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**Curative activity of postharvest GRAS salt treatments to control citrus sour rot
caused by *Geotrichum citri-aurantii***

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Abstract

The effectiveness of the "generally recognized as safe" (GRAS) salts potassium sorbate (PS), sodium benzoate (SB), sodium ethylparaben (SEP) and sodium methylparaben (SMP) to control sour rot, caused by *Geotrichum citri-aurantii*, was assessed by dipping economically important citrus species and cultivars in aqueous solutions for 30, 60 or 150 s at 20 °C, followed by examination after 8 d of storage at 28 °C. Curative activity was determined because the fruit were inoculated 24 h prior to treatment. Dipping fruit for 60 s in SMP (200 mM), SEP (200 mM) or SB (3% w/v) were very effective and reduced sour rot incidence and severity by up to 90%. Their effectiveness was similar or superior to that of the conventional fungicide propiconazole (PCZ). In contrast, PS (200 mM) did not control sour rot on 'Oronules' or 'Ortanique' mandarins, but it reduced sour rot incidence on 'Barnfield' oranges by 50% compared to inoculated, water-treated control fruit. Sour rot was better controlled on oranges than on mandarins. Furthermore, heating the solutions to 50 °C enhanced their effectiveness, while post-treatment rinsing of the fruit with tap water reduced their effectiveness. Dipping 'Valencia Late' oranges in SB (3% w/v) or SMP (200 mM) for 60 s followed by long storage for up to 8 weeks at 5 °C and 90% RH, reduced sour rot incidence from 55% among water-treated control fruit to 2 to 6%, and matched the effectiveness of PCZ. No fruit in any test were visibly harmed. Both SB and SMP salts could be potential alternatives to conventional fungicides, such as PCZ or guazatine, for the integrated postharvest management of citrus sour rot.

Keywords: orange; mandarin; food additives; alternative non-polluting postharvest disease control

1. Introduction

Citrus sour rot, caused by *Geotrichum citri-aurantii*, is the most prevalent postharvest disease of citrus exports after green and blue molds caused by *Penicillium* spp. (McKay et al., 2012; Smilanick et al., 2020). In citrus regions with Mediterranean-type climate, sour rot has increasingly become an important cause of economic losses, especially during wet and rainy seasons (Duan et al., 2016).

Conventional fungicides commonly used to control postharvest diseases of citrus fruit include thiabendazole, imazalil, fludioxonil, pyrimethanil and sodium *o*-phenylphenate (Liu et al., 2019; Palou, 2018). However, they are usually applied to control *Penicillium* spp. and none is satisfactorily effective to control *G. citri-aurantii*. After the definitive withdrawal of guazatine in the European Union (EU) in 2011, the only postharvest fungicide highly effective for sour rot control on citrus fruit is the ergosterol demethylation inhibitor (DMI)-triazole propiconazole (PCZ) (McKay et al., 2012). Nonetheless, the Commission Implementing Regulation (EU) 2018/1865 of 28 November 2018 denied the renewal of the approval for the commercial use of this active substance, in accordance with Regulation (EC) No 1107/2009. This was due to the risks associated with the presence of metabolites of PCZ in groundwater and the harmful effects on human health. The lack of effective and approved fungicides for sour rot control is a serious problem for European citrus exporters since they are forced to ship the fruit without the guarantee of adequate antifungal protection. Therefore, safe, effective and commercially viable alternative strategies for citrus sour rot control are urgently needed.

Some naturally occurring alternatives for controlling sour rot of citrus fruit, such as the use of yeasts as biocontrol agents (Klein and Kupper, 2018), plant extracts (Karim et al., 2016), essential oils (Serna-Escolano et al., 2019) and antimicrobial peptides (Liu et al., 2019) have been proposed. However, low efficacy, inconsistency, instability of

formulated products or incompatibility with existing application equipment and chemical sanitizers generally limit the commercial use of these approaches (Feng et al., 2011; Moraes Bazioli et al., 2019).

The use of organic and inorganic salts classified as ‘Generally Recognized as Safe’ (GRAS) compounds or as food additives by national or international legislations is a promising non-polluting alternative to conventional fungicides for the control of postharvest diseases of fresh horticultural produce, including fresh citrus fruit (D’Aquino and Palma, 2020; Palou, 2018). As GRAS compounds, salts such as carbonates, sorbates, benzoates, paraben salts, etc. are exempt from residue tolerances on all agricultural commodities by the United States Food and Drug Administration (US FDA), which makes them candidates to employ to remediate issues of fruit residues, improve process water quality discharged from packinghouses, and even to comply with some organic agriculture rules. Furthermore, additional advantages of these salts, if compared to other GRAS compounds, are their high solubility in water, easy synthesis and great availability at relative low cost, ease of application as postharvest aqueous solutions, and suitability for commercial implementation using the currently existing facilities in fresh produce packinghouses (Palou et al., 2008, 2016). Although, the use of acids can also show antifungal activity, salts are typically preferred for postharvest treatments because of their superior solubility and additional curative activity of cations such as Na⁺, K⁺ and NH₄⁺ (Smilanick et al., 1999).

Several GRAS salts were shown to control citrus green and blue molds, caused by *Penicillium digitatum* (Pers.:Fr.) Sacc. and *Penicillium italicum* Wehmer, respectively, the most economically important postharvest diseases of citrus fruit worldwide. These include sodium bicarbonate (SBC) and sodium carbonate (SC) (Palou et al., 2001, 2002a; Smilanick et al., 1999), potassium sorbate (PS) (Montesinos-Herrero et al., 2009;

Smilanick et al., 2008; Youssef and Hussien, 2020), potassium silicate (PSi) (Moscoso-Ramírez and Palou, 2014), sodium benzoate (SB) (Montesinos-Herrero et al., 2016; Palou et al., 2002b), sodium methylparaben (SMP) and sodium ethylparaben (SEP) (Moscoso-Ramírez et al., 2013a,b), among others. In general, aqueous solutions of these salts provided significant curative activity and were compatible or even synergistic with other alternative control methods, such as heat or biological control agents, that would allow their use as hurdle technologies in postharvest integrated disease management programs (Palou et al., 2016; Wisniewski et al., 2016). Nevertheless, few studies have reported the application of GRAS salts to control citrus postharvest sour rot. Smilanick et al. (2008) showed that sour rot was reduced from 94.5 to 37.0 and 15.7% by immersing artificially inoculated lemons in PS or SBC solutions at 1% (w/v) for 30 s at 50 °C, respectively. Furthermore, among 32 salt compounds previously tested in vitro against *G. citri-aurantii*, only sodium salicylate, boric acid and EDTA exhibited considerable antifungal activity against sour rot on mandarin fruit (Talibi et al., 2011). In other studies, sodium dehydroacetate as stand-alone treatment or in combination with sodium silicate reduced sour rot incidence on ‘Satsuma’ mandarins (Li et al., 2019), and induced an increase in the activities of the enzymes peroxidase (POD), superoxide dismutase (SOD) and phenylalanine ammonia-lyase (PAL) within the fruit rind (Duan et al., 2016).

In general, the practical implementation of the use of GRAS salts to control fruit postharvest decay relies on the evaluation on in vivo laboratory tests with fruit artificially inoculated with the target pathogens of the control ability of the salts at selected concentrations under controlled conditions simulating those applied in the packinghouse. In vivo semicommercial or commercial trials can be conducted afterwards to assess the commercial value of the potential implementation of postharvest treatments with effective salt solutions at optimal concentrations (Palou, 2018). In the present work, we evaluated

in laboratory trials the curative activity of different GRAS salt aqueous solutions, not tested before for this purpose, to control sour rot on economically important citrus species and cultivars. The effective postharvest treatment conditions (dip temperature and immersion time) were optimized and the effect of rinsing the treated fruit with tap water and the effectiveness of salt treatments on long-term cold-stored fruit also were assessed. Some preliminary results from this research have been summarized (Palou et al., 2018).

2. Materials and methods

2.1. GRAS salts

The following organic salts, classified as GRAS by the USA FDA, PS (3% w/v, C₆H₇O₂K; Merck KgaA, Darmstadt, Germany), SB (3% w/v, C₇H₅NaO₂Na; Guinama S.L., Alboraiia, València, Spain), SEP (200 mM, C₉H₉NaO₃; Merck KgaA) and SMP (200 mM, C₈H₇NaO₃; Merck KgaA), were used in this study. These salts and concentrations were selected after preliminary in vivo primary screenings conducted with mandarins or oranges artificially inoculated with *G. citri-aurantii* (data not shown).

2.2. Fruit

Depending on the experiment, ‘Clemenules’ and ‘Oronules’ clementine mandarins (*Citrus reticulata* Blanco), ‘Ortanique’ [*C. reticulata* x (*Citrus sinensis* x *C. reticulata*)] hybrid mandarins, and ‘Barnfield’, ‘Navelina’ and ‘Valencia Late’ oranges (*Citrus sinensis* L.) were used. Fruit were collected from commercial orchards in the Valencia area (Spain) and used the same day or stored up to 1 week at 5 °C and 90% relative humidity (RH). Before each experiment, fruit were selected, randomized, washed and disinfected superficially by immersion for 2 min in a 0.5% sodium hypochlorite solution, rinsed with tap water and allowed to air-dry at room temperature.

2.3. Fungal inoculation

G. citri-aurantii isolate NAV-1, from the fungal culture collection of the IVIA Postharvest Technology Center (CTP), had been isolated from a decayed orange found in a Valencian packinghouse, purified, molecularly identified and deposited in the Spanish Type Culture Collection (CECT, University of Valencia, Valencia, Spain), where it was accessioned as CECT 13166. The fungus was cultured on potato dextrose agar (PDA, Scharlab S.L., Sentmenat, Barcelona, Catalonia, Spain) plates at 25 °C. Arthroconidia from 7 to 14-d-old PDA cultures were harvested by adding 9 mL of sterile aqueous solution of 0.05% Tween 80® (Panreac, S.A.U., Barcelona, Catalonia, Spain) to the petri dish. Arthroconidia were then rubbed with a sterile glass rod, and the conidial suspension concentration was measured with a hemocytometer and adjusted to 1×10^7 arthrospores per mL. To increase the virulence of the fungal inoculum, filtered orange juice (0.1 L L^{-1}), thiabendazole fungicide (0.05 g L^{-1}) and cycloheximide (0.005 g L^{-1}) were added to the arthrospore suspension (Smilanick et al., 2008). The tip of a stainless steel rod, 1 mm wide and 2 mm in length, was immersed in the inoculum suspension and a single puncture was done once in the equator of each fruit. Inoculated fruit were kept in a temperature-controlled room at 28 °C and 90% RH for 24 h, until treatment. These conditions favor the development and the infection rate of *G. citri-aurantii* (Smilanick et al., 2020).

2.4. Effectiveness of GRAS salts on different citrus cultivars

The effectiveness of GRAS substances to control sour rot existing infections (curative activity) was evaluated on citrus fruit inoculated with arthroconidia of *G. citri-aurantii* about 24 h before treatment, as previously described. Studied citrus cultivars were ‘Clemenules’ and ‘Oronules’ clementine mandarins, ‘Ortanique’ hybrid mandarins, and ‘Barnfield’, ‘Navelina’ and ‘Valencia Late’ oranges. Treatments were as follows: (i) water (negative control), (ii) SMP, 200 mM (w/v), (iii) SEP, 200 mM (w/v), (iv) PS, 3% (w/v), (v) SB, 3% (w/v) and (vi) PCZ fungicide, 0.6% (v/v) (positive control, Melanite®,

10% a.i., Decco Ibérica Postcosecha SAU, Paterna, Valencia). Stainless steel buckets containing 10 L of aqueous solution of GRAS salt were used. Fruit of each cultivar were placed into 18 L multi-perforated wall stainless steel containers, exactly fitting in the above-mentioned buckets, and completely immersed in 10 L of the treatment solution for 60 s at room temperature (20 °C). Each treatment included 4 replicates of 10 fruit each. Treated fruit were incubated at 28 °C and 90% RH for 8 d, at which time the percentage of infected wounds (disease incidence) and lesion diameter (disease severity) caused by *G. citri-aurantii* were assessed.

2.5. Influence of dip temperature

Metallic grid baskets containing ‘Barnfield’ navel oranges puncture-inoculated 24 h before with *G. citri-aurantii* were submerged for 60 s in a solution of SMP (200 mM), SEP (200 mM), PS (3%) or SB (3%) at 50 or 20 °C. Solutions at 50 °C were heated by placing the buckets in a 250-L stainless steel water tank fitted with two electrical resistances (4.5 kW each), a thermostat and an automatic water-recirculating system. Control fruit were dipped in deionized water alone at 20 °C for 60 s. After the dip treatment, fruit were incubated at 28 °C and 90% RH for 8 d, after which sour rot incidence and severity were determined. Fruit trials were carried out in 4 replicates using 5 fruits per replicate. The experiment was conducted twice.

2.6. Influence of immersion time

In order to evaluate the influence of immersion time on the control of sour rot by GRAS salt aqueous solutions, the following treatments were selected: water (control); SMP, 200 mM; SEP, 200 mM; and SB, 3%. In this assay, ‘Oronules’ clementine mandarins were used 24 h after artificial wound inoculation. Fruit were completely immersed in the treatment solution for 30, 60 or 150 s at room temperature (20 °C). After dipping, 20 fruit per treatment (4 replicates of 5 fruit each) were placed in cavities in plastic fruit trays,

followed by incubation at 28 °C and 90% RH for 8 d, at which time disease incidence and severity were assessed. The experiment was conducted twice.

2.7. *Influence of low-pressure fruit rinsing*

Oranges ('Navelina' and 'Valencia Late') and mandarins ('Oronules') were inoculated with *G. citri-aurantii* and submerged 24 h later, as described above, in water (control), SMP (200 mM), SEP (200 mM) or SB (3%), all at 20 °C for 60 s. The experiment with each cultivar was conducted in duplicate with one set of dipped fruit rinsed afterwards for 5 s with tap water at low pressure using a hose and the other set of fruit not rinsed. Then, all fruit were incubated for 8 d at 28 °C and 90% RH and the incidence and severity of sour rot were assessed. All treatments were carried out with 4 replicates and 5 fruit per replicate.

2.8. *Effectiveness on long-term cold-stored fruit*

'Valencia Late' oranges were artificially inoculated with *G. citri-aurantii* and then dip-treated for 60 s with water (negative control) or aqueous solutions of SMP (200 mM), SB (3%) or PCZ (0.6%) (positive control) at room temperature (20 °C). Each treatment was applied to 4 replicates of 5 fruit each. Once dried, the fruit were arranged in cavity sockets on plastic trays and wrapped with a plastic bag to avoid external contaminations during the cold storage period. Treated fruit were stored for 8 weeks at 5 °C and 90% RH simulating common commercial storage of 'Valencia Late' oranges. Sour rot incidence and severity were assessed after 2, 4, 6 and 8 weeks at 5 °C.

2.9. *Statistical analysis*

Data on disease incidence (%) were transformed to the arcsine of the square root of the proportion of infected fruit to improve the homogeneity of variances. In some cases, reductions with respect to control treatments were calculated as percentages. Transformed data were analyzed by analysis of variance (ANOVA) with Statgraphics Centurion XVI

(Manugistics Inc., Rockville, MD, USA). The level of significance was established at $P = 0.05$. When appropriate, Fisher's Protected Least Significant Difference (LSD) test was used for means separation. Values displayed in graphs are non-transformed means.

3. Results

3.1. Effectiveness of GRAS salts on different citrus cultivars

The effectiveness of GRAS salt treatments applied by immersion for 60 s at 20 °C to control *G. citri-aurantii* was significantly higher on oranges than on mandarins (Fig. 1). After 8 d of incubation at 28 °C, treatment with SMP at 200 mM significantly reduced the incidence and severity of sour rot on mandarins and oranges by approximately 35 and 85%, and 75 and 80%, respectively.

The effectiveness of SEP at 200 mM was significantly higher on 'Navelina' oranges and lower on 'Ortanique' mandarins than on the rest of cultivars. This treatment reduced both sour rot incidence and severity by 97% on 'Navelina' oranges, but it only reduced disease incidence on 'Ortanique' mandarins by 27%.

Similarly, treatment with SB at 3% was more effective on 'Barnfield' oranges, followed by 'Navelina' and 'Valencia Late' oranges, with incidence reductions of 85, 74 and 53%, respectively. Contrastingly, the lowest reductions of incidence of sour rot were observed on 'Ortanique' and 'Oronules' mandarins (27 and 7%, respectively). Likewise, a similar pattern was observed for the severity reduction obtained with SB treatment, and values of reduction ranged from 90 to 75% on oranges and 55 to 28% on mandarins.

Among all treatments, PS at 3% was the least effective to control sour rot. On 'Barnfield' oranges, PS treatment reduced the incidence and severity of sour rot by 48 and 55%, respectively, whereas these reductions were 22 and 5%, respectively, on 'Oronules' mandarins and both below 9% on 'Ortanique' mandarins.

Finally, the fungicide PCZ used as a positive control showed a reduction in disease incidence and severity of 80 and 89%, respectively, on ‘Navelina’ oranges, and these reductions did not significantly differ from those obtained with SMP, SEP and SB treatments. On ‘Barnfield’ oranges, PCZ treatment reduced sour rot incidence and severity by 58 and 64%, respectively. In addition, PCZ showed high effectiveness to inhibit the growth of *G. citri-aurantii* on ‘Oronules’ mandarins in comparison with other treatments, but it was not effective on ‘Ortanique’ hybrid mandarins.

3.2. Influence of dip temperature

Sour rot incidence and severity reductions obtained on ‘Barnfield’ oranges dip-treated with SMP, SEP, SB or PS at 50 or 20 °C are shown in Fig. 2. Average results from two repeated experiments showed that heating solutions to 50 °C increased the effectiveness of the salt treatments. When the treatments were applied at 20 °C, SMP and SB were significantly more effective than SEP and PS treatments. Likewise, SMP and SB treatments were not significantly different from each other, and both treatments reduced the incidence and severity of sour rot by approximately 80 and 85%, respectively. SEP and PS treatments showed a disease incidence reduction of 63 and 48%, and a severity reduction of 72 and 55%, respectively. On the other hand, SMP and SB dip treatments at 50 °C reduced the incidence of sour rot by almost 100% on oranges artificially inoculated with *G. citri-aurantii*. SEP and PS treatments heated to 50 °C reduced the incidence of sour rot by about 90% on oranges incubated for 8 d at 28 °C. Although PS treatment showed higher effectiveness at 50 than at 20 °C, this treatment was less effective than the rest; therefore, it was discarded in further experiments.

3.3. Influence of immersion time

Dip immersion periods of 30, 60 or 150 s with SMP (200 mM), SEP (200 mM) or SB (3 %) at 20 °C were tested with ‘Oronules’ mandarins artificially inoculated with *G. citri-*

aurantii about 24 h before dip application (Fig. 3). Unexpectedly, average results from two repeated assays showed very little influence of immersion time. Dips in 200 mM SMP solution for 30, 60 or 150 s reduced the incidence and severity of sour rot by 53 and 61%, 34 and 51%, and 15 and 39%, respectively. Treatment with SEP at 200 mM for 30, 60 and 150 s reduced sour rot incidence by 48, 33, and 43%, respectively, on mandarins incubated for 8 d at 28 °C. These incidence reductions were not statistically different. A similar trend was observed for severity reduction with the same salt treatment. On the other hand, incidence reduction of sour rot on fruit dipped in SB at 3% for 30, 60 and 150 s was 2, 10 and 6%, respectively. Regardless of immersion time, severity was reduced about 30% on mandarins treated with SB.

3.4. *Influence of low-pressure fruit rinsing*

‘Navelina’ and ‘Valencia Late’ oranges and ‘Oronules’ mandarins were used to investigate the effect of rinsing the treated fruit with tap water after GRAS salt dip applications (Fig. 4). In general, the effectiveness of GRAS treatments to control sour rot on rinsed citrus fruit was lower than on non-rinsed fruit, but this was dependent on the experiment and the citrus cultivar.

On ‘Navelina’ oranges, SMP treatment followed by a water rinse reduced the incidence and severity of sour rot by 65 and 74%, respectively, and there were no significant differences with the reductions obtained on non-rinsed fruit. Similarly, water rinsing after treatment did not significantly affect the effectiveness of SEP treatment. The incidence and severity reduction of sour rot on SEP-treated and rinsed oranges were 70 and 77%, respectively. In contrast, the curative activity of SB treatment was significantly lower when the fruit were rinsed after dip treatment. Disease incidence and severity of sour rot on SB-treated and non-rinsed fruit were 59 and 68%, respectively, while they were only 35 and 44%, respectively, on rinsed oranges.

On ‘Valencia Late’ oranges, SMP dips were less effective when the fruit were rinsed, but this difference was not statistically significant. On the contrary, this difference was significant on SB-treated fruit, in which sour rot incidence and severity reductions were 53 and 75%, respectively, on non-rinsed oranges while they were 35 and 52%, respectively, on rinsed fruit.

Finally, the rinsing effect on sour rot control was even higher on ‘Oronules’ mandarins treated with SMP and SB salt solutions. While both treatments reduced disease incidence and severity by 35 and 45%, respectively, on non-rinsed mandarins, these reductions decreased to 8 and 18%, respectively, on rinsed fruit.

3.5. *Effectiveness on long-term cold-stored fruit*

The incidence of sour rot on ‘Valencia Late’ oranges stored for up to 8 weeks at 5 °C and 90% RH was effectively reduced by the application of SMP, SB and PCZ dips at 20 °C for 60 s (Fig. 5). Regardless of treatment, sour rot incidence on control fruit was significantly higher than on treated fruit from the second week and until the end of the storage period. After 8 weeks of storage at 5 °C, disease incidence on fruit treated with SMP, SB and PCZ was 12, 20 and 15%, respectively, while it was 55% on control fruit (Fig. 5A). On the other hand, no significant differences in disease severity were found among SMP, SB, and PCZ treatments during the whole storage period at 5 °C, but all these treatments significantly reduced the severity with respect to the control fruit. Sour rot severity after 8 weeks of cold storage on control, SMP, SB and PCZ-treated oranges were about 18, 4, 7 and 2 mm, respectively (Fig. 5B), representing a severity reduction of 80% for SMP, 62% for SB, and 86% for PCZ, as compared to the control.

3. Discussion

In some citrus producing areas, such as the EU countries, PCZ was the only available postharvest fungicide for managing citrus sour rot. However, its non-renewal as an active ingredient for postharvest applications in these areas has become a serious problem for the citrus industry, making marketing of conventionally grown, high quality citrus fruit considerably more difficult (Smilanick et al., 2020). Thus, the search and implementation of effective alternative methods for controlling sour rot have become urgently needed. After repeated and comprehensive research conducted into their use, both many years ago and more recently, GRAS salts such as SBC, SC, or PS, are employed in some citrus packinghouses as alternatives to conventional fungicides for the management of *Penicillium* decay (Palou et al., 2016). Therefore, our goal was to assess the effectiveness and consistency of similar postharvest treatments with GRAS substances as a practical strategy for the control of citrus sour rot caused by *G. citri-aurantii*. The present research work implies a sequential procedure to optimize the conditions of application of GRAS salts as postharvest treatments for the curative control of sour rot in a wide range of citrus cultivars of commercial importance. It is well known that the effective commercial control of citrus sour rot, as well as that of other wound diseases such as citrus green and blue molds, requires the use of postharvest antifungal treatments with high level curative activity. In these cases, preventive activity, that is, the protection of fruit against fungal infections that will occur after the antifungal treatment is applied, is of secondary importance (Palou et al., 2008). The reason is that the vast majority of infections by *G. citri-aurantii* able to colonize the fruit and develop disease take place through rind wounds that occur before the fruit arrive to the packinghouse, especially from arthrospores present in airborne soil particles deposited on the fruit surface by water splash or through soiled harvest containers (Baudoin and Eckert, 1982; Smilanick et al., 2020). On the other hand, in this research, the efficacy of the best GRAS treatments was

also assessed over a long cold storage period of up to 8 weeks, which, depending on the market situation, could take place under the current commercial handling of ‘Valencia Late’ oranges, arguably the most important late-season citrus cultivar.

Our findings indicated that the tested GRAS salts were more effective on oranges than on mandarins. A similar trend was also observed by Smilanick et al. (2008) in their studies with PS treatments, as they reported that the curative effect of PS on citrus fruit artificially inoculated with *G. citri-aurantii* was lower on mandarins than on lemons. Likewise, in previous research by our group, SBC, SMP and PS aqueous solutions proved to be less effective on mandarins than on oranges for the control of citrus postharvest decay caused by *Penicillium* spp. (Montesinos-Herrero et al., 2009; Moscoso-Ramírez et al., 2013a). It was discussed in these studies that such differences in the effectiveness of GRAS salts were related with the fruit susceptibility to infection by *P. digitatum* or *P. italicum*, and our present results show that the same principle can be of application for infections by *G. citri-aurantii*. Some citrus cultivars have been identified and categorized into three groups according to their level of susceptibility to sour rot (Nazerian and Alian, 2013): (i) most susceptible: mandarin cvs. ‘Onshiu’ and ‘Ponkan’; (ii) moderately susceptible: mandarin cvs. ‘Clementine’, ‘Page’ and ‘King’, orange cvs. ‘Thomson Navel’ and ‘Washington Navel’, sweet lime and grapefruit cv. ‘Marsh’; and (iii) the least susceptible: sweet orange cv. ‘Valencia’ and sour orange. These observations are in accordance with the results obtained here, as we observed that the curative activity of GRAS salts was lower on mandarins, the most susceptible fruit to sour rot. Thus, in this work, PS treatment did not effectively control sour rot on ‘Oronules’ and ‘Ortanique’ mandarins, but it reduced by half the disease incidence on ‘Barnfield’ oranges. Similar findings were reported by Montesinos-Herrero et al. (2009), who observed that a dip treatment with 3% PS at 20 °C for 30 s did not exert a significant curative activity against green and blue molds on

369 'Nadorcott' and 'Clemenules' mandarins. In contrast, the same PS treatment applied to
370 'Ortanique' mandarins, 'Fino' lemons, and 'Valencia Late' oranges reduced the incidence
371 of green and blue molds by up to 95%. In other research with lemons artificially
372 inoculated with *G. citri-aurantii*, PS solutions at concentrations of 0.5-2.0% successfully
373 controlled sour rot (Abd-El-Kareem and Saied, 2015; Smilanick et al., 2008).
374 Furthermore, we found in this work that PS salt solution improved significantly its
375 curative activity when it was heated to 50 °C, with reductions of sour rot severity on
376 'Barnfield' oranges of up to 94%. This finding corroborates those of Abd-El-Kareem and
377 Saied (2015) showing that 1.5% PS at 55 °C for 30 s reduced sour rot severity on lemon
378 fruit by up to 90%. Therefore, the synergy observed between PS application and heat (50
379 °C) could be used as a better practical approach to control citrus sour rot on particular
380 citrus species and cultivars. On the other hand, the antifungal activity of SMP, SEP and
381 SB treatments against *G. citri-aurantii* was similar or even higher than that of the
382 conventional fungicide PCZ on different citrus cultivars. Previous work also reported the
383 curative ability of these salts against postharvest disease caused by *Penicillium* spp. on a
384 variety of citrus species and cultivars (Montesinos-Herrero et al., 2016; Moscoso-
385 Ramírez et al., 2013a,b).
386 To date, the mechanisms by which some GRAS salts inhibit the development of *G. citri-*
387 *aurantii* on infected citrus fruit are unclear. Working in vitro, Talibi et al. (2011) reported
388 inhibition of arthrospore germination and mycelial growth in culture medium and found
389 that the antifungal activity was affected by the pH modification induced by the salt
390 solution, but this was not the only direct cause. They discussed that a reduction of the cell
391 turgor pressure with shrinkage and collapse of the hyphae and arthrospores or the
392 alteration of the cell-transport function with inhibition of enzymes involved in the
393 glycolytic pathway may explain the antifungal activity of the salts. Nevertheless, it has

been repeatedly demonstrated that the in vitro inhibition of postharvest fungal pathogens by salts cannot anticipate their actual efficacy as postharvest treatments to control disease in in vivo conditions. Besides the direct effects on pathogens, the indirect effects of the treatment on the citrus fruit host need to be considered and, in this sense, multiple interactions between salt residues and constituents of the citrus rind in the infection courts may have an important role in creating unfavorable environmental conditions for the pathogen, such as osmotic stress or biosynthesis of active antifungal compounds, and possibly increasing the tissue resistance to disease (Smilanick et al., 2020; Youssef and Hussien, 2020). Both direct effects on the pathogen and indirect effects on the fruit host have also been described as the mode of action of heat to control citrus postharvest diseases, particularly green and blue molds on oranges and mandarins (Palou et al., 2001, 2002a). Additionally, such effects have also been reported as the cause of the synergistic activity observed between heat and GRAS salts such as sodium carbonates and PS for disease control (Montesinos-Herrero et al., 2009; Palou et al., 2001, 2002a; Smilanick et al., 2008). Moreover, heating aqueous GRAS salt or food additive solutions to high temperatures improved the efficacy of postharvest dip treatments by increasing the permeability of the membrane and facilitating the entrance of the antifungal active ingredients (Montesinos-Herrero et al., 2016). In the present study, GRAS dip treatments heated to 50 °C improved the control of citrus sour rot compared with dips at 20 °C in a synergistic effect similar to that previously observed for PS combined with heat (Smilanick et al., 2008) and also for the control of citrus *Penicillium* molds with different GRAS compounds (Palou, 2018). Nevertheless, this benefit is not general, but dependent on the properties of each particular GRAS salt and the application conditions. For instance, Moscoso-Ramírez et al. (2013a,b) found that SEP and SMP dips at 50 °C for 60 or 150 s were equally effective to control green and blue molds on oranges than dips at

20 °C. However, the efficacy of these SEP and SMP dips significantly increased when applied at 62 °C, but fruit dipped at this temperature showed minor heat phytotoxic injuries on the rind surface. Indeed, the use of heat needs to be precisely controlled because there is generally a very narrow range between effective and phytotoxic temperatures (Fallik and Ilic', 2020; Palou et al., 2001). Solutions heated to excessive temperatures could easily damage the fruit tissue, causing rind browning and/or pitting. Besides, excessive heat has also been associated with the development of off-flavors on citrus fruit (Irtwange, 2006). Moreover, from a practical point of view, application at high temperatures may complicate the commercial adoption of postharvest treatments in citrus packinghouses, since implementation and application costs of heated solutions containing GRAS salts or conventional fungicides would be considerably higher than those of non-heated solutions. Other important factors that may play a role on the feasibility to commercially adopt GRAS salts as alternative antifungal treatments in citrus packinghouses are dip treatment duration time and the convenience or not of rinsing the fruit after dip treatment. Our results with SMP, SEP and SB solutions applied at 20 °C showed no clear effect of prolonging the dip length from 30 to 60 and 150 s on the effectiveness of these salt applications. Similarly, Moscoso-Ramírez et al. (2013a,b) observed that the curative activity of SMP and SEP solutions against citrus green and blue molds was similar after dips at 20 °C for 60 and 150 s. Working with sodium carbonates for *Penicillium* decay control, Smilanick et al. (1997) found that, although the influence of dip duration was significant, the solution temperature was an even more important factor. In the present work, the influence of rinsing the fruit after treatment with tap water on the efficacy of GRAS salts was dependent both on the salt and the citrus cultivar and it was not significant in the case of the most effective treatments (SMP and SEP on oranges). Conversely, it was very significant in the case of SB on mandarins. As observed

in prior work, if the effectiveness is not considerably affected, rinsing the fruit with tap water after dip treatment is a good practice to prevent deposition of salt residues on the fruit surface and avoid potential phytotoxicities and adverse effects on fruit quality, especially during long-term storage (Larrigaudiere et al., 2002; Montesinos-Herrero et al., 2016).

Regarding disease development during long-term cold storage, SB (3%) and SMP (200 mM) dip treatments at room temperature (20 °C) for 60 s were as effective as commercial PCZ treatment to reduce sour rot on artificially inoculated ‘Valencia Late’ oranges, treated and stored at 5 °C for 2 months. This is an interesting result that shows the value of these GRAS treatments to replace the conventional fungicide treatments used for sour rot control on late season cold-stored citrus fruit. It is clear from these results that the in vivo growth of *G. citri-aurantii* at 5 °C is rather limited, as disease incidence and severity on control fruit (wound inoculated with a high inoculum density) after 7-8 weeks at this temperature was only 50-60% and 15-20 mm, respectively; and this fact surely plays a definitive role on the high in vivo efficacy of GRAS salts on these conditions. Sour rot incidence results obtained with PCZ were similar to those obtained by McKay et al. (2012), who reported that dip treatments with PCZ at 256 µg/mL on grapefruit 18 h after artificial inoculation with *G. citri-aurantii* were highly effective, with only 10% of treated fruit showing typical symptoms of sour rot.

In summary, this study showed that dip treatments with aqueous solutions of SMP (200 mM) or SB (3%) for 60 s at 20 or 50 °C have potent curative activity to control sour rot of citrus fruit. Although this antifungal effect is highly dependent on the citrus cultivar and these GRAS treatments show limitations related to their limited fungicidal activity and their non-polluting nature if compared to conventional fungicides, they could be conveniently tailored for particular commercial applications. Another approach to

counteract such limitations could be their application in combination with other alternative physical, biological or non-polluting chemical disease control methods to obtain additive or synergistic activities and control disease through a multiple hurdle strategy (Wisniewski et al., 2016). Since citrus sour rot is a disease that at room temperatures can rapidly spread from decayed fruit to adjacent healthy fruit, it would be interesting to assess in further research the ability of these GRAS salts to stop or slow down fruit-to-fruit spread. Furthermore, another aspect that deserves further research is the ability of aqueous solutions of these salts to kill or inactivate microorganisms of food safety concern potentially present on the surface of citrus fruits or within the solutions themselves. Currently, this is especially important in the USA, under the Food Safety Modernization Act (FSMA) established by the US FDA. Thus, it would also be interesting to determine the compatibility of the solutions with common fruit sanitizers such as chlorine, chlorine derivatives and peracetic acid. Besides their potential as substitutes of the fungicide PCZ, recently banned in the EU, other advantages are that these salts are also able to significantly reduce other important citrus postharvest diseases, such as green and blue molds, and that their commercial application in citrus packinghouses as aqueous solutions would not require significant changes in the facilities and equipment currently in use by the fresh citrus fruit sector. Being classified as GRAS compounds or food additives, the specific registration of these salts as postharvest antifungal treatments for citrus could be more easily pursued by the industry and, if this is the case, they could be successfully deployed in citrus packinghouses according to the regulations and safety standards of fruit for human consumption.

Declaration of interest

None

CRedit authorship contribution statement

Lourdes Soto-Muñoz: Investigation, Formal analysis, Validation, Visualization, Writing - Original draft preparation. **Verónica Taberner:** Investigation, Formal analysis, Visualization. **Beatriz de la Fuente:** Investigation, Formal analysis. **Nihed Jerbi:** Investigation, Formal analysis. **Lluís Palou:** Conceptualization, Methodology, Resources, Supervision, Writing - Review and Edition, Project administration, Funding acquisition.

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Figure captions

Fig. 1. Reduction of incidence (A) and severity (B) of sour rot on citrus species and cultivars artificially inoculated with *Geotrichum citri-aurantii*, dipped 24 h later in aqueous solutions of sodium methylparaben (SMP, 200 mM), sodium ethylparaben (SEP, 200 mM), sodium benzoate (SB, 3% w/v), potassium sorbate (PS, 3% w/v), or propiconazole (PCZ, 0.6% v/v) for 60 s at 20 °C, and incubated for 8 d at 28 °C and 90% RH. Disease reductions were determined with respect to control fruit treated with water (incidence of $90 \pm 10\%$ and severity of 50 ± 13 mm for all cultivars). Values are means of 4 replicates of 10 fruit each per treatment. For each variable and GRAS salt, mean values with different uppercase letters indicate significant differences among cultivars. For each variable and cultivar, mean values with different lowercase letters indicate significant differences among GRAS salts, according to Fisher's protected LSD test ($P \leq 0.05$) applied after an ANOVA to the arcsine-transformed values. Non-transformed means are shown. Asterisks indicate non-registered data.

Fig. 2. Effect of dip temperature on the effectiveness of aqueous solutions of sodium methylparaben (SMP, 200 mM), sodium ethylparaben (SEP, 200 mM), sodium benzoate (SB, 3% w/v) and potassium sorbate (PS, 3% w/v) to control sour rot on 'Barnfield' oranges artificially inoculated with *Geotrichum citri-aurantii*, treated 24 h later and incubated for 8 d at 28 °C and 90% RH. Inoculated fruit were immersed in the treatment solution for 60 s at 20 (▣) or 50 °C (▤). Reductions of disease incidence (bars) and severity (solid diamond shape) were determined with respect to control fruit treated with water (incidence of $73 \pm 17\%$ and severity of 40 ± 14 mm). Values are means of two experiments with 4 replicates of 5 fruit each per treatment. For each variable and GRAS salt, mean values with different uppercase letters indicate significant differences among

dip temperatures. For each variable and temperature, mean values with different lowercase letters indicate significant differences among GRAS salts, according to Fisher's protected LSD test ($P \leq 0.05$) applied after an ANOVA to the arcsine-transformed values. Non-transformed means are shown.

Fig. 3. Effect of dip duration on the effectiveness of aqueous solutions of sodium methylparaben (SMP, 200 mM), sodium ethylparaben (SEP, 200 mM), and sodium benzoate (SB, 3% w/v) to control sour rot on 'Oronules' mandarins artificially inoculated with *Geotrichum citri-aurantii*, treated 24 h later, and incubated for 8 d at 28 °C and 90% RH. Inoculated fruit were immersed in the treatment solution for 30 s (▣), 60 s (□) or 150 s (▤) at 20 °C. Reductions of disease incidence (bars) and severity (solid diamond shape) were determined with respect to control fruit treated with water (incidence of 100% and severity of 57.8 ± 4.4 mm). Values are means of two experiments with 4 replicates of 5 fruit each per treatment. For each variable and GRAS salt, mean values with different uppercase letters indicate significant differences among dip durations. For each variable and dip duration, mean values with different lowercase letters indicate significant differences among GRAS salts, according to Fisher's protected LSD test ($P \leq 0.05$) applied after an ANOVA to the arcsine-transformed values. Non-transformed means are shown.

Fig. 4. Reduction of incidence (bars) and severity (solid diamond shape) of sour rot on 'Navelina' and 'Valencia Late' oranges and 'Oronules' clementine mandarins artificially inoculated with *Geotrichum citri-aurantii*, dipped 24 h later in aqueous solutions of sodium methylparaben (SMP, 200 mM), sodium ethylparaben (SEP, 200 mM) or sodium benzoate (SB, 3% w/v) for 60 s at 20 °C, and non-rinsed (▣) or rinsed (▤) with tap water

at low pressure for 5 s after dip treatment. Evaluations were performed after of 8 d of incubation at 28 °C and 90% RH. Disease reductions were determined with respect to control fruit treated with water (incidence of $90 \pm 12\%$ and severity of 60 ± 9.0 mm for all cultivars). Values are means of 4 replicates of 5 fruit each per treatment. For each citrus cultivar and GRAS salt, mean values with different lowercase (incidence) and uppercase (severity) letters are significantly different according to Fisher's protected LSD test ($P \leq 0.05$) applied after an ANOVA to the arcsine-transformed values. Non-transformed means are shown.

Fig. 5. Incidence (A) and severity (B) of sour rot on 'Valencia Late' oranges artificially inoculated with *Geotrichum citri-aurantii*, dipped 24 h later in water (control:●) or aqueous solutions of sodium methylparaben (SMP, 200 mM:▲), sodium benzoate (SB, 3% w/v:■) or propiconazole (PCZ, 0.6% v/v:◆) for 60 s at 20 °C, and cold stored at 5 °C and 90% RH for 8 weeks. Values are means of 4 replicates of 5 fruit each per treatment. For each variable and evaluation date, means with different letters are significantly different, according to Fisher's protected LSD test ($P \leq 0.05$) applied after an ANOVA. Incidence values (%) were arcsine-transformed. Non-transformed means are shown.

Figure 1

Soto-Muñoz L.

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Figure 2

Soto-Muñoz L.

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Figure 3

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Figure 4

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Figure 5

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