Research Article

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Starch-based antifungal edible coatings to control sour rot caused by *Geotrichum citri-aurantii* and maintain postharvest quality of 'Fino' lemon

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Abstract

BACKGROUND: Two edible coating (EC) emulsions based on potato starch (F6 and F10) alone or formulated with sodium benzoate (SB, 2% w/w) (F6/SB and F10/SB) were evaluated to maintain postharvest quality of cold-stored 'Fino' lemons and control sour rot on lemons artificially inoculated with *Geotrichum citri-aurantii*. Previous research showed the potential of these ECs to improve the storability of 'Orri' mandarins and reduce citrus green and blue molds caused by *Penicillum digitatum* and *Penicillium italicum*, respectively.

RESULTS: The coatings F6/SB and F10/SB significantly reduced sour rot incidence and severity compared to uncoated control samples on lemons incubated at 28 °C for 4 and 7 days. The F6/SB coating reduced weight loss and gas exchange compared to uncoated fruit after 2 and 4 weeks of storage at 12 °C plus a shelf life of 1 week at 20 °C, without adversely affecting the lemon physicochemical quality.

CONCLUSION: Overall, the F6/SB coating formulation, composed of pregelatinized potato starch, glyceryl monostearate, glycerol, emulsifiers and SB, with a total solid content of 5.5%, showed the best results in reducing citrus sour rot and maintaining the postharvest quality of cold-stored 'Fino' lemons. Therefore, it showed potential as a new cost-effective postharvest treatment suitable to be included in integrated disease management programs for citrus international markets with zero tolerance to chemical residues.

Keywords: *Citrus limon*; food additives; GRAS salts; postharvest quality; sour rot control

INTRODUCTION

Citrus postharvest sour rot, caused by *Geotrichum citri-aurantii* (Ferraris) E.E. Butler, has increasingly become a major cause of economic losses in the Mediterranean region, especially when fruits are harvested after wet and rainy periods and packinghouse sanitation is inadequate. Moreover, compared to oranges or mandarins, which are typically stored at 3–5 °C, sour rot development is particularly significant on mature lemons often stored for extended periods at relatively high temperatures of 12–14 °C to avoid chilling injury.[1]

Citrus sour rot cannot be efficiently controlled by conventional chemical fungicides, such as imazalil and thiabendazole, which are widely used to control citrus green and blue molds caused by *Penicillium digitatum* and *Penicillium italicum*,[2] respectively. After the withdrawal of guazatine and propiconazole in the European Union (EU) in 2011 and 2018, respectively, no postharvest fungicides that effectively control sour rot are available in EU countries, which is currently of great concern among European citrus packers and exporters. Furthermore, because the general use of conventional fungicides is becoming increasingly restricted as a result of risks associated with toxicity in humans and environmental contamination, research is highly encouraged to seek new, safe and effective alternatives to control citrus sour rot.[2,3]

The use of organic and inorganic salts, classified as food additives or 'generally recognized as safe' (GRAS), as antifungal postharvest treatments has been suggested as a promising non-polluting alternative to conventional fungicides. The main

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advantages of GRAS salts are their high water solubility, availability, low cost and potential for controlling postharvest diseases as aqueous solutions or ingredients of composite edible coatings (ECs).[4] Many studies have demonstrated the effectiveness of GRAS salts, such as carbonates, sorbates, benzoates and metabisulfites, amongst others, to control citrus green and blue molds.[3,5,6] However, few studies have reported the application of GRAS salts to control citrus postharvest sour rot. Smilanick *et al.*[6] showed that sour rot incidence was significantly reduced by immersing artificially inoculated lemons in potassium sorbate or sodium bicarbonate solutions at 1% (w/v) for 30 s at 50 °C. Talibi *et al.*[7] tested 34 different salts and found that only sodium salicylate, boric acid and ethylenediaminetetraacetic acid were effective for controlling sour rot on mandarins. Moreover, sodium dehydroacetate combined with sodium silicate reduced sour rot incidence on 'Satsuma' mandarins.[8] Previous work in our laboratory at the IVIA CTP showed the significant curative activity of dips in aqueous solutions of sodium benzoate to control sour rot on commercially important cultivars of oranges and mandarins, with an effectiveness similar to that of propiconazole, especially if heated solutions were used.[2] Therefore, it is worthy to explore the performance of ECs containing this salt as an antifungal ingredient for sour rot control. Compared to aqueous treatments, the use of antifungal ECs has significant advantages because they provide important physiological functionalities to fruit devoted to prolonged storage. Among them, the regulation of water and gas exchange to reduce fruit respiration and transpiration, and consequently extend the fruit postharvest life, is the most important.[9]

The postharvest use of ECs containing GRAS salts consists of coating the fruit with a thin layer of edible material that provides antifungal activity through a slow diffusion of the salt from the coating matrix to the fruit rind. At the same time, composite ECs allow to maintain the fruit physicochemical quality through the action of the matrix components, typically a hydrocolloid that provides a barrier to gases and a lipid that provides a barrier to water vapor.[4,9,10] Previous works by our group have reported the effectiveness of ECs based on hydroxypropyl methylcellulose (HPMC)-lipid matrixes containing antifungal GRAS salts to improve fruit quality during storage and control citrus postharvest diseases.[9,11,12]

For each target pathogen and host, the effectiveness of antifungal ECs depends on the interaction between the added active agents and the film-forming materials because they can subsequently affect either positively or negatively the mechanical and structural properties of the resulting films and coatings.[13] Thus, it is necessary to optimize the formulations for each target pathogen, fruit host species and even fruit cultivar.[9] In this regard, our group has recently developed and optimized antifungal ECs based on potato starch (PPS) and glyceryl monostearate formulated with the GRAS salt sodium benzoate as antifungal agent to maintain the physicochemical and sensory quality attributes of 'Orri' mandarins during storage at 20 °C.[14] The antifungal ability of the optimized PPS-based ECs containing sodium benzoate has been tested with good results with respect to controlling green and blue molds on different citrus species either incubated at 20 °C or stored at low temperatures.[15] However, to our knowledge, no information is available about the use of GRAS salts as ingredients of starch-based ECs for controlling sour rot caused by *G. citri-aurantii* on lemons and preserving fruit quality during cold storage. Therefore, the present study aimed to (i) evaluate the effect of the optimized antifungal PPS-based ECs on the quality attributes of 'Fino' lemons during long-term cold storage and (ii) assess their curative activity to control sour rot on 'Fino' lemons artificially inoculated with *G. citri-aurantii* and incubated at 28 °C.

MATERIALS AND METHODS

Fruit

The present study was carried out with 'Fino' lemons [*Citrus limon* (L.) Osbeck] obtained from commercial orchards in the Murcia area (Spain) at commercial maturity and used the next day in the experiments. No commercial postharvest treatments were applied before the experiments. Lemons were selected for uniformity of size and shape and diseased or damaged fruits were discarded. Selected fruits were disinfected superficially by immersion for 4 min in a sodium hypochlorite solution (5 g L^{-1}), rinsed with tap water and allowed to air-dry at room temperature before EC application or fungal inoculation. Fruits were randomized before each experiment.

Preparation of edible coating formulations

The EC formulations were prepared by combining pregelatinized potato starch (PPS) (Quimidroga, S.A., Barcelona, Catalonia, Spain), glyceryl monostearate (GMS) (Italmatch Chemicals Spa, Barcelona, Catalonia, Spain) and glycerol (Panreac-Química S.A., Barcelona, Catalonia, Spain) suspended in water. Sodium benzoate (SB) (Sigma-Aldrich Química S.A., Tres Cantos, Madrid, Spain) was incorporated as antifungal GRAS salt in the formulations at 20 g kg^{-1}. The PPS, GMS and glycerol were combined in different proportions, as detailed in Table 1, to prepare four different ECs named as F10, F6, F10/SB and F6/SB based on the optimized stable emulsions described by Soto-Muñoz *et al.*,[14] where F10 and F6 were the ECs without the GRAS salt and F10/SB and F6/SB were the same ECs formulated with 20 g kg^{-1} SB. All formulations included sunflower lecithin and diacetyl tartaric acid esters of mono-diglycerides (Lasenor S.A., Barcelona, Catalonia, Spain) as emulsifiers at a constant ratio of GMS:emulsifier of 2:1 (dry basis, d.b.). The emulsions were kept overnight at 5 °C before use.

Table 1. Composition of experimental composite starch edible coatings

Components	F10	F6	F10/SB	F6/SB
PPS (g kg^{-1}, d.b.)	483	286	320	182
GMS (g kg^{-1}, d.b.)	132	286	88	182
Glycerol (g kg^{-1}, d.b.)	252	143	167	91
Sodium benzoate (SB; g kg^{-1}, w.b.)	—	—	20	20
SC (g kg^{-1})	39	35	59	55

Abbreviations: d.b., dry basis; GMS, glyceryl monostearate; PPS, pregelatinized potato starch; SC, solids content; w.b., wet basis.

Effect of coatings on quality of cold-stored fruit

Fruit preparation, coating application and storage conditions

A set of randomly distributed 60 lemons per treatment was manually coated by immersion for 15 s in the corresponding emulsion at room temperature. Coating treatments were F10, F6, F10/SB and F6/SB (Table 1). Control fruits were dipped for 15 s in tap water at 20 °C. One set of fruit was also dipped for 15 s in a 20 g L^{-1} SB aqueous solution at 20 °C. Coated lemons were drained and dried at room temperature (23 ± 3 °C). Treated fruits were placed into plastic open boxes and cold-stored at 12 °C, as recommended for this chill sensitive cultivar, for up to 4 weeks, followed by a shelf-life period of 7 days at 20 °C. The following fruit quality attributes were determined at harvest and after 2 and 4 weeks of cold storage plus 1 week of shelf life.

Weight loss

Twenty fruits per treatment were used to evaluate lemon weight loss during storage. After treatment, each fruit was individually numbered and weighed with a calibrated analytical balance (model P30; Alessandrini, Modena, Italy). Measurements were repeated at the end of each storage period.

Fruit firmness

Firmness of 20 lemons per treatment was determined as the percentage of rind deformation, related to initial diameter, with an Instron Universal testing machine (model 3343; Instron Corp., Canton, MA, USA) after application of a load of 10 N to the equatorial region of the fruit, according to Valencia-Chamorro *et al.*[11]

Color

Skin color was evaluated with a Minolta Colorimeter (model CR-300; Minolta Co. Ltd, Osaka, Japan) on samples of 20 fruit per treatment, using the CIELAB parameters lightness (L^*), a^*, b^*, chroma (C^*) and hue angle ($h°$). Only hue values are shown.

Juice quality

Soluble solids concentration (SSC) (°Brix), titratable acidity (TA) (g L^{-1} of citric acid) and pH were determined as described by

Table 2. Quality attributes of 'Fino' lemons coated with pregelatinized potato starch (PPS)-based composite edible coatings (ECs) formulated with or without sodium benzoate (SB, 20 g kg^{-1}) and stored for 2 and 4 weeks at 12 °C followed by 1 week of shelf life at 20 °C

		Treatments‡					
Quality attributes†	Storage conditions	Control	SB (20 g L^{-1})	F10	F6	F10/SB	F6/SB
Weight loss (%)	At harvest						
	2 weeks at 12 °C + 1 week at 20 °C	3.62 ± 0.11 a	3.62 ± 0.13 a	3.74 ± 0.25 a	3.81 ± 0.23 a	3.59 ± 0.15 a	3.63 ± 0.17 a
	4 weeks at 12 °C + 1 week at 20 °C	5.73 ± 0.18 b	5.59 ± 0.23 b	5.46 ± 0.27 b	5.04 ± 0.15 a, b	5.58 ± 0.25 b	4.51 ± 0.23 a
Firmness (% deformation)	At harvest	2.49 ± 0.06					
	2 weeks at 12 °C + 1 week at 20 °C	2.50 ± 0.06 a	2.35 ± 0.09 a	2.43 ± 0.11 a	2.45 ± 0.11 a	2.42 ± 0.11 a	2.65 ± 0.15 a
	4 weeks at 12 °C + 1 week at 20 °C	3.28 ± 0.10 a	3.10 ± 0.12 a	3.03 ± 0.16 a	2.91 ± 0.12 a	3.64 ± 0.27 a	3.17 ± 0.17 a
pH	At harvest	2.83 ± 0.00					
	2 weeks at 12 °C + 1 week at 20 °C	2.84 ± 0.02 b	2.85 ± 0.01 b	2.67 ± 0.06 a	2.88 ± 0.01 b	2.95 ± 0.01 c	2.85 ± 0.02 b, c
	4 weeks at 12 °C + 1 week at 20 °C	3.03 ± 0.10 a	3.16 ± 0.05 a	3.13 ± 0.00 a	3.12 ± 0.01 a	3.14 ± 0.00 a	3.15 ± 0.00 a
Titratable acidity (g L^{-1} citric acid)	At harvest	51.6 ± 1.3					
	2 weeks at 12 °C + 1 week at 20 °C	48.2 ± 0.7 a	49.1 ± 1.6 a	48.2 ± 0.9 a	46.0 ± 0.9 a	41.4 ± 1.4 a	50.5 ± 2.6 a
	4 weeks at 12 °C + 1 week at 20 °C	48.5 ± 0.3 a	49.7 ± 1.5 a	47.2 ± 1.2 a	46.5 ± 1.0 a	48.2 ± 0.4 a	47.2 ± 1.0 a
Soluble solids content (°Brix)	At harvest	7.03 ± 0.09					
	2 weeks at 12 °C + 1 week at 20 °C	7.05 ± 0.09 a	7.00 ± 0.10 a	6.80 ± 0.06 a	7.05 ± 0.09 a	6.95 ± 0.13 a	6.93 ± 0.08 a
	4 weeks at 12 °C + 1 week at 20 °C	7.15 ± 0.05 a	7.07 ± 0.02 a	7.38 ± 0.26 a	7.02 ± 0.13 a	7.30 ± 0.03 a	7.13 ± 0.13 a
Hue angle ($h°$)	At harvest	92.26 ± 0.37					
	2 weeks at 12 °C + 1 week at 20 °C	92.39 ± 0.31 b	91.62 ± 0.21 a	92.98 ± 0.26 b	92.84 ± 0.22 b	91.64 ± 0.27 a	92.28 ± 0.25 a, b
	4 weeks at 12 °C + 1 week at 20 °C	91.78 ± 0.27 a	91.59 ± 0.18 a	91.34 ± 0.34 a	91.07 ± 0.30 a	91.28 ± 0.29 a	91.67 ± 0.28 a

† For each attribute and storage period, means in rows with different lowercase letters are significantly different according to Fisher's protected LSD test ($P < 0.05$) applied after an ANOVA.
‡ Data are the mean ± SE. Treatments applied were: (i) uncoated control (immersion in water); (ii) coating F10; (iii) coating F6; (iv) 20 g L^{-1} SB aqueous solution; (v) coating F10/SB; and (vi) coating F6/SB.

Table 3. Internal concentration of CO_2, O_2, ethanol and acetaldehyde in 'Fino' lemons coated with pregelatinized potato starch (PPS)-based composite edible coatings (ECS) formulated with or without sodium benzoate (SB 2%) and stored for 2 and 4 weeks at 12 °C followed by 1 week of shelf life at 20 °C

Internal atmosphere†	Storage conditions	Treatments‡					
		Control	SB (20 g L^{-1})	F10	F6	F10/SB	F6/SB
Acetaldehyde ($mg\ L^{-1}$)	At harvest	1.3 ± 0.03					
	2 weeks at 12 °C + 1 week at 20 °C	2.3 ± 0.01 ab	1.5 ± 0.019 a	6.0 ± 0.07 c	6.4 ± 0.14 c	3.2 ± 0.03 ab	3.8 ± 0.04 b
	4 weeks at 12 °C + 1 week at 20 °C	1.8 ± 0.05 a	2.3 ± 0.04 a	2.6 ± 0.07 a	3.2 ± 0.03 a	2.3 ± 0.03 a	3.7 ± 0.05 a
Ethanol ($mg\ L^{-1}$)	At harvest	18.4 ± 0.24					
	2 weeks at 12 °C + 1 week at 20 °C	9.4 ± 0.10 a	4.1 ± 0.10 a	60.6 ± 0.85 b	117.3 ± 0.78 d	111.6 ± 6.31 cd	71.9 ± 0.54 bc
	4 weeks at 12 °C + 1 week at 20 °C	33.7 ± 0.86 a	28.6 ± 2.63 a	140.5 ± 1.69 bc	201.0 ± 5.45 d	162.8 ± 4.87 cd	115.1 ± 3.23 b
CO_2 concentration (kPa)	At harvest	1.65 ± 0.25					
	2 weeks at 12 °C + 1 week at 20 °C	1.40 ± 0.09 a	1.69 ± 0.13 ab	1.96 ± 0.07 bc	2.27 ± 0.15 d	2.18 ± 0.10 cd	2.06 ± 0.03 cd
	4 weeks at 12 °C + 1 week at 20 °C	1.36 ± 0.04 a	1.32 ± 0.07 a	1.75 ± 0.04 a	2.47 ± 0.37 b	1.53 ± 0.10 a	1.48 ± 0.05 a
O_2 concentration (kPa)	At harvest	17.96 ± 0.98					
	2 weeks at 12 °C + 1 week at 20 °C	18.14 ± 0.74 d	16.82 ± 0.80 cd	13.51 ± 1.23 ab	11.62 ± 1.04 a	14.65 ± 0.65 bc	15.20 ± 0.66 bc
	4 weeks at 12 °C + 1 week at 20 °C	16.66 ± 0.58 d	17.86 ± 0.63 d	12.43 ± 0.12 b	9.27 ± 0.73 a	14.63 ± 0.95 c	12.47 ± 0.53 b

† For each gas and storage period, means in rows with different lowercase letters are significantly different according to Fisher's protected LSD test ($P < 0.05$) applied after an ANOVA.
‡ Data are the mean ± SE. Treatments applied were: (i) uncoated control (immersion in water); (ii) coating F10; (iii) coating F6; (iv) 20 g L^{-1} SB aqueous solution; (v) coating F10/SB; and (vi) coating F6/SB.

Valencia-Chamorro *et al.*[11] in 5-mL juice samples from squeezed lemons (three replicates of five lemons each per treatment). TA and pH were determined with an automatic titrator (Titrator T50; Mettler Toledo, Greifensee, Switzerland) and SSC was measured using a digital refractometer (model ATC-1; Atago Co., LTD, Tokyo, Japan).

Internal gas concentration

Concentrations of CO_2 and O_2 (kPa) in the internal cavity of 10 lemons per treatment were determined using a gas chromatograph (Thermo Trace, Thermo Fisher Scientific, Inc., Waltham, MA, USA) following the methodology described by Valencia-Chamorro *et al.*[11]

Ethanol and acetaldehyde contents

Both compounds were analysed from the headspace of juice from three replicates of 10 lemons per treatment by gas chromatography according to Valencia-Chamorro *et al.*[11] Results were expressed as mg L^{-1}.

Effect of coatings on sour rot development

Fungal inoculum

Arthrospores from 7–14-day-old cultures of *G. citri-aurantii* strain NAV-1 [strain of the IVIA CTP fungal culture collection, deposited in the Spanish Type Culture Collection (CECT, University of Valencia, Valencia, Spain) with the accession number CECT 13166] were taken from the polydopamine surface of Petri dishes with a sterilized inoculation loop and transferred to 0.5 g L^{-1} Tween® 80 solution (Panreac-Química S.A.). Arthrospores suspensions were filtered through two layers of cheesecloth. The final inoculum suspension was adjusted to 10^7 arthrospores mL^{-1} using a hemocytometer, and included fresh lemon juice (10%), thiabendazole (50 mg L^{-1}) (Textar® 60T; Decco Ibérica PostCosecha, S.A.U., Paterna, Valencia, Spain) and cycloheximide (5 mg L^{-1}) (Carl Roth GmbH+Co. KG, Karlsruhe, Germany) to enhance the virulence of the arthrospore suspension.

Fruit inoculation, coating application and disease assessment

Lemons were artificially inoculated with *G. citri-aurantii* by immersing a stainless-steel rod with a probe tip of 1 mm wide and 2 mm in length into the conidial suspension and wounding each fruit once on the equatorial zone. Inoculated lemons were kept at 28 °C and 90% relative humidity (RH) for 24 h before coating application. Afterwards, inoculated fruits were coated by immersion (15 s at 20 °C) with the corresponding formulation, then drained and allowed to air-dry at 20 °C. Treatments applied were: (i) control = uncoated (15 s immersion in water); (ii) F10 coating; (iii) F6 coating; (iv) (15 s immersion in 20 g L^{-1} SB aqueous solution; (v) F10/SB coating; and (vi) F6/SB coating, as described in above. Each treatment was applied to four replicates of five fruits each and each assay was carried out in triplicate. Treated fruits were placed on plastic trays and stored at 28 °C and 90% RH. Disease incidence was assessed as the percentage of decayed fruits and disease severity was determined as the diameter of the lesion (mm). For severity, all wounds, including the asymptomatic ones (diameter = 0 mm) were considered. Both parameters were evaluated after 4 and 7 days of incubation at 28 °C.

Statistical analysis

Data analyses were performed with the software Statgraphics Centurion XVII (Statgraphics Technologies Inc., The Plains, VA, USA). Data from all experiments were subjected to analysis of variance (ANOVA). Because the experiment was not a significant factor, means of repeated experiments are presented. Disease incidence was transformed to the arcsine of the square root of the proportion of infected fruits to improve the homogeneity of variances. When appropriate, Fisher's protected least significant difference (LSD) test was used for means separation. $P < 0.05$ was considered statistically significant. Non-transformed means are reported.

RESULTS

Effect of coating on quality of cold-stored lemons

The quality attributes of uncoated (control) and coated 'Fino' lemons at harvest and after 2 and 4 weeks of cold storage at 12 °C followed by a shelf-life period of 1 week at 20 °C are shown in Table 2. Weight loss was in the range of 3.6–3.7% and 4.5–5.7% after 2 and 4 weeks of cold storage, followed by 1 week of shelf life. The ECs F6 and F6/SB were the most effective to reduce weight loss compared to uncoated lemons after 4 weeks of storage, and the weight of F6/SB-coated lemons was significantly different from that of control samples ($P < 0.05$). After 2 weeks, fruit firmness was similar compared to the value at harvest and ranged from 2.3% to 2.6%, without significant differences between treated and control fruits. After 4 weeks, fruit firmness decreased (higher rind deformation) compared to the value at harvest, with values of deformation in the range 2.91–3.64%, without significant differences between treated and control fruits. Regarding juice quality, pH significantly increased after 4 weeks of cold storage. After 2 weeks, pH was significantly higher in lemons treated with F10/SB (2.95) compared to the control (2.84), whereas average values were significantly lower on fruits coated with F10 (2.67). However, after 4 weeks of storage, no significant differences were found among treatments, with values ranging from 3.03 to 3.16. Concerning SSC and TA, there were no significant differences between coated and uncoated fruit, with similar values after both storage periods. After 2 weeks, TA ranged from 41.4 to 50.5 g L^{-1} acid citric and SSC ranged from 6.80 to 7.05 °Brix, whereas, after 4 weeks, TA was in the range 46.5–49.7 g L^{-1} and SSC varied from 7.02 to 7.38 °Brix. Hue values slightly decreased with storage time compared to the value at harvest, although without significant differences ($P > 0.05$). After 2 weeks of storage, significantly lower hue values were found for lemons treated with SB alone or incorporated into the ECs (F6/SB and F10/SB) compared to the control and the ECs without the GRAS salt. However, no significant differences were observed among treatments after 4 weeks followed by 1 week of shelf life, with values ranging from 91.07 to 91.78.

Table 3 shows that, after both storage periods at 12 °C followed by shelf life of 1 week at 20 °C, the volatile content of lemons was low and not affected by coating application. After 2 weeks, acetaldehyde ranged from 1.5 to 6.4 mg L^{-1} and ethanol ranged from 4.1 to 117.3 mg L^{-1} and significant higher values than the control were obtained with coatings F6 and F10. After 4 weeks, acetaldehyde ranged from 1.8 to 3.7 mg L^{-1}, without significant differences among treatments, whereas ethanol ranged from 28.6 to 201.1 mg L^{-1}, with higher contents in coated fruits than in control and SB-treated fruits. After 2 weeks of cold storage plus shelf life, CO_2 values inside coated fruits were higher than in control and fruits dipped in 20 g L^{-1} SB, whereas, after 4 weeks, only lemons coated with F6 differed significantly from the control. However, O_2 values in coated fruits were significantly lower than those in control and SB-treated fruits for both storage periods, with average values in the range 9.27–17.86 kPa.

Effect of coatings on sour rot development

Figure 1 shows the development of sour rot after 4 and 7 days of incubation at 28 °C on 'Fino' lemons artificially inoculated with *G. citri-aurantii*. After both incubation periods, severity and incidence values of sour rot did not differ between uncoated control lemons and fruits treated with the coatings not containing the GRAS salt SB (F10 and F6). By contrast, lemons coated with F10/SB or F6/SB significantly reduced sour rot severity (by 47% and 61%, respectively) and incidence (by 42 and 41%,

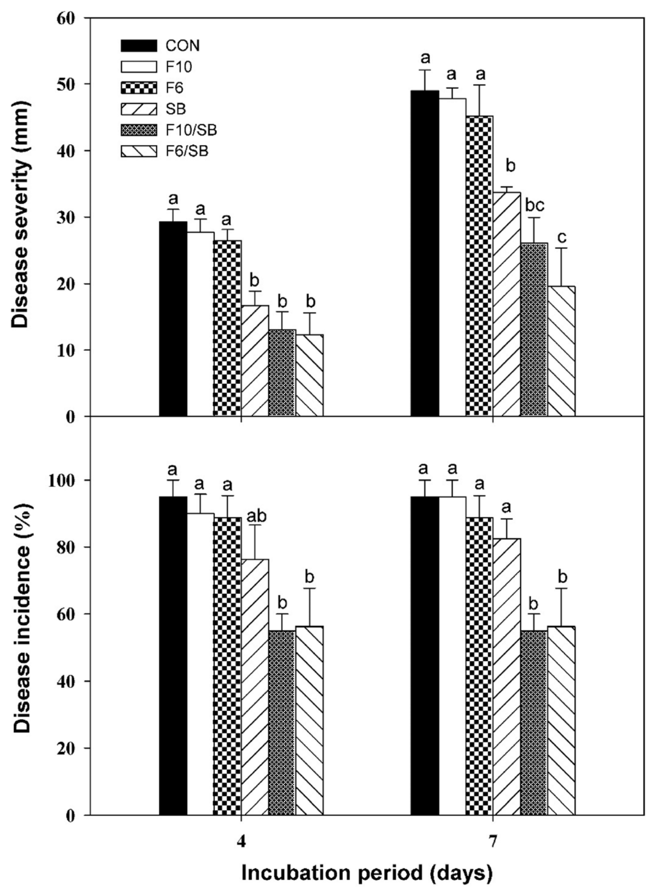

Figure 1. Disease incidence and severity of sour rot on 'Fino' lemons artificially inoculated with *Geotrichum citri-aurantii*, treated 24 h later, and incubated for 4 and 7 days at 28 °C and 90% RH. Treatments applied were: (i) control (CON) = uncoated (15 s immersion in water); (ii) F10 coating; (iii) F6 coating; (iv) 15 s immersion in 20 g L^{-1} SB aqueous solution; (v) F10/SB coating; and (vi) F6/SB coating. F10 and F6 are pregelatinized potato starch (PPS)-based composite edible coatings, which were formulated with or without sodium benzoate (SB, 20 g kg^{-1}). Values are means of three repeated experiments. For each time of incubation, columns with different lowercase letters indicate significant differences according to Fisher's protected LSD test ($P < 0.05$). For disease incidence, ANOVA was applied to arcsine-transformed values. Non-transformed means are shown. Thin vertical lines above columns indicate the SE.

respectively) with respect to the control treatment. In general, no significant differences were observed among the three treatments containing SB except for incidence levels after 7 days of incubation, which were significantly higher on lemons treated with the SB aqueous solution than on lemons coated with the emulsions F10/SB and F6/SB.

DISCUSSION

The results obtained in the present study showed that only the coating F6/SB significantly reduced the weight loss of lemons at the end of cold storage and shelf life, whereas the same coating without SB, although giving low values, resulted in values not significantly different from those obtained with the coating F10 and the control. In general, the incorporation of lipids into starch or polysaccharide-based coatings contributes to a reduction in the amount of water lost.[10] However, the success of ECs in reducing fruit water loss during storage is determined not only by the type of bipolymer matrix and emulsion composition (e.g. the hydrophilic–hydrophobic ratio of the emulsion components), but also by other factors, such as commodity and cultivar, fruit physiological stage at harvest and storage conditions.[9] In addition, several studies have confirmed that the addition of GRAS salts or food additives, as SB, to ECs greatly affects the moisture barrier properties of stand-alone films or coatings on a variety of fresh fruits.[12,14] In this sense, using response surface methodology, our group optimized an antifungal EC formulated with 20 g L^{-1} SB as an antifungal ingredient and different ratios of PPS, GMS and glycerol to maintain the physicochemical and sensory quality of 'Orri' mandarins during storage, with F6/SB being the most effective coating to reduce weight loss, followed by F10/SB.[14] Therefore, the results of the present study confirm the effectiveness of this coating with respect to reducing the weight loss of other citrus fruits such as 'Fino' lemons.

The results also showed that fruit firmness was not affected by the application of PPS-based ECs amended with SB. Conversely, in other studies, it was found that the firmness of strawberries treated with starch-coatings amended with SB was retained for longer periods compared to that of uncoated fruits.[16] In general, the effect of the ECs on the maintenance of citrus firmness is usually related to their control of weight loss. However, contradictory results have been reported for the relationship between weight loss and fruit firmness. Indeed, different citrus species and cultivars coated with HPMC-based ECs amended with different GRAS salts were reported to perform differently. Thus, although coating application did not affect the firmness of cold-stored oranges, mandarins treated with the same type of coatings were significantly firmer after cold storage and shelf life than uncoated control fruits, indicating the intervention of multiple factors.[11,12] Regarding juice quality and fruit color, the present results showed that these parameters were not affected by the application of the PPS-based ECs. By contrast, starch-based coatings amended with SB developed in previous works[16,17] were confirmed to extend the storage life of strawberries, decreasing water losses and improving fruit quality. Moreover, color changes were delayed, weight and firmness losses were lower, and changes in anthocyanin content, SSC, TA and pH of fruits were slowed down. This can be directly attributed to the important physiological differences between these two types of fruits.

By contrast to our expectations, in the present study, after 4 weeks of cold-storage, the internal CO_2 levels did not significantly differ between coated and uncoated fruits, except for the coating F6. However, the internal O_2 levels were lower in coated than in uncoated fruits, indicating that the tested coatings slightly modified the fruit internal atmosphere. This was confirmed by the higher ethanol level of coated samples after storage because 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.[11,18] Nevertheless, the levels of these volatiles were very low compared to the values reported for other citrus species and cultivars in accordance with the slight modification in the internal atmosphere.[11,12,18] The capacity of an EC to create an effective gas barrier depends on many factors, such as the coating composition and properties (including the addition of GRAS salts), the fruit species and cultivar, and the storage conditions.[12]

On the other hand, the results obtained show that the coatings without SB did not exhibit antifungal activity, confirming that SB was responsible for the antifungal effect of the emulsions F6 and F10 with respect to controlling sour rot. These findings are in agreement with previous studies reporting that starch-based coatings and films can control pathogenic fungi and bacteria only if amended with antifungal ingredients.[19] In a previous study conducted by Ratnawaki and Afifah,[20] arrowroot starch-based films alone did not show any inhibition of foodborne pathogenic bacteria, whereas films amended with GRAS salts, such as SB, potassium sorbate and calcium propionate did demonstrate inhibition. Among them, SB was the most effective antibacterial salt, and its antimicrobial effect was attributed to a decrease of external pH, an alteration of the integrity and permeability of the bacteria cell membranes, and a disturbance of nutrient transport.[21] Overall, this work demonstrates a significant curative activity of PPS-based ECs for controlling sour rot on 'Fino' lemons. There is little information available on the performance of antifungal starch-based ECs applied to horticultural produce. Nevertheless, some studies have reported significant antifungal activity when starch-based matrices were amended with different antimicrobial ingredients.[22] Recently, our group evaluated the antifungal curative activity of PPS-based ECs containing SB (i.e. the same as those used in the present study) with respect to controlling green and blue molds, obtaining significant decay reductions on fruit artificially inoculated with *Penicillium* spp. on mandarins, oranges and lemons stored at either 20 °C or low temperature.[15] The present results are very positive in the sense that the significant reduction of sour rot on artificially inoculated lemons satisfactorily broadens the spectrum of action of these ECs for the postharvest treatment of citrus fruits. To our knowledge, this is the first report on the effectiveness of this type of ECs to control citrus sour rot.

In general, the present results confirm that the functionality of ECs based on polysaccharide matrixes that do not exert direct inhibitory effect against spoilage microorganisms can be increased by the incorporation of antifungal ingredients such as GRAS salts or food-grade preservatives.[4] The amendment of SB as antifungal ingredient to the EC matrix effectively provided antifungal properties to the resulting coating, which was able to control sour rot on lemons to the same extent as dips in a SB aqueous solution. The use of antifungal agents as ingredients of ECs may facilitate a slow diffusion of the active agent from the matrix, regulating its temporal and spatial release and also facilitating its continuous and effective contact with the target pathogen.[23]

In summary, the present study has shown the potential of PPS-based ECs containing SB as a non-polluting alternative for controlling citrus postharvest sour rot and maintaining the postharvest quality of 'Fino' lemons. These coatings significantly reduced the incidence and severity of sour rot on 'Fino' lemons artificially inoculated with *G. citri-aurantii* and, overall, the F6/SB coating formulation showed the best performance for the physiological preservation of cold-stored lemons without adversely affecting the physicochemical quality parameters of the fruit. Further research should mainly focus on the evaluation of additional secondary ingredients with respect to improving the physical characteristics of this coating and enhancing its general performance, especially the barrier properties by a reduction of gas exchange. Information gathered from the present study provides a basis for further research into the commercial application of these antifungal PPS-based ECs in citrus packinglines. Their application would not require significant changes in the facilities and equipment currently in use because the coatings may be applied in the waxing points in the packingline. Thus, such treatments would not only perform as an alternative to synthetic chemical fungicides, such as propiconazole (as recently banned in the EU), for sour rot control, but also effectively substitute the use of conventional waxes, reducing the number of required postharvest treatments and consequently reducing operations and costs. Because SB is classified as a food additive (E-211) by the European Food Safety Authority (EFSA) and the rest of the coating ingredients are all food-grade, the specific registration of starch ECs such as F6/SB could be pursued by the industry as a new cost-effective postharvest antifungal treatment suitable for citrus international markets with zero tolerance to chemical residues. However, further research is required to assess their efficacy on a larger scale using naturally infected fruit, including an evaluation of visual appearance and sensory traits, as well as to explore their compatibility with other alternative postharvest treatments regarding potential use in integrated disease management programs.

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CONFLICT OF INTERESTS

The authors declare that they have no conflicts of interest.

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