. The evaluation was based on the assessment of fruit flavor and coating appearance by a trained panel of 8–10 members. Fruit flavor, evaluated in a 1–9 scale, was very slightly modified by the application of HPMC-BW based coatings containing salts. After the first storage period of 21 d at 5 °C, no significant differences were observed among control and coated oranges, while after 42 d, the coating containing PS was rated as with the poorest flavor, although with no significant differences with control fruit. It is well known that citrus off-flavor is due to the accumulation of volatile components associated to anaerobic fermentation (Ke and Kader, 1990). Among the different volatiles that may be present, ethanol has been found to be the component undergoing the greatest change occurring in citrus during storage and the application of fruit coatings can lead to enhanced anaerobic respiration as they restrict gas exchange through the rind surface (Tietel et al., 2011). Prior research showed that off-flavor production from modified EC in citrus is cultivar dependent. In general, mandarins are more sensitive to anaerobic conditions than other citrus fruit, which has been attributed to differences in enzymatic activity and in peel permeability to gases (Shi et al., 2007). Thus, for example, minimum EC associated with off-flavor has been reported to be 2000 mg/L in 'Valencia' oranges (Ke and Kader, 1990), whereas EC of 1000 mg/L and 500–600 mg/L have been reported in 'Clemenules' (Navarro-Tarazaga and Pérez-Gago, 2006) and 'Murcott' mandarins (Shi et al., 2005), respectively. In this work, EC levels were lower than those reported by other authors to induce off-flavor in oranges and in some mandarin cultivars (Table 4), which can explain the sensory scores.

Table 5
Sensory evaluation of flavor and coating appearance of 'Barnfield' oranges coated with HPMC-BW edible composite coatings containing antifungal GRAS salts, stored at 5 °C followed by 7 d of shelf life at 20 °C.

GRAS salts	21 d 5 °C + 7 d 20 °C		42 d 5 °C + 7 d 20 °C	
	Flavor (1–9 scale)	Coating appearance (1–3 scale)	Flavor (1–9 scale)	Coating appearance (1–3 scale)
Control	6.90a	3.00a	6.63ab	3.00a
PS 2%	7.00a	1.29c	5.90b	1.36b
SEP	6.72a	1.86b	6.09ab	1.63b
SB 2%	6.81a	1.00d	7.00a	1.00c

HPMC, hydroxypropyl methylcellulose; BW, beeswax.
PS, potassium sorbate; SEP, sodium ethylparaben; SB, sodium benzoate.
Columns with different letters are significantly different according to Fisher's protected LSD test ($P < 0.05$) applied after an ANOVA.

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 coated oranges was not optimal because scores after shelf life were 1–2, while they were 3 for uncoated control oranges. Coatings containing SB at 2% were the worst qualified in terms of external appearance ($P < 0.05$). In general, HPMC-BW emulsion coatings are not characterized for providing significant gloss to coated fruit such as citrus and tomatoes, mainly due to the macro emulsion character of the coating formulation (Fagundes et al., 2015; Valencia-Chamorro et al., 2009a, b, 2011a, b). Furthermore, Valencia-Chamorro et al. (2010) also reported the presence of small white spots on the surface of coated mandarins that reduced the general good appearance of the samples when the HPMC-based coatings were amended with some GRAS salts.

4. Conclusion

In this work, GRAS salts and concentrations were selected according to their in vitro antifungal activity against *L. theobromae* NEU-1, a strain of the pathogen isolated from citrus fruit in Valencia (Spain). The potential of these salts as ingredients of antifungal HPMC-BW edible coatings applied for stem-end rot control and quality preservation of citrus fruit was highlighted. Coatings containing PS, SEP, and SB were selected and significantly reduced the severity of Diplodia stem-end rot on artificially inoculated ‘Ortanique’ mandarins and ‘Barnfield’ oranges. Application of the coatings containing SEP and SB significantly reduced weight loss of coated ‘Barnfield’ oranges. All the coatings increased internal CO_2 concentration compared to uncoated oranges, but did not adversely affect EC and AC in the juice, the flavor, and the production of off-flavors. Further research should focus on the improvement of physical characteristics of the coatings to enhance water loss control and gloss of coated citrus fruit. Likewise, the combination of these antifungal coatings with other alternative nonpolluting control methods to synergistically improve the control of Diplodia stem-end rot in citrus packinghouses should also be explored.

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